

**Fourth MIM Pan-African Malaria Conference:
Pregnancy-Associated Malaria
Yaounde, Cameroon
November 17, 2005**

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[START RECORDING]

DIANE TAYLOR, Ph.D.: [Inaudible] Yaounde. I thought I would present to you the results for studies from here in Cameroon. I will begin with talking about something about the epidemiology of malaria here and then move on to talk about studies. We've been doing on placental malaria. I believe that we need to know something about the epidemiology of the disease we're studying in pregnant women in order for us to be able to interpret our unillogical results. So, that's why I'm beginning there.

The epidemiology of P-fal suffered in pregnant women here in Cameroon is similar to that in all other African countries where malaria is transmitted throughout the year. Malaria, at least in terms of being asymptomatic infections, slide positive for malaria, is higher in pregnant than non-pregnant women. The prevalence is also higher in primi gravidas than in multigravida woman. This we often think of as due to being pregnancy-associated immunity and also younger women are more susceptible to malaria than older woman, so that here, at least in Cameroon, we are finding a fairly large age-dependent immunity as well.

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Let me show you what I mean about age and parody-dependent immunity. Here in Cameroon, P-falciparum is transmitted throughout the year where we have two wet and two dry seasons. In the city, we have approximately one infectious bite per month. In the outer areas where transmission is much higher, it is actually equal to approximately one infectious bite per day.

Now [inaudible] the literature on malaria and pregnancy has taken place in high-transmission areas, so the [inaudible] data in the city where transmission is low may look a little bit different.

This literature summarizes a great deal of information and the citations of the publications are down here in the lower right-hand corner. What we see here, we have in white squares that would be the slot prevalence of slide-positive malaria in non-pregnant individuals. You can see there that pregnant women have a higher prevalence of malaria than non-pregnant women during the first, second and third trimester and that the highest prevalence is during the first trimester, at least under high-transmission conditions, but in the city, the situation is similar, but it is not statistically significant.

The full parody and age we believe are risk factors here. If we look at our high-transmission area, we can see that

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primi gravidas have about a 2.1-fold increased risk of having malaria compared to multi gravida and that women under 20 years of age have about a 3.4-fold risk of infection. In the study, the situation's a little bit different. We did not find a significant difference between primis and multies in their risk factor, and there are number of reasons to explain that; we can talk about that at the end if you choose. Certainly always we find that women who are younger are at an increased risk of infection.

To illustrate this point, we can see here is that this is the percent that are slide positive for malaria be it in the placenta, the peripheral blood or either the peripheral or placental blood. You can see that this increases with age such that under age 20, 35 percent or more of the women will be slide positive at the time of delivery, whereas you can see a decrease with age.

The situation is clear that malaria does have an impact on the mother and on the developing baby. If we look at the impact of malaria on maternal anemia, we can see that women who have malaria at the time of delivery, the prevalence of anemia is approximately twice that of women who do not have placental malaria. However, we find that the anemia is through all different pregnancies and all pregnant women are more susceptible to anemia. This [inaudible] a little bit with from

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low birth weight and preterm delivery, PTD, in that women with placental malaria are at about a two-fold risk or the prevalence is approximately two-fold higher in those who are malaria positive than those who are from malaria-negative at delivery.

Therefore in summary, both parity and gravidity in age are independent risk factors for placental malaria here, and that's both pregnancy-associated and we believe age-dependent immunity may be involved and that malaria does have an impact on maternal anemia, low-birth-weight babies and preterm deliveries.

Now the prevalence of infection is actually higher than that shown in those previous slides in that, if you look at the time of delivery, approximately 82 percent of the women were PCR-positive for plasmodium falciparum. Of those, 27 percent were slide positive, i.e. parasites were detected in either the peripheral blood or the placenta. Therefore, 54 percent had some microscopic infections. Roughly about twice as many women actually are infected as we detect in our prevalent studies. By PACR, we have approximately 7.6 percent of the women having pre-malaria infections in addition to P. Falciparum and about 2.5 percent of the women having P. ovale in addition to P. falciparum.

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If you look in the peripheral blood and placenta of women at delivery, and you ask, "How many different parasite genotypes do they have?" Including in the PCR-negative women, we find that they have approximately [inaudible] but shown on the slide based on genetic typing for MSP1 and MSP2. On average, we have about 2.4 parasite genotypes in peripheral blood and 2.5 parasite genotypes in the placenta at delivery, but this is not gravidity dependent; we did not find a decrease in the number of circulating genotypes, so it appears that pregnancy-associated immunity reduces parasitemia to submicroscopic levels in many cases but does not eliminate different genotypes.

This has served in some [inaudible] of our epidemiological information that we're going to move from is that many women in Yaounde, even though we have one infectious bite per month, half of these from infections many have submicroscopic infections. They average about 3.4 parasite genotypes in the peripheral blood to 3.5 in the placenta, but the genotypes and the parasites in the placenta and the peripheral blood are different. So, one is actually harboring somewhere around six to seven different parasite genotypes, and I found this to be frankly interesting because when indeed the transmission is in one infectious bite per month, that means women are actually getting infected and maintaining their

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parasitemias, keeping them at a low level, but it doesn't look they were really eliminating them completely.

There are actually two major areas of research that are currently going on today looking at placenta. Of course everyone knows placenta malaria leads to an increase in adverse pregnancy outcomes including preterm deliveries and low-birth-weight babies. Those two major areas are sort of the path of physiology associated with low birth-weight babies, and this is going to be the bulk of my talk today. I'm going to talk about the histology and cytokine changes that take place in the placenta as it relates to low-birth-weight babies and preterm deliveries. I'm going to just have three slides on the sequestration of parasite, and this is the VAR CSA story, and Steve Rogerson as actually going to talking about this in his invited lecture.

Now this is the immunohistological section of placentas. This one is an uninfected placenta; I've actually drawn these in so we can some orientation. These are the syncytiotrophoblasts, the fetal tissue; these are red blood cells in the intervillous space, and here is one lonely macrophage that has stained the CD68 [misspelled?]. Over here however, we see all the different parasites within the red blood cells, and each of these are macrophages that have accumulated in response to infection.

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So what are the hallmarks of placental malaria? Is the accumulation of monocytes macrophages in the intervillous space? To try to understand how this was happening, we asked the question. If you take maternal white blood cells from the intervillous space, and you take these from placentas that are negative for malaria at delivery, so this you might think of as normal maternal white cells within the placenta. We took this and we place those in culture, and we added malarial antigen. We asked the question, "Would those cells in the intervillous space respond to malarial antigen and would they produce Chemokines, and would they produce cytokines?" When we did that, we found that they secreted highly-significant amounts of beta chemokines. Beta chemokines are attractants for macrophages and monocytes, so that made sense, but we did not find alpha chemokines, which are neutrophils. We also found that these cells would respond by secreting TNF alpha and IL10.

While we know that maternal cells are not the only cytokine players in the placenta that fetal cells or trophoblasts can also secrete cytokines. So in this part of the study, we took a piece of fetal tissue here; we took the X gland of fetal tissue, we placed it in culture, and we added malarial antigen. We asked, "What would be the response from the fetal component?" We found that the fetal cells

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trophoblasts would produce interfering gamma, but not the other cytokines that are shown above.

So those were essentially our findings from in vitro. We wanted to look in vivo, so we studied as many in this room have done, cytokine levels in the plasma of the intervillous plasma, and we found a significant increase in TNF alpha, interfering gamma and Il10. This is very similar, but many of you here have also found. We certainly know that one source of the interfering gamma is the trophoblast, and we think that other T-cells can be involved as well. We believe the macrophages another primary source of TNF alpha and the IL-10.

So now we knew that malaria would give us some change in pathology. We knew it would give us change in cytokines. How does this relate to adverse pregnancy outcome? This is the very first study we did which was published in 2001 in which we had placenta histological sections and plasma from a number of consecutively-collected placentas, and we selected all of those we had at the time which was 65 from low-birth-weight babies under 2,500 grams. We did get a frequency matching of the normal-birth-weight babies and looked at these, coded and looked at them. What we found from the malaria-positive, low-birth-weight group that they had evidence of chronic infection, macrophage infiltration and hemozoa pigment and increasing macrophage and increasing TNF alpha. That was the only

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cytokine we actually looked at in this study. So, this is what we saw in the low birth-weight babies compared to the normal birth weight babies. Then we went on to look further at the histological section in that when the placenta is under stress, the placenta will respond by angiogenesis and increase the diameter of blood vessels and increase the number of blood vessels in order to support transport of nutrients and wastes across the barrier. We looked at the number of fetal blood vessel per villi and the diameter of fetal blood vessels. What we found was a significant increase in both the number and the diameter of the fetal blood vessels, but they were present equally in all malaria-infected placentas compared to non-malaria-infected placentas. It appears that the placenta has the ability to respond and remodel its architecture to respond to the malaria and that it is not associated with low birth-weight babies.

