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DOES A SINGLE PERIPHERAL BLOOD SAMPLE FROM A MALARIA-INFECTED INDIVIDUAL CAPTURE ALL PARASITE GENOTYPES PRESENT IN AN INFECTION?

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There is contradicting data about whether or not a single peripheral blood sample accurately captures all parasite genotypes present in a malaria infection. Previous studies have demonstrated a rapid turnover of parasite genotypes during the course of an asymptomatic infection with some genotypes appearing while others disappearing from the peripheral blood. This rapid turnover of genotypes suggests that a peripheral blood sample taken at a single time-point contains only a subset of parasite genotypes present in the entire malaria infection. Parasite genotypes not detected in the peripheral blood are thought to sequester in deep tissues precluding their detection in the peripheral blood. However, recent studies have shown that parasites sequestered in deep tissues are genetically identical to those circulating in the peripheral blood. This suggests that a single peripheral blood sample effectively captures all the parasite diversity present in the infection. These discrepant findings may have resulted from the poor resolution of msp1/2 genotyping methods used to determine the genetic composition of infections. To resolve problems associated with msp1/2 measures of infection complexity, we have employed a more-sensitive and less ambiguous 24-SNP Taqman assay to obtain the DNA fingerprint of malaria parasites sampled from adults with asymptomatic malaria over the course of seven consecutive days. We have used this approach to examine whether or not the within-host parasite genetic diversity in asymptomatic individuals remains constant or changes over seven consecutive days. Results from the first set of asymptomatic infections will be presented and discussed at the meeting.

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COMPARISON OF TWO GENOTYPING METHODS FOR DISTINGUISHING RECRUDESCENCE FROM RE-INFECTION IN ANTIMALARIAL DRUG EFFICACY/EFFECTIVENESS TRIALS

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In areas of intense malaria transmission, individuals treated for malaria may encounter new episodes of malaria parasitaemia during the period of follow-up. Without comparing the genetic identity of pre-treatment and post-treatment parasites, it is difficult to resolve whether the recurrence is as a result of treatment failure (recrudescence) or a new infection. Genotyping of the merozoite surface proteins 1 and 2 (msp 1 and 2) is the current gold standard for genotyping infections to correct drug efficacy/effectiveness data. However interpretation of msp1 and msp2 data is often ambiguous and subjective. Therefore, new and better methods for distinguishing recrudescence from re-infection are urgently needed. We compared the performance of the msp1 and msp2 genotyping with a high sensitivity and high resolution 24 single nucleotide polymorphism (SNP) Taqman assay in a cluster-randomized effectiveness trial in an area of high malaria transmission in Malawi. Filter paper samples were collected on day 0 and day 42 of follow-up from children with malaria aged 4-11 (n=106) treated with either artemether-lumefantrine or dihydroartemisinin-piperazine. Parasite DNA was extracted from pre-treatment (day 0) and

post-treatment (day 42) and genotyped using msp1 and msp2 genotyping method and 24-SNP Taqman assay as previously described. The agreement between the two methods was 86%. Discordant results were often due to false positive msp1/2 results. We found a high rate of re-infection with 33% of new episodes of parasitemia detected on day 42 of follow up. Rate of treatment failure based on SNP barcoding of day 0 and d42 filter paper blood samples was 3%. A full comparison of the two genotyping methods for distinguishing re-infections and treatment