Effect of parasite diversity and age on antibody responses

This study was aimed at investigating the effect of parasite diversity and age on the levels of antibody responses to *P. falciparum* in individuals living in an area of seasonal malaria transmission. Two blood stage antigens (MSP1 $_{(19)}$, AMA1) and two liver stage antigens (CSP and celTOS), making a total of four antigens were selected to be used for the study. These antigens were tested in archived plasma samples with ages ranging from 1year to 70 years. The samples were collected close to the end of the rainy season in the months of November and December (wet season), and April the following year at the end of the dry season (dry season).

Of the samples collected in the wet season, 34. 1% had parasites whiles those collected in the dry season was 15. 5% (table 1). Appawu (2004) reported the seasonality of malaria transmission in a neighbouring district (Kasena Nankana District), being high in the wet season and low in the dry season. The high transmission in the wet season could be due to favourable breeding grounds for mosquitoes as a result of several collections of water when it rains and in turn increase the number of vectors and hence the high number of parasites individuals are exposed to (Ahmed, 1989). This data is in agreement with an earlier finding in northern Ghana by Apawu and his group where they found high parasite carriage, and high multiplicity of infection (MOI) in the wet season compared to low carriage and MOI in the dry season. Parasite carriage here means the proportion of participants with *P. falciparum* at enrolment. There was however no statistically significant difference in the parasite densities when compared between the seasons (*p= 0529*) table 1. There was no difference in the overall ages between the

wet and the dry season. (p= 0. 937). This was expected as most of the samples collected were from the same individuals in the two seasons.

To explore age related pattern of *P. falciparum* infection, participants were categorized into three (3) age groups: under 5 years, between 5 and 15 years, and over 15 years. The data shows the 5-15 year olds having higher infected proportion in the two seasons (table 2). Parasitaemia was also determined using a more sensitive method, the polymerase chain reaction (PCR). The PCR data confirmed the microscopy data because the 6-15 year group also had higher proportion of individuals carrying parasites at enrolment. This could be due to the fact that they are the more exposed group with incompletely developed immune systems. In contrast, under-fives who are much younger are likely to receive better care from parents thus preventing them from being infected with the parasites. Bed net usage could be one way of protecting the children. Also because of their younger ages they may not be allowed roam around freely. Adults on the other hand despite having possible similar exposure as 5-15 year olds, seem to have less parasites because of their developed anti-malarial immunity. This partial immunity is gained with age and repeated exposure thus making the younger individuals more susceptible to infection (Pratt-Riccio *et al.,* 2005; Dodoo *et al.,* 2008). Thus the over 15 year group who are older, had the least proportion of infection (table 2).

The study also sought to determine the relationship between IgG levels and age. Figures fig 7 and 8 shows the correlation plots between age and IgG levels in the wet and dry season respectively. There was a significant positive correlation between and IgG levels raised against AMA1-FVO, MSP1,

CSP and CelTOS in the wet season. And for the dry season, the significant correlation was between age and IgG levels to AMA1-FVO, MSP1, CSP, and Celtos. The correlation coefficient (r^2) and the p values are indicated on each plot. There was however no correlation between age and AMA1-3D7 (r^2 0. 0142, *p 0. 0699*) for the wet season, and AMA1-FVO (r^2 = 0. 0114 *, p= 0. 0594*) for the dry season. The significant correlations were however very weak. This indicates that though IgG levels increased with age, the association between the antibody levels and age was not so strong and that the influence of age on the amount of antibodies produce against most of the antigens in the population studied was not that much. However the age dependent increase in IgG response to the antigens tested may be due to the mature immune system in adults, and could also be due to cumulative exposure to infection over time (Nebie 2008). It is however not known what might be the reason for the lack of correlation with age to AMA1-3D7, and AMA1-FVO, in the wet and dry season respectively.

IgG responses to the two AMA1 alleles (3D7, and FVO) was plotted against each other to determine which is more predominant in the study population. There was a strong correlation between the antibody levels of AMA1 alleles of 3D7 and FVO (r^2 = 0. 8382, *p <0. 0001*) implying that both strains are circulating in the study site during the wet season. A similar trend was observed in the dry season but with a lower correlation coefficient compared to the wet season (r^2 = 0. 5302, *p <0. 0001*).