The next issue we wanted to look at was malaria in preterm deliveries, and here again we had different samples from 83 mother-infant pairs with preterm delivery, and we again get a case interest study design where we pair these with full-term deliveries, FTD. When we did that, our group that was premature, the average length of gestation was estimated to be 33 weeks versus 40 in the full-term group. When we looked at these groups cross-wise, they were the same for age, for

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secundi and primigravidas and use of anti-malarial drugs, but the striking feature was the percentage of women who had anemia in the malaria-positive preterm delivery group.

This slide summarizes what we think was the major risk factor for preterm delivery in that anemia and parasitemia is greater than 1 percent. Thesis of the lower axis is these are unaffected women with anemia and non-anemia, less than 1 percent and greater than 1 percent. Those who are not anemic are about the same, but those with high preplacental parasitemias who are anemic were at a 3.5-fold increase risk for having preterm deliveries. So we believe that here it means those are the biggest risk factors. We also found elevated IL-10 and a very high IL-1- to TNF alpha ratio, and the data supporting that statement is shown on this slide.

In this case we measured cytokines in the intervillous space plasma. If you look at full-term, malaria-positive and negative, preterm delivery, malaria-negative for TNF alpha, they have approximately 45 picograms. There is a significant elevation in TNF alpha to about 65 picograms, so yes it is statistically elevated, but I think you might argue that you can question maybe if it is indeed biologically relevant. There is a minor increase in TNF alpha, but what is amazingly striking is in women with malaria positive at preterm delivery is the big spike in IL-10. I think you can see it [inaudible].

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There is a tenfold increase in IL-10 and placental plasma of women with preterm deliveries who have parasitemias.

What do we think has happened? What we think is the antigen stimulate macrophages to make beta chemokines, which have trapped macrophages, or at least retained them in the site with the intervillous space. They secrete TNF alpha and IL-10 as over production of IL-10 in preterm deliveries, there is an association we know between IL-10 and anemia which is one of our risk factors and the fact that IL-10 could indeed partially down regulate inflammatory cytokine-type responses, and if some of these are down regulated, this could lead to increase in parasitemias.

I put this on to remind myself to lead to the next slide. That is just to say that 45 percent of women at delivery in our study period had parasitemias over 1 percent. Certainly not all of these women are going to have preterm deliveries, and even if you factor in anemia, we wanted to ask the next question which was, "Why do some women have preterm deliveries and others do not?" In this case, we calculated that possibly promotor polymorphism was important. So, we looked at TNF alpha as well as IL-10 promotor polymorphisms, and if you have a change in G to A, you have an increase here in TNF alpha production. If you have the GCC/GCC haplotypes, you have an increased production of IL-10. What we did was typing of women

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who had preterm deliveries and full-term deliveries with and without placental malaria to see if this correlated with preterm delivery and these outcomes. We actually found that for TNF alpha that the frequency of the -308 was higher and those were malaria positive with preterm deliveries compared to the other study groups, but only 2.2 percent of the population actually had this genotype. In some ways, it seems that the TNF alpha is not that much over produced; it seems like with this it is a very small fraction. We were kind of feeling that TNF alpha may not be the predominant player here but that IL-10 is.

Looking at all ten from the standpoint, if you look at women who have the GCC/GCC polymorphism, the frequency is statistically higher in women with preterm deliveries and malaria positive, and it is in the other three groups. So the frequency of this is significantly higher.

Also when you look in the serum, the serum level of IL-10 is higher by fivefold, and those who have malaria compared to those who do not have malaria for preterm deliveries, thus if you have a preterm delivery, it's higher in those women who have placental malaria, not in those who do not. The same thing is a fourfold increase in malaria-positive preterm delivery over full-term delivery. This is not just pregnancy

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associated; it is just not malaria associated; it is malaria-pregnancy associated.

Also I had said that IL-10 can feedback and induce anemia, and the women who have the GCC/GCC haplotypes, have lower packed cell volumes. The packed cell volume was 29 versus 34 in the other groups. Therefore, there is an association here with anemia as well.

That is sort of the cytokine story and histology story, but I wanted to also bring in a little bit about more CSA from a slightly different standpoint than you are used to hearing. You all know that CSA coats the surface of the fetal villi, and as malarial parasites circulate into the intervillous space, they bind to CSA and antibodies can block this binding. Well some of us incisally kind of wondered about if CSA was supposedly associated with this [inaudible] trophoblast, which are now here circled in yellow; if these express CSA but the parasites all are out here. We often find the parasites in the intervillous space, so the ligand and the parasite didn't quite seem to get into the same position. This is some work we did with Chonia Gad [misspelled?] as well. Even on histochemistry, using antibodies against CSA, he demonstrated that CSA is associated with insitional trophoblast; you can almost see spots of this looking like it maybe in caveoli, at least the trophoblast membrane. Also, it is associated with a fibrinoid

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ventral in between in the intervillous spaces where the red cells are circulating. Therefore, now the parasite and the ligand are in the same location.

Last but not least, I made a major statement at the beginning that age-associated immunity was important as well as gravidity-dependent immunity in placental malaria. So the question is then antibodies in addition to those VAR CSAs importance. What we did was we look at antibodies immune responses to other antigens and ask the question, "Did they help control or reduce placental malaria?" In the study, we used an inhibition of binding assay to CSA and found that the more antibodies you have, the lower your placental parasitemias are, which was great. We also found a hit of that if you looked at antibody responses against a crude extract of malaria suggesting that there could possibly be some other antigens there as well, because this extract was not produced from a VAR-binding parasite. On the other hand, we found that MSP1-19 antibodies against that correlated with infection. Women who do not have antibodies, and I repeat this, do not have antibodies against MSP1-19, are in a significantly higher risk of having placental malaria.

In my final [inaudible], what we think is happening here then is as these motor-nucleus macrophages migrate into the intervillous space and start feeding cytokines, TNF alphas

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know to regulate the disposition of fibrinoid material. Then, as CSA is being produced, it binds to this material allowing the parasites to bind. Antibodies that against the VAR genes either keep the parasites from being bound by trophoblasts or the fibrinoid deposits or possibly mediate killing of the parasite in the placenta. I don't think any of us are totally sure that the parasites are actually eliminated by leaving the placenta and then being carried in the spleen after being killed in situ. We also believe then that antibodies that block the other invasion steps, that of parasites could be important as well, and we're looking for other antigens that might be able to produce antibodies against that could be important in controlling malaria.

Thus in conclusion, I think that the field has moved greatly. Many of you have very similar data to these for your study sites. We have learned a lot in the last five years, but on the other hand, I believe we have a very, very long way to go.

I would like to acknowledge and thank the organizers for inviting us to present our material today and share it with you about malaria here in Yaounde. I would like to thank all the women who participated in these studies. We would like to thank NIH for their financial support and their advice and for the [inaudible] National Center for Maternal and Child Health

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Grant that help support us and others to continue their career in malaria and pregnancy. Thank you.

[Applause]

FEMALE SPEAKER 1: [Inaudible]

MALE SPEAKER 1: [Inaudible] –and you said that the genotypes of plasmodium in the peripheral and the placenta are different genotypes.

DIANE TAYLOR, Ph.D.: MSP1 and MSP2.

MALE SPEAKER 1: And you [inaudible] there is a separate punctuation in a sense—a genotype that separates the punctuation of parasites in the placenta from peripheral -

DIANE TAYLOR, Ph.D.: –I’ve discussed interpretation of these results, and if you look at about four or five other papers, they’ve have found the same thing: that genotypes in the placenta are different from those in the peripheral blood. The question is, “Are they two different populations, or is it just in a sampling error, that you have different genotypes?” I have no idea which way that works, but I just know that the women have more parasites than it appears.

MALE SPEAKER 1: [Inaudible]—a moment to conduct a test. [Inaudible] and we looked at the data and we looked at the data to different datas that the [inaudible] of [inaudible]. [Inaudible].

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DIANE TAYLOR, Ph.D.: First of all, this discussion does not take me by surprise. When you do multi-varied analysis of all of our data, the biggest correlation with preterm delivery is anemia. Bar none. Anemia is the biggest correlation with preterm delivery. A multi-varied analysis in placental malaria drops out. So anemia is the key factor. What we believe, is that malaria is one of the things that is contributing to the anemia. Micronutrients, nutrition, diet, pregnancy—a number of things are contributing to anemia as well. That is what we think is happening. The only time we see this in a case-controlled study design where we have matched for as many variables as we possibly can, we've drawn from the same pool of individuals matching these factors.

Now I'm going to continue one thing: In the literature, just in terms of pregnancy, anemia is a risk factor for preterm delivery, and there's currently now thought to be an association between IL-10 and promotor polymorphisms in preterm deliveries in general. It may not be malaria-specific, but the only data we have is for malaria, so we don't want to push this further beyond malaria, but it may be a specific finding in general.

MALE SPEAKER 2: I would like to come back to the middle of preterm delivery because the conditions here show that TNF alpha was [inaudible], and we know that TNF alpha is a

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prostaglandin cytokine. We know that during inflammation, we can have an invasion of [inaudible], and [inaudible] and know being principle of erythro constructions at any time of the prostaglandins. The only thing that can have an influence on that preterm delivery?

DIANE TAYLOR, Ph.D.: I missed one word and you said it twice, so I'm a little short on the question. Basically if you look on spontaneous abortions, TNF alpha is elevated, and it doesn't matter if it's malaria-positive or malaria-not. So, elevated TNF alpha has been associated with spontaneous abortions. What we think is probably happening in the situation that you have TH1 TH2, sort of modulation going on to give you a successful pregnancy, and we honestly has believed that we would find high TNF alpha in our preterm deliveries; we though that was just going to be the answer. So when we had marginal elevation in TNF alpha, we were initially surprised when we saw the big spike in IL-10, we were flabbergasted. This data was a little bit different than we though of initially until we started developing the model after we got the data. I'm not sure that answered the question, but I tried.

MALE SPEAKER 2: I wanted to associate that to the traditional prostaglandins.

DIANE TAYLOR, Ph.D.: That's the word I can't understand. What's the last word?

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MALE SPEAKER 2: Prostaglandins-

DIANE TAYLOR, Ph.D.: Prostaglandins. Thank you. Thank you. I have absolutely no data of prostaglandins, but that would be something we obviously want to do. Thank you.

MALE SPEAKER 2: [Inaudible]-is that true only for low- and intermediate-transmission areas or also for high-transmission?

DIANE TAYLOR, Ph.D.: Could you just repeat the front half of that question? I just couldn't hear it.

MALE SPEAKER 2: Are age and parity both risk factors in just low- and intermediate-transmission areas or also high-transmission areas.

DIANE TAYLOR, Ph.D.: In all transmission areas as far as I know.

[Interpose]

MALE SPEAKER 2: [Inaudible].

DIANE TAYLOR, Ph.D.: Certainly, I don't believe there's a study out there that has not shown that primigravidas are no more susceptible. Every study out there has shown primigravidas are more susceptible than multigravidas with a few exceptions. Age has been the big question, and many people feel that age is not involved and it appears here that it is.

[Interpose]

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MALE SPEAKER 1: [Inaudible] were gathered from areas [inaudible].

[Interpose]

MALE SPEAKER 1: [Inaudible]. Transmission is and inability to [inaudible].

DIANE TAYLOR, Ph.D.: Is that because the transmission is so low that they simply don't have enough immunity for the second time they're infected, because they're not infected in the first pregnancy? So if you're in your third pregnancy, but you're still a primigravida unilogically.

MALE SPEAKER 1: Yes.

DIANE TAYLOR, Ph.D.: Okay.

MALE SPEAKER 1: [Inaudible]

DIANE TAYLOR, Ph.D.: I think we are going to give it to Adrian Luti [misspelled?].

ADRIAN LUTI: It's the first defense, try and try again. I'd like to extend my congratulations to those [inaudible]. I wanted to come back to this whole story about the pathophysiology and immunology of anemia and how you think that might be related to elevated IL-10. As you probably are aware, in acute symptomatic infections in children in Africa, the common denominator there is elevated TNF and reduced IL-10. I know that there are pathophysiological explanations for how

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that might be working with TNF. I have absolutely no idea how it my be working for IL-10.

DIANE TAYLOR, Ph.D.: To be honest with you, standing up here on a box that is wriggling that I cannot give you that answer, because it doesn't come to mind, that I have thought it through and the post-doc who worked on this did. It is actually in that publication with our discussion of why we think it works this way. So, I'll refer you to our paper. I'm sorry.

[Applause]

DIANE TAYLOR, Ph.D.: Actually, I think I've been doing it this way.

MALE SPEAKER 4: Thank you very much. Thank you.

SABINE GIES: Thank you very much. Good afternoon everybody. I'm very pleased to present some preliminary data from an intervention study, actually ongoing in Burkina Faso.

In Burkina Faso, malaria is [inaudible] endemic with highly seasonal transmission. The rainy season is from June to October, and for the moment, we keep kloequine is used for malaria prevention in pregnant women even if the national strategy is going to change next year. They are going to implement, but until know kloquine is used.

The next slide.

In intervention studies, we are actually studying new approaches—how to get IPT to break the limit in rule settings.

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Based on the local knowledge on malaria and pregnancy, we decide the promotional messages targeted to pregnant women and trying to convince them to get to antimalaria clinics early and to get the required two doses of SP. We randomly selected twelve health centers in the ruler [misspelled?] district and attributed them to three studies arms. One is IPT and promotion. The second study is IPT alone, and the third one with the kloequine. We started these interventions in April 2004. IPT was given in the health center since April 2004. The development of the promotional campaign took a bit longer, so the promotion started only around in August-September in the communities and in the health centers.

On the whole we follow a population of 75,000 people, and we make the follow up only of first and second pregnancies. To identify the target population, the field workers do monthly visits in the villages and try to identify all pregnant women and apply a questionnaire and then choose the first and second pregnancies to enroll in the study. They take at the first visit, fundal height to estimated gestational age and kypholage dates for the follow-up visits. The biological samples we take are at two levels. We take them at the health centers at the first antenatal visit of the woman. Substantially, we take a thick film at antenatal clinics just before giving an anti-

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malarial drug. So, they get the thick film and then get either SP or kloequine treatment doses.

When the woman delivers in the health center, we also take samples from the woman, thick film, PCV and placental smears from the maternal side of the placenta. At community level, the field assistants do home visits around 32 weeks gestation to take also blood film and PCV. In case of the home delivery, the field workers go to the home as soon as possible after delivery; sometimes they are there at delivery, so we have blood samples from the mother and sometimes from the placenta from home deliveries. All these samples are organized in the district hospital and Boromo which is from the farthest village about 80-kilometers away, so distance and transport is a big issue. All women found positive for malaria at 32 weeks of delivery get a treatment after examination of the slides.

Next slide please.

The results I want to share with you today are baseline data and preliminary data; I just got the database about three weeks ago, so the analysis is very fresh. It's about 472 women who have delivered before November 2004. This table is just to show where the symptoms come from. It's just to give an idea about the numbers of symptoms we have, so in antenatal clinics, we have 243 slides from antenatal clinics and most of them before June, actually from the dry season and samples from 32-

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weeks gestation and delivery, most of them from the rainy season starting in July.

Next slide please.

In this population, median age was 19, very young population, first and second pregnancies. More than half of them were less than 20 years old, more than half of them first pregnancies, most of them were married. About a quarter had any school attendance. They were asked whether the household had a bed net. So, bed nets were present in about 20 percent of the households. Less than ten percent of the women said having slept under a bed net the previous night of enrollment, not of [inaudible] pregnant data from the dates we have blood samples; it's just at enrollment. About 60 percent of the deliveries took place in a health center, 40 percent were home deliveries.

Next slide.

So if you look at all age-prevalence, malaria-prevalence data at first antenatal visit, we have an overall parasitemia prevalence of 30 percent, which is highly different between the dry season and the rainy season. We have 16 percent in the rainy season and more than 50 percent during the dry season. At the time of delivery, most of the women, almost 85 percent, had at least one antenatal visit.

Next slide.

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The prevalence of malaria at 32-weeks gestation was 32 percent overall and is represented in the right bars. In the dry season, it was about 24 percent and is going up straight to 44 percent during the rainy season. The blue box shows anemia prevalence; overall anemia is defined by PCV below 33 percent, and the overall anemia prevalence was 52 and this is going up in the dry season from 42 to 65 in the rainy season. If you just look at moderate and severe anemia, which is PCV below 30 percent, the red bars included in this numbers of overall anemia. So, the proportion of moderate and severe anemia is about one-third in the dry season and is going up to more than half of the anemia cases in the rainy season. Severity of anemia is increasing with the season.

Next slide.

No. That's not the next slide. No. This one.