Antibody titres against recombinant antigens have often been linked to protection from clinical disease (Polley 2004, Nebie 2008, Dodoo 2008,

Dodoo 1999, Cavanagh 2004). Where high anti-malaria antibodies is interpreted to mean protection from clinical disease. The important role of antibodies was demonstrated by the passive transfer of purified IgG from immune donors to individuals with *P. falciparum* infection, which reduced parasitaemia (Cohen 1961, sabchareon 1991). Apical membrane antigen-1 AMA1 and MSP1 $_{19}$ have been associated with reduced risk of clinical malaria (Branch 1998, Osier *et al* 2008). Anti-CSP antibodies have also been found to be partially protective where in Kenya high anti-CSP, anti-LSA, and anti-TRAP (pre-erythrocytic) antibodies were demonstrated to be associated with relative protection from reinfection (John CC 2005, 2008). The most advance malaria vaccine, RTS'S which is a subunit of CSP, has been found to protect to about 35-55% in children 5-17 months for about 8 months (Alonso 2004,) . Also anti-CelTOS antibodies have been shown to inhibit invasion of herpatocytes by sporozoites in mice (Bergmann-Leitner 2010). In this study, antibody responses to the antigens, AMA1-3D7, AMA1-FVO, MSP1 $_{19}$, CSP, and CelTOS were determined using indirect ELISA. The optical densities (OD) of the antigens were converted to arbitrary units (AU) where the highest OD for each antigen was awarded an arbitrary unit of 4, to allow for comparison between antigens the antigens tested. Antibodies to AMA1 in individuals living in malaria endemic regions have been reported to be high, (Thomas, 1994, chelimo, 2005), and this study thus reports high antibodies to AMA1-3D7, and AMA1-FVO compared to MSP1 $_{(19)}$, CSP, with anti-CelTOS antibodies being the least. (Fig: 3) in both wet and dry seasons. The data also shows total IgG responses in the wet season was higher than the in the dry season for all the antigens ($p < 0.0001$) except MSP1 $_{19}$ where no

statistical differences was found between the two seasons (*p= 0. 85*)
(Figure 3). The seasonal changes in antibody response could be as a result of
higher number of vector (mosquitoes) in the community in the wet season
which could in turn increase the exposure of the population to parasites and
also introduce new parasite clones/strains in the population and hence the
high anti-malaria antibodies found in the wet season. In contrast, the dry
season cause a reduction in the number of breeding sites for the vectors
reducing their population resulting in minimal exposure and hence the low
antibody levels.

In malaria endemic regions, the number of different clones of *Plasmodium*
falciparum parasites infecting a person could be a transmission indicator, an
indicator of the hosts immune status, and a useful parameter in evaluating
malaria control interventions (Babiker 1999, Arnot 1998 , mayengue 2009) .
It has also been reported that parasite diversity in high malaria transmission
areas are high and that individuals could carry multiple genotypes (clones)
but the opposite pertains in low endemic areas with most infections being
monoclonal (Peyerl-Hoffmann 2001, Bakiker 1997, Haddad 1999).
Genotyping was done using block 3 region of the *MSP 2* gene (Smythe *et al.*
1990) because of its high polymorphic nature (Felger et al., 1994 1999;
Robert et al., 1996). Also MSP2 was selected to be used for this study
because of the high allelic diversity observed in Ghana and other countries
bordering Ghana with as many as 154 alleles in Ghana and about 50
genotypes in Côte d'Ivoire. (Silue 2006, falk 2006). The results from the two
seasons using the 3D7 allelic family primers showed high mean multiplicity
of infection in the wet season compared to the dry season (1. 76, and 1. 46,

p= 0. 001) table 3. In both seasons, the number of infections per person ranged from 0 to 4. A similar degree of multiple infections has been reported in other African settings (Ntoumi et al., 1995, Beck 1997, Engelbrecht 1999). The samples used for this study was collected in asymptomatic individuals and the high multiplicity of infection found in the wet season could probably be due to high rate of exposure as a result of favourable breeding conditions compared to the dry season. This study did not find any significant correlation between MOI and age in both seasons (*p= 0. 5768* for wet season, and *p= 0. 4158* for the dry season). The samples were then grouped into two based on whether positive or negative using PCR to detect *Plasmodium* parasites. No differences were detected in the IgG levels between the antigens tested in both seasons when compared based on the PCR data. This result is similar to that found when microscopy was used except that anti-CelTOS IgG in the parasitaemic group was higher than the non-parasitaemic group when microscopy was used. The disparity in the CelTOS report could be due to the lower sample size analysed using PCR compared to the microscopy.

Apical membrane antigen AMA1 has been found to be a promising blood stage vaccine candidate antigen but this potential has been dampen due to extensive polymorphism (Remaque 2008).