The findings at delivery are similar, but even more marked for the parasitemia data in the rainy season. So, you still have peripheral blood parasitemia in white, so it's around 10 percent in the dry season at delivery and is going up to 50 percent in the rainy season. Placental malaria is following the same trend, less than 10 percent in the dry season and almost 50 in the rainy season. Anemia is also getting more severe and more frequent in the rainy season.

Next slide.

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If you look at birth rate, birth weight from health-facility deliveries where we have birth weight data included, you find that babies from primigravidae have a lower birth weight, and the proportion of low birth-weight babies from primigravida is almost the double of those in secundigravidae. I think this indicates that malaria is a great contributor to this low birth weight.

Next slide.

To come to an end, we found that malaria burden is very high in first and second pregnancies in our area. We have relatively good coverage of antenatal care, but we still have very high parasitemias—about 50 percent in the peripheral blood and in the placenta at delivery. Anemia prevalence is also high and severity increases during the rainy season, and [inaudible] data indicate that malaria is a big problem.

Our group, we hope, that the IPT and promotional campaign will lead to much better research, which I may be presenting in another talk, another time. Thank you very much.

[Applause]

MALE SPEAKER 5: [Inaudible].

SABINE GINES: Yes.

MALE SPEAKER 5: [Inaudible] age and also malaria infection because then you have for primigravidae and

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secundigravidae. You are talking about malaria, the roll of malaria in low birth weight.

SABINE GIES: You want to know if birth weight data linked to age of the woman and to malaria slides.

MALE SPEAKER 5: I would like to know if -

SABINE GIES: No, but as I told, I've just got the data set and did not have much time to analyze data. So, it's not finished.

MALE SPEAKER 5: My next question is about anemia, and do your studying in Burkina Faso, so what can you say about the element? Is there eminance in these populations? This can play a role in it as well; that's what I am doing. You think about it.

SABINE GIES: I'm very sorry. I don't hear you very well.

MALE SPEAKER 5: I'm talking about [inaudible] elements which can play a role in anemia during pregnancy as well compared to malaria. I don't know if you got that for this [inaudible].

MALE SPEAKER 6: Thank you for good presentation. I actually have no question, but the questions need to be asked. [Inaudible] has seen her in France, last September. [Inaudible] need to ask you used koloequine as a prophylactic. [Inaudible]. How did you treat it? [Inaudible]. How did you

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treat it? Another question: If it had been treated by [inaudible]. [Inaudible] less weight, low birth weight in a primigravidas. Is this from statistical [inaudible]. If you get a good, live baby, the mother cannot do you any good. [Inaudible].

[Laughter]

MALE SPEAKER 7: [Inaudible] or your nurse's assistant then you are going to deliver these women in their homes. I just kind of wanted to get clarification in just this one area. I was curious to know what that meant. Thank you.

SABINE GIES: Just to answer some of the questions. I think it's a truly a point that [inaudible] infection contributes to anemia in this region. I don't have data; we don't collect stool samples, but I think it's one of the co-factors which we may find. If I cultured all women, and in this baseline data, they actually find 472 women where it's close to a mixture of prevention. Most of them started pregnancy before our intervention, so they may have started antenatal before kloequine and then got SP some time later or even did not get the SP. So, the prevention isn't this group is a bit mixed. The kloequine group, they were treated with a kloequine treatment dose, and in the SP group women were treated with quinine treatment dose. The issue about increasing birth weight and increasing problems at delivery is a big issue

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in the population actually found in the preliminary studies that women are afraid to deliver a big baby, so there's a big issue if you explain that you give another drug to increase the growth weight, they actually won't take it. In the promotional campaign, we don't emphasize birth weight, but emphasize a healthy baby. They would deliver a healthy baby, and we had no major problems for the moment with the women included in the study.

The home deliveries actually home without trained assistants. There are women delivering at home sometimes with Tva, sometimes just with a neighbor or parent, and if we have data, it's because out field workers live in the same villages, and they would be there for the samples. But, they are not trained for medial issues. Thank you.

[Applause]

A KFUTWAH: Thank you very much. I would like to thank the presenters for giving me this opportunity to talk on something very different.

In 2000, Centra Pasteur in collaboration with some other national bodies here in Cameroon allowed us permission of the minister of public Health to initiate a program to start the reduction mother-to-child transmission of HIV using Nevirapine. We showed that nevirapine could reduce mother-to-child transmission by more than 50 percent. However failure of

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[inaudible] to reduce mother-to-child transmission was related to one of three factors. That factor was related to the mother, which was the high viral loads. The other factors are related to baby which has a low birth weight and [inaudible]. Interestingly, we had a factor that was related to the environment, which was rainfall. We observed in this study that the relationship between mother-to-child transmission of HIV and rainfall, as shown in this chart from 2000 up to 2002. The peaks in rainfall are shown here in the book. Three months after the peaks of this rainfall, we observed that there were peaks in mother-to-child transmission of HIV. We therefore suspected the implication of a pathogenic agent that is endemic in Cameroon related to the rains and the role of malaria was highly suspected. Further more, studies have showed that plasmodium falciparum stimulates the secretion of peripheral metacytokines such as TNF alpha and IL-8 in the placental environment. We therefore [inaudible], plasmodium falciparum in the placental environment conceived the cytokine profile to more important [inaudible] cytokines. This could increase HIV of the patient in the placenta and increase HIV local viral load, and therefore increase the risk of mother-to-child transmission of HIV.

In order to better understand the cytokine profile, HIV transmission and malaria in the placenta, we recruited a group

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of HIV positive and negative women, and from this we made just before delivery, we collected maternal blood and then immediately after delivery, we got the placenta. From both maternal blood and the placenta, we did take thin blood smears to look for malaria parasite. Also from the maternal blood, we did a whole blood count searching for the viral load for the HIV positive and also sort for other co-infections. We also looked for cytokines in the placenta both analyzed on the [inaudible]. These results are seen and we're not able to present here today.

Next slide.

However, we already observed that there were similar chances in distribution of malaria between the HIV positive and HIV-negative group. This suggests that both HIV positive and HIV-negative women are equally exposed to malaria and also that there was high parasitemia in the HIV-positive compared to the HIV-negative group both in the peripheral as well as in the placenta. We also observed that there were reciprocal discrepancies that is there were women, some women, who were positive for malaria in the periphery and negative in the placenta and also visa-versa. Also there was a tendency of a higher mother-to-child transmission of HIV in women who were co-infected.

Next slide.

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We therefore went ahead to do in vitro studies. We used a recently-developed system on the placenta is a culture system. This system was developed in 86 percent [inaudible] capable of getting placenta fragments in culture for up to ten days. Previous studies already show that these cells of placental origin are resistant to cell-free viruses. This, however, using a [inaudible] virus that has an HIV genome, and that is variable of the fecal hepatitis virus. We could bypass this resistance and also sell acetated viruses [inaudible] on infected placenta fragments. Most of roles [inaudible] is rated to be some of the harmful effects of placental malaria. We, therefore, using the placenta is a culture system, was to study the effects of TNF alpha on HIV replication in the infected placenta.

Next slide.

This new system, the placenta histoculture, after extensive [inaudible], the infected placental fragments, HIV virus, the sclerotic virals, and these virals contain an HIV genome and in cooperating in this HIV genome is a reporter gene quoting for [inaudible], which we used for quantification for HIV replication. The envelope of vesiculas to matites virus are felt overnight infection, placental fragments were washed and cultured as seen here on [inaudible]. In the medium we're having final concentration on TNF alpha 5 and 59 of them

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respectively meet a control without TNF alpha. These cultures were left for 48 hours, 96 hour and 120 hours.

We had two systems of revelation of how HIV replication in these placenta fragments. The first system, all fragments from the serum were put in the tube and ten lies homogenized, and using an illuminator, we looked for luminescence in the tissue lysis. Also we adopted and imaging system known as the imaging system. This system consists of a camera and a series of optical lenses that is used to observe bioluminescence in living animals.

Next slide.

Our results showed that over time, dependent effect of TNF alpha on this placental fragments as noted by our luminescent results here, and at [inaudible] TNF alpha showed increased suspicion of HIV. In this imaging system, as shown by [inaudible] shows here, those who were infect with HIV [inaudible] envelope, these were [inaudible] kind of control where HIV id deficient, and the envelope was used on the infected virus. Here, there was no TNF alpha and here, we had found no amounts of TNF alpha. We observed that there was significant increase where there was five noted on the TNF alpha and HIV [inaudible]. In fact, about threefold increase.

We therefore concluded by seeing that TNF alphas significantly increase HIV replication the placenta. Going by

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these results we went ahead to use [inaudible] plasmodium falciparum that is CSA binding. Briefly, [inaudible] we infected just [inaudible] HIV [inaudible] envelope, and we kept the fragment in contact with infected red blood cells and a control of uninfected blood cell in a shaker for about one hour. After this, we learned that only histocultures helped to find these. [Inaudible] preliminary results were not consistent, and were not really permissible. We have a representative of ETF of 0.2 nanogram of HIV and also with defenders we could see some effect of uninfected red blood cells, and we did not think that there was a lot of viral ability between blood cell type and red blood cells that we used in the study. These studies are ongoing and we're trying to standardize and enable to study the effect of Plasmodium on HIV replication directly.

Next slide.

In conclusion, therefore, we can see that mild activation by co-infection stimulated the secretion of TNF alpha, and we are shown here that TNF alpha increases HIV replications in human placenta, and these could increase mother-to-child transmission of HIV. These can decrease the efficiency of preventative studies that are used to prevent mother-to-child HIV as observed in Cameroon. Therefore, HIV

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mother-to-child transmission and malaria prophylaxis could be adjusted according to local and environmental irregularities.

Next slide.

I want to thank our collaborators both nationally and internationally, especially the Unite de Regulations des Infections Retrovirals and also Unite d'immunologie Moleculaire des Parasites, and IPP. Thank you.

[Applause]

MALE SPEAKER 8: Nice studies that may eventually lead to something very interesting. What I missed in your presentation was associated with the apparently elevated risk of mother-to-child transmission and the presence of falciparum parasites, and I missed the data on viral loads. Were the viral loads in the women with infected placentas also elevated?

A KFUTWAH: The first part of the presentation?

MALE SPEAKER 8: You had on you list of data that you looked at viral loads as one of your parameters. So, I wonder if the viral loads in the women who had infected placentas who were also associated with higher risk of mother-to-child transmission were higher.

A KFUTWAH: Yes. Several studies have showed the viral load is associated with mother-to-child transmission to begin with. This study, that part of it as I said, is analysis as to our statistician, and there are a lot of correlations that are

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supposed to be made such as the viral load and placenta infection and the cyto cine expression.

[Applause]

STEPHEN ROGERSON: I would just like to thank the organizers for inviting me to come in and out of the year and over a day where the wild things are to get here, but it's been a really enjoyable making and I'm glad I made the trip. What I'd like to do is to talk to you about some work we've done recently looking at two aspects of the parasites causing placental malaria, the genes they're expressing into the protein on the surface and how it's recognized.

Next slide. Thanks.

We know that there's a large burden of malaria in pregnancy in Africa with over 20 million at risk each year. A number of reasons for that, and these include an increased risk of mosquito bites when you're pregnant. This reactivity-dependent susceptibility which is elegantly showed is associated with lack of any energy to placental parasites is one possible cause for it. There are other immunological factors such as the alterations acquired and accelerated immunity. We haven't paid much attention to hormonal changes as one of the questions suggested nor to some of the mechanical factors operating in the placenta.

Next slide. Thanks.

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We know that you can frequently find parasites in the placenta, many of them looking like they may be adhering to the syncytiotrophoblasts, others that are out here in the intervillous spaces as Diane has showed us.

Next thanks.

For some years, we've been able to study the phenomenon of adhesion in different ways in vitro. This is a Chinese woman's ovary still expressing CSA with malaria-infected cells binding to it.

Next, thanks.

Then when we come down to the molecular level, we've been able to learn a lot about the receptor-ligand interactions that may be mediating this process, and this is a cartoon of the infected red cell with the knobs in the membrane, and this PfEMP1 molecule expressed at the knobs which has been shown in many studies by many different groups for about 20 years now to be able to interact with different receptor molecules in which the most important that you probably can't see are CSA and sialic acid which we think are important in this interaction in the placenta.

Next slide thanks.

So, we've heard from [inaudible] yesterday about PfEMP1, and he's come up with a very similar slide to this one for his talk yesterday. I'll just run through it again for

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those of you that weren't there. To PFEMP1 is responsible both for adhesion and for antigenicity on the surface of the red cell or is a major cause of both of those properties, and it's the most clearly defined we have.

Go back please.

It's encoded by these VAR genes of which there are up to 60 per genome, and we know that if you take on parasite clone, it has a rate of switching between different VAR genes which we can make use of another VAR tree by selecting the parasites which expressing different adhesive genotypes such as these shown down here. When we do that, we find that we get different VAR genes, including those adhesive types. A certain VAR genes or set of VAR genes has been associated binding to CSA.

Next slide, thanks.

So this is the sort of a diagram of a representative of VAR genes showing that there is a two-axon domain, one representing the intracellular portion of the molecule, the other the extracellular part that's responsible for the adhesion. In this gene, there's a DBL1 alpha and CIDR region which are conserved in almost all, all but one VAR gene in the 3d7 gene have this. This factor was a stumbling block for a long time to understanding malaria in pregnancy.

Next slide, thanks.

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It wasn't until Alley Salante [misspelled?] showed that there was another gene which increased VAR2csa which was up-regulated in placental parasites that we suddenly, what we hadn't been able to find out before, started to make a lot more sense.

Next slide, thanks.

This has worked for Mike Duffy in our lab looking at VAR2csa using two different techniques, that of real time PCR and northern blot. What we did was to take the 3d7 parasite line and select it in two different ways for CSA adhesion. Both time we found that we got very high levels of transcription of VAR2csa without any VAR gene being increased in its [inaudible] numbers, and that when you look by northern blot in the CSA-selected lines of the red circles, you can see that the main VAR gene is this one here, and when you use a specific probe to VAR2csa only the CSA-binding parasites show this VAR gene.

Next slide, thanks.

We were interesting having went with these laboratory mice to go on and say, "What VAR genes are expressed in placental malaria?"

Next, thanks.

So, Mike Duffy designed some degenerate oligonucleotides with could ampliphy both the DBL gamma region;

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this was done in the CS-2 VAR gene that John Rite [misspelled?] described and also a DBL-3x domain of VAR2csa.

Next slide, thanks.

This shows us the primers, and most of the data's been obtained with these two primers which, when you see these stars here, that means there's a very similar sequence between VAR2csa and various DBL gammas sequences that have previously been associated with CSA binding. With these primers and also this set of primers, when we couldn't get any results from the other ones which don't react with VAR2CSA, so we don't amplify that CSA with those.

Next, thanks.

This shows even these outside primers on 3d7 genomic DNA where we expect to get 13 DBL gamma domains plus VAR2CSA, and we found with 40 different clones, we got nine of those 13 plus one across the VAR2CSA which is this one here, which suggests that they're reasonably unbiased primers for these purposes.

Next, thanks.

To look at the expression of VAR genes in [Inaudible] from malaria, we took peripheral blood from a number of children and placental blood from pregnant women. We didn't put them through intervening culture because we wanted to see what was there in the samples we got straight out of the patient. We expected

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RNA and to perform reverse transcription PCR. In some instances, we also looked at DNA just to show our primers weren't selectively amplifying some specific genes. When we got our assay PCR product, we cloned it and then sequenced a number of clones to see which genes we could find being expressed. When we'd found those sequences, we looked to see what they related to in the database and also used the sequences, if they were new ones, to design oligonucleotides we could use for the realtime PCR.

Next, thanks.

So this is the result of the reverse transcription which is a sort of a more qualitative examination of the samples. Using those outer primers, we found we could detect VAR2CSA in ten out of ten placental isolates and in nano for childrens isolates. All together we found DBL gamma domains in a total of 19 placental isolates which included some of the candidates that other people have put forward over the years, and I'm including the VAR CS2 gene, the FCR-3 VARCSA or VAR1CSA gene and this 3d7 chromosome-5 which is quite similar to the 720 isolate that [inaudible]. We also found a number of other different DBL gamma sequences. The finding of this diversity of sequences doesn't necessarily mean they're all important, and so we went on to try and work out which were the most abundant using realtime PCR. We did that on 13 placental isolates and

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six childrens isolates. In 12 of the 13, we found VAR2CSA to be the most abundant VAR gene. We also, interestingly and unexplained, found VAR2CSA to be the most abundant of the VAR genes we found using this approach in two of six children isolates. Overall, we found a dominance of VAR2CSA in placental isolates and of the DBL gamma sequences in the children.

We were also interested in the possibility with increasing gravidity there might be other VAR genes that could be responsible for placental sequestration in multigravidity who would have perhaps early-developed immunity to VAR2CSA, and when we looked at primi gravidity and multigravidity we found that both had VAR2CSAs, the most predominant VAR gene.

We also found that low levels of this VAR CS2, interstingling, in many of the placental isolates and in none of the children. We found the FCR-3 VAR in 12 of 13 placental isolates, so it probably relates to its unusual transcription or profile that it continues to be transcribed in [inaudible] stages.

Next, thanks.

This shows the VAR genes that we detected, looking at DBL-3x and DBL gamma showing the three main candidates which were the VAR2CSA, the SCR-3 VAR and the VAR CS2 plus any other, what we call indogenous gene, a gene that was specific to that

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particular patient and showing the relative abundance of the different transcripts in the circle. These are placental samples along here, and in all of these, the black boasts the highest one, except for one here, which has the VAR CS2 as the more dominant VAR gene, although VAR2CSA is also detected. These numbers here show the differences in gravidity, so both gravida ones and gravida five or sixes had VAR2CSA being predominant. In the children's isolates, in four of them, there were other genes—none of these candidates, but other than that VAR genes were apparently dominant, and in two of them, there was VAR2CSA as the dominant gene. I should say we did not look at DBL alpha sequences, and they could be genes, which have DBL alpha and not DBL gamma, which were more dominant than any of the genes we did have to detect.

Next, thanks.

This is expressing that data in a different way. Looking at the expression of these different genes and trying to control for parasites state because we know that [inaudible] express much less VAR transcription than to ring stages, and so we controlled for this by finding a gene, which is the skeletal binding protein, which has a very similar profile to the VAR genes and normalizing for that gene. When you do that, you find that there's no real change in the relationships that

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VAR2CSA is dominant in placental isolates, and it is still the dominant gene in these two children's isolates.

Next, thanks.

You probably can't read it or see anything much of this spider's web or bullet mark in the windshield, but the main point this makes is that you can see the little tiny red spots which are sequences derived from placental C DNA, and you'd see many of these red spots are busted here. There's a green blurb which is the VAR2CSA gene. This really shows how the sequences relate to VAR2CSA, so many placental isolates clustering here, whereas children's isolates, which are in blue, are sort of rather spread around. There are quite a lot of these red symbols sort of representing placental sequences scattered around the whole of this tree, which aligns with different sequences we found. Many VAR2CSA-like genes caught a lot of others we could find.

Next, thanks.

Unfortunately, I couldn't make a proper sort of pictorial representation of the sequence alignments so I could shine on Alley Salante's poster outside, but overall we found that if you look for lining at the VAR2CSA sequences and looking for at least 80 percent identity, there was around about 60 percent conservation in this region of the gene that

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we looked at. So, it's not highly conserved when you compare it to vaccine candidates like MSP119 or AMA-1 even.

Next, thanks.

I'd like to move on now to talk a bit about PFEMP1-related work.

Next, thanks.

This is work from James Theeson [misspelled?] primarily in the paper that's coming out shortly in the *Journal of Infectious Disease* looking at western blots to look at the proteins that we can find, the PfEMP1 proteins that we can find. Firstly, three different CSA-binding laboratory lines are shown here, and all of them have this same, not necessarily same, but assay of this high-molecular-weight transcript which is characteristic of PfEMP1, and they all look like they're about the same size, whereas this isolate which binds to CD36 and I-CAM1 has a different pattern on western blot. So, all our lab lines look the same, but when we look at some placental isolates here, you can see that here's the CS2 line, which is sort of positive control here, and there are bands that look pretty much the same as CS2 in all of these lines, and there are also other bands with different migration. We don't know whether that represents different sequences that have a VAR2CSA that migrate differently or it represents different PfEMP1 molecules.

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Next, thanks.

We've also looked at trypsin and digestion-like modern Neilson has placed around outside looking at this because we found very early on that VSA binding unlike binding to most receptors was crimson resistant. So this looks at the CS2 isolate in which we first described that, and when you do trypsin digestion, followed by western blotting, you frequently find some PfEMP1 remains because some of the PfEMP1 is inside the red cell. When we try psin [misspelled?] digest the CS2 we can find that these two bands appear. When we take this line, HCS3, we had a different pattern with much more bands and three of these seven CSA lines we get a different pattern again from CS2, so differences in the trypsin digestion pattern.

Next slide, thanks.

On the right here, this is looking at the effects of trypsin digestion on binding to CSA with the same three lines. Again, you can see there's a diversity in the affects of trypsin digestion on how much binding remains with the CS2 being limitedly resistant up to 100 micrograms with very little change. The 3d7 selected on CSA is showing a dose-dependent reduction and poor old HCS3 having hardly any even at a low concentration of trypsin. There are obviously difference in epitypes that are being expressed, and the susceptibility of the molecule to trypsin digestion.

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This is a different bit of data, which is looking at these two isolates again, S2 and HCS3, and using an antiserum, we reacted against CS2 which recognizes the surface of the infected red cell in about 95 percent of CS2 parasites and about 85 percent of the HCS3. So, it shows cross reactivity at the level of antigenicity. When we incubate parasites with this antiserum, only the CS2 is blocked from binding. This shows that one in ten CS2 has no binding at all in the presence of the antiserum, and as we dilute it out, we do see binding coming, but the HCS3 doesn't show that. Again, despite recognizing antigens on the surface, the antibody is having quite different effects on binding which may be more important for preventing placental sequestration.

Next, thanks.

This is looking again at these same two isolates in relation to Agglutination, which is a test, which shows sort of [inaudible] since [inaudible] invented the Fex [misspelled?] assay that does correlate quite well with the Fex assay [misspelled?]. This is looking at those samples from women of different gravidity that recognize one or other of these two isolates, and you can see that in the black as gravidity goes up, more and more women recognize both of the isolates. Whereas here, in ray and the stifle bars respectively, samples which were recognized either only CS2 or only HCS3. Particularly in

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first pregnancy, some women recognize one of these isolates, but not the other.

Next slide, thanks.

I think this is perhaps more encouraging that there is a reasonable level of conservation between the two in the terms of functional effects of antibody, and this is looking at adhesion in addition, so if there's no inhibition, you see a line across here.

The main point I wanted to make is that in many of these sera, there's a relatively similar level of adhesion inhibition for the two different isolates using the same serum, but there are a few outliers such as this one here where there's low, quite marked, inhibition of the VS2 isolate and very little of the HCS3, and these two here, which show the opposite pattern. Again, there is evidence of probably maybe conserved approaches, and a lot of these isolates fit in some diversity.

Next, thanks.

This states a relatively small number of samples but looking at placental isolates, direct from the placenta without intervene culture, and the pairing them to CS2 and we can see that here, there's a much more nephrogenous sort of picture. I think there may be factors other than the serum we incubated, the parasites that are coming into play here. We take parasites

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out of the placenta without intervening culture, but I think this is an area for perhaps warrants a bit more exploration.

Next, thanks.

I'd like to summarize by saying that firstly we found like others have that VAR2CSA is the most abundant gene expressed in the placenta, and we've done this by trying to take a relatively unbiased look based on looking at all the possible candidates that had been previously describes as potentially mediated placental adhesion. It's not the only placental VAR gene to be expressed however. We've also found that some children's parasites, and I think the Copenhagen found the same thing, expressed VAR2CSA, and the reason for this is still, to me, unclear. The VAR2CSA sequence is quite highly conservative; it's not dramatically highly conservative. How important this is, I guess we really don't know at the moment, but looking at Alley Salante's poster outside will shed a bit more light on that, I think for those of you who haven't seen it.

When we look at the protein level, there does seem to be some differences in PfEMP1 proteins expressed by placental isolates. Unfortunately, we don't have gene sequence information on those same isolates; we haven't been able to do the two on the same thing to see what that actually means. There seems to be both diversity and conservation in terms of

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the epitopes expressed by parasites expressing VAR2CSA and of the host responses to them, and I think for now perspective is probably still quite a bit of work to do in terms of unraveling that. I can see from talks and presentations here, that a number of groups are really making good progress in doing that. So, thanks for you attention.

[Applause]

MALE SPEAKER 9: A couple of things that occur to me that may be part of an explanation. Maybe you can comment on it. Maybe there's an element of hierarchy in terms of the expression of these isolates that may bind in the placenta, because for the most part what we see at the end stage of delivery which is where we usually collect those parasites. That's one thing.

Just going back to what Diane told us about the amount of multiplicity that we see in the placenta versus periphery are not always, or maybe not ever, the same could also be that a certain part of the parasite population in the placenta represents those from the periphery that just happen to be trafficking through, and you pick them up on the microbe.

STEPHEN ROGERSON: Okay. To us to answer the second part of your question first, we mostly in these studies, we used purcol [misspelled?] purifying mature stages for the placental examination. So, you wouldn't expect any rings with passes of

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the placenta which would confuse things quite a bit as you say because they have a lot more VAR expression to have been present in out samples after the purcol purification. To come back to the first part of your question, the hierarchy I think is an interesting question, and I don't know; I'm presuming people are trying to look at switching rights and why that CSA does seem to be so successful in placental malaria. Presumably it is a VAR gene that is turned on quite frequently in parasites to that the parasite is in the right place and switches to this gene and has that ability to infect a placenta and accumulate there. Is that your question basically?

MALE SPEAKER 9: Yes, thanks.

MALE SPEAKER 10: I wanted to ask you. Did you study the surface proteins of the placenta [inaudible] for reactivity with VAR2CSA specific regions? You know that these VAR2CSA proteins—is the slice the correct one? Does it react with VAR2CSAs probes.

STEPHEN ROGERSON: Right. I think James has done a little bit of work in collaboration with Alley Salante and a lot more on this topic that I think to me it seems as though the stiff proteins are expressing; these proteins do react with VAR2CSAs, specific reagents. I guess that answers your question, I hope.

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MALE SPEAKER 11: Thank you for a good talk. Regarding the transmission that you find in the serum parasites, have you also found children isolates and also some laboratory isolates that has a high level of-

STEPHEN RICHARDSON: I can't hear you very well.

MALE SPEAKER 11: It's true that we have also found serum parasite and laboratory isolates that have a high VAR2CSA transcription but no translation. You can't find the protein on the surface and can't find any CS2 blocks. I think it's because VAR2CSA is the only [inaudible] has the oxygen over reading frame that makes a translation control of this protein. I think it is important also to look at, as Matt says, specific protein [inaudible] instead of only transcription because there is a translation control of this particular gene.

STEPHEN ROGERSON: Okay, thanks.

[Applause]

NICAISE TUIKUE-NDAM Thank you, Mr. Chairperson. My talk on [inaudible] to focus on malaria during pregnancy and particularly on parasite, fetal characterized in those pregnant infected women. [Inaudible] numerous studies [inaudible] adapted parasite lines in order to understand [inaudible] sequestrations. Although these 30 studies found out several features characterizing placental isolate, fresh placental isolate, fresh isolate collected from pregnant women had never

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been studied in depth for [inaudible] characteristic of VAR2 transcriptions. The aim of our study was to analyze fresh placental isolates in order to figure out the ability to interact with human placental human CSP chondroitin sulfate [inaudible] that we previously purified from human placenta as well as to study the VAR genes transcription in fetal isolates.

Next slide.

The study was conducted in Segal in the Adolf [inaudible] in the suburb of [inaudible], which included 50 pregnant women, 50 women at delivery and 36 nonpregnant women were also included in the study. We collected parasite delivered from the placenta by fluid, infected red blood cells from the placenta, and those parasites collected from the peripheral blood were allowed to mature. Then to obtain the mature infected red blood cells. Those major parasites, we injected a low to bind to CSA or CSBG and we also from the surface we got surface antigens using assay of plasma sample that we obtained in a previous study conducted in [inaudible]. This set of plasma samples was composed of plasma samples from women of different gravidities. At the same time, samples were also stored as [inaudible] or in [inaudible] for subsequent RNA and DNA extraction that were used for genotyping, sequence analyses and quantification of gene expressions.

Next slide.

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What we obtained at the level of MSP genotyping was that all the samples from pregnant women as those from nonpregnant women were [inaudible]. The multiplicity of infection was roughly the same between the sample collected from the gene sample belonging to different groups. A high [inaudible]infection was much more frequent in pregnancy-associated malaria as compared to isolate from nonpregnant women. When we quantify each genotype using fluorescent PCR, we also observed that it was always a dominant of the MSP2 genotypes, and samples from pregnant women did more fighting. Some kind of sequestration during pregnancy [inaudible] malaria as compared to infection in nonpregnant women. [Inaudible] all the samples obtained from the placenta or [inaudible] extract showed CSA, but when chondroitin sulfate [inaudible] we produce it [inaudible] and the ability to bind each receptor was [inaudible] isolates and in analyzing these variations, parasites, with regard to the ability to bind to CSA was not [inaudible] demise as those were classified as high binders from the median line. We could devise from the median as high binders and low-bind parasites. We observed that parasite isolated from pregnant women the event of low birth weight were more likely to interact with CSPGs or CSA as compared to those parasites isolates from women who developed normal birth-weight babies. This suggests that ability of the parasite to interact

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with CSPG might be an important risk factor for the occurrence of low birth weight.

We also studied the recombination of [inaudible] antigens on some of the isolates. Six isolates were used in this study, and they're representative from three out of the six isolates we used. For all six isolates, by measuring the antibody anti-VSA and the study of infected erythrocyte, the level anti-VSA antibody was [inaudible] as for all the six isolates there was a parity dependent profile of the anti-VSA antibodies. We also measured the ability of those plasma to interact, to interfere, with adhesion to CSBG and for six isolates we used to measure this only one out of the six isolates showed a parity dependency, which interfered with the CSA binding possibly suggesting that the antigenic targets [inaudible] and floccytometry are probably not the same.

The correlation between the level of antibody measured by floccytometry and the ability to interfere with adhesion to CSA was not often correlated. The only correlation was found on in two out of the six isolates we used. We were interested in studying the VAR transcription in isolates, and many further studies are being prepared to study the families of the VAR genes, the VAR1CSA and the VAR2CSA has been involved in the pathogenesis of pregnancy-associated malaria. Most of these studies rely on laboratory-adopted lines and the qualification

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of each of these genes, the genes collected in each of the PfEMP1 was not quantified in natural isolates prior to these studies.

It is specific primers [inaudible] PCR that could target with limited bias the VAR1 genes in PfEMP1 and the VAR2CSA genes. We designed a primary in the region of the genes. What we observed was that the transcription of the VAR2CSA was consistent in all the placental isolates as compared to isolates from nonpregnant women. This high-developed VAR2CSA transcription was also found in the [inaudible] peripheral blood suggesting that secreting end stage in pregnant women to a large extent was rated from the major parasite localized in the placenta. For the VAR1CSA the transcription level was similar between parasites from nonpregnant women and parasites from pregnant women, and this does not argue for the possible involvement of the VAR1CSA in the mechanism of binding CSA or in the pathogenesis of pregnancy-associated malaria as opposed to the VAR2 transcription, which has significantly high transcription by [inaudible] placental isolates [inaudible] from the peripheral from the [inaudible] as compared to [inaudible] isolates.

We also analyzed the level of VAR2CSA of those parasites as compared to the ability to bind to CSPG, the high binders were more likely to transcribe high level of VAR2CSA

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but not VAR1 suggesting the direct involvement of the VAR2CSA mechanism of interacting the CSPGs. We also produced au] from three of the VAR2CSA domains, DBL-1, DBF-5 and DBS-6 and looked for specific antibodies in this domain. In the plasma sample of the women were donated to the parasites, and we found an association between the level of the VAR2CSA transcription and the level of specific antibodies to VAR2CSA measured to all three domains. This specify of this relation was allotted by the lack of relation by the VAR2CSA transcription label and the majors of the MSP1 specific antibodies and the corresponding samples.

To summarize this study, I would like to contribute to the characterization of the pregnancy-associated malaria by showing that polychromal infections like in those infections of nonpregnant individuals, they were characterized by more dominant genotypes than in nonpregnant women. This could be explained by the selection these parasites [inaudible] pregnancy-associated malaria, and in the study [inaudible], women at that time were under chloroquine prophylaxis that can also help tear down some genotypes. The specific transcription of VAR2CSA is pregnancy-associated malaria is related to the ability of isolates to bind to placental receptors, so the product of this subfamily of the VAR genes are probably involved in the mechanism of interaction with CSPGs. The

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present detection of specific antigens to VAR2CSA in corresponding pregnant women, such as that this protein, this VAR genes are capable, the product of VAR2CSA are capable to induce specific immune response in pregnant women. Involvement of these antibodies to the mechanism of production is not widely studied. Taken together VAR2CSA PfEMP1 is involved in placental frustration and that [inaudible] in all our studies, it became that the rationale for making the vaccine to protect women from placental malaria using VAR2CSA is most rational way to develop these strategies. Thank you for you kind attention.

[Applause]

FEMALE SPEAKER 2: [Inaudible].

NICAISE TUIKUE-NDAM In transcription of VAR2CSA was [inaudible] dependent, the study wasn't designed for that as we selected women on the fact that they were infected at delivery, and we didn't find any association between the level of VAR2CSA transcription and the parady.

MALE SPEAKER 13: Thank you for your talk. That was very good.

I see you've made a strong case for VAR2CSA, but your data on VAR1CSA seemed to demonstrate transcription, both in placenta and from peripheral blood, so you excluded it. Why can't you make a hypothesis that VAR1CSA can bind in the

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placenta and in elsewhere? I don't think that data excludes it as another possibility.

NICAISE TUIKUE-NDAM: I forgot the conclusion on the amount of the VAR2CSA transcription accounting to the nonpregnant women group and pregnant women group. There was a significant difference between the two groups only for VAR2CSA, but no for VAR1CSA. This to me said that VAR1CSA doesn't show any specificity in pregnancy-associated malaria.

MALE SPEAKER 13: But we know that you don't need to have—it doesn't necessarily have, to be specific. When you take parasites from placenta you see this CSA binding. True, but you add a very large number of cells to your assays and only a small percent bind. Better to describe that the placental malaria contains some cells that bind to CSA, and I agree that CSA is clearly predominant, I think we'd all agree. But your data suggests that there are other genotypes presents, and it doesn't exclude the possibility that others may also be contributing.

NICAISE TUIKUE-NDAM: According to some data, the VAR1 genes most of time lack a specific silencing domain, and this transcription has been the most credited by other things to be common and [inaudible] about parasites, and it is doubtful that the transcription of VAR1 results in the protein translation.

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MALE SPEAKER 13: Well I think I would say that you've said that VAR2CSA appears sufficient for placental binding, but not necessary.

NICAISE TUIKUE-NDAM: Yes. Yea.

FEMALE SPEAKER 3: [Inaudible] in the peripheral blood of the pregnant lady is much more higher than that found in the peripheral blood of nonpregnant ladies which means to be that the complexity of infection is a [inaudible] of nonpregnant ladies and much more higher than the pregnant ones, which means that you'd be able to see all four-side pictures as the peripheral blood of nonpregnant ladies and those who are pregnant these others chances of [inaudible] or sequestered in the placenta. Would you agree with that or?

NICAISE TUIKUE-NDAM: Could you repeat please? I didn't catch the question.

FEMALE SPEAKER 3: You said that you find eight of the pregnant ladies or eight quality patients. Why do you find only two of the peripheral blood of nonpregnant? Which means that the complexity of infections of peripheral blood of nonpregnant is much more higher than those ones, which means [inaudible] of strains that could be found in sequestered in the placental due to VAR CSA binding.

NICAISE TUIKUE-NDAM: Yea. That's the conclusion of them, that peripheral [inaudible] in pregnancy-associated

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malaria much more probably the [inaudible] of sequestration in the placenta. I could side that maturation sites in pregnancy-associated malaria, it's much more placental which is not probably the same in infections occurring in nonpregnant women or nonpregnant individuals.

MICHAEL OFORI: Thank you. I will be discussing with you about some of the antigenic or antibody reactive for isolated [inaudible] collected from pregnant women in Guana.

As we have heard this afternoon, varieties of antigens are produced by the parasite, but expressed [inaudible] and then in these new studies of pregnancy malaria have been shown to correlate and stem from other diseases which we see in the children and also nonpregnant individuals. This has as we have heard made it possible for parasite [inaudible] to accumulate in the placenta. Studies have shown that this stays specific to placental malaria [inaudible] antigen. Fortunately also the VAR2CSA [inaudible] and is highly conserved between genomes. [Inaudible] suggest that there [inaudible] of having [inaudible]. So, that consisted out study question in this light.

To answer this question, we collected parasites from our [inaudible] where we collected parasites from the woman depending on gestation. There we cultured this parasite and did some addition [inaudible] so this could [inaudible]. Then

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we look at plasma antibodies and plasma from different time points through gestation, that is plasma correlated through recruitment. That is when women develop [inaudible] disease. [Inaudible] as the plasma correlated from when they are [inaudible] viewpoint as well as plasma from six months. Notice we are trying to look at the antibodies [inaudible] various antigens at this point. So we used [inaudible] we thought we'd measure the [inaudible] of this antigen-specific antibodies in the slice.

We are looking at the recruitment plus samples. And for this type of figure, what we are measuring is for each particular [inaudible] and CSA-specific IGG levels and these antigen levels are [inaudible] with increasing [[inaudible] from my [inaudible] and then you'll see that. What that means is that those we [inaudible] indicated very low levels of the [inaudible] was when you have a very [inaudible] and present [inaudible] antigen that was very highly recognizing.

On my diagram, you see that I have here [inaudible] 42 pregnant women at different time points. [Inaudible] recruitment by then doing gestation through a different, and then on [inaudible] I have parasite cultured from women at time points. [Inaudible]. So what I want to show is that I want to see how [inaudible] would recognize parasites at different time points during gestation to see if there were changes or

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[inaudible] variations in their [inaudible]. And on the same [inaudible], you see there was a very [inaudible] because there were half primigravida and [inaudible]. Though you have 70 gravida and half multigravida down the graph. So I want to show you, if you look at the graph, we realize that [inaudible]the graph and the diagram, you get more [inaudible]. Antigen [inaudible] this parasite is [inaudible]. Why is it fighting the disease, you get more antibodies [inaudible]?

Again, when you go across you realize then you have different parasite and then the levels of the colors of the [inaudible] down and that indicates that [inaudible] also aligned by the gestation collected in part [inaudible] gestation of [inaudible]. So we realized that when you get a parasite at any stage of gestation, many of the [inaudible] antibodies are able to recognize that parasite and [inaudible] as compared to parasite [inaudible]. So we have my recruitment [inaudible] realize that [inaudible] depends on a five [inaudible]. In recognizing that parasite depend on gestational age at which the parasite was collected.

So then I also look at the episode plasma realizing the [inaudible]. There again, you see that you'll have [inaudible] samples, samples here, but we hoped to increase to fill all these out. Then you realize that here again, you see that as you go down, the [inaudible] doesn't change much. But as you

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move along this line, I realize it's usually that the antibodies of this parasite is producing with the increase in gestation period from the time that we cultured the parasite.

Then also look at the plasma we collected at delivery. We see whether this [inaudible] but then but here we realize that we don't get any [inaudible], and the reason is that [inaudible] gestation [inaudible]. Even the primigravida women who experience [inaudible] malaria develop sufficient amount of antibodies, and therefore the antibodies [inaudible].

Then we went on to see [inaudible] plasma samples to see how the antibodies holds the key. Here again, if you compare these to the previous one, the one I just showed at delivery, you realized the pattern is now showing back again. So the question was why did this, so I think the idea is that most of the primigravidas seem to have [inaudible] really fast, and that [inaudible] probably is from primary immune response by then. [Inaudible] as compared to multigravida [inaudible] of these parasites pregnancies. The key is quite more or less villous a compared to [inaudible] from multigravidas just like this.

We also went onto see some of the ideas [inaudible] some of this process that we are looking at and realized that parasite collecting in pregnancy seems to adhere most [inaudible] collect at any part of pregnancy.

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So in completion now see that the antigen [inaudible] of the [inaudible] and then also this depends [inaudible] when gestational age was fully recognized like this. TO be sure that [inaudible] tested this [inaudible] note to CD54, CD66 and [inaudible]. [Inaudible]. We therefore [inaudible]variance line. Not always I will see nulligravida [inaudible] academic [inaudible]. The expression of these molecules for what we can do by the [inaudible] immunity and remind us [inaudible].

So I'll conclude by [inaudible] all this for who to guide for this study was done. I also want to say I thank the [inaudible] for providing [inaudible] for this day. Thank you very much for this.

[Applause]

MICHAEL OFORI: Yea, I think I [inaudible] some point. Looking at the binding [inaudible] and then also some of this actually [inaudible]. We also tried to see whether this [inaudible] part has been seen near completion as well as were they sex related so that you see if even men [inaudible] men. If the men are able to recognize this parasite and see [inaudible] of them, the men did not which shows this is really related to the pregnancy.

FEMALE SPEAKER 4: I just wanted I guess a comment and a question. Your conclusion was that maybe parasites have more than one VAR gene. You had some anecdote or evidence to suggest

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that by doing MSP1 and 2 typing we found some placentas where we had one clone to take to them; we could find more than one but to CSA transcript, but do you have any evidence also to suggest that these parasites actually exist because the genotyping in one patient is prone to all sorts of errors?

MICHAEL OFORI: [Inaudible]. We've had some [inaudible] and things to do that, but we haven't done that yet, so I cannot give you a good answer.

[Applause]

[END RECORDING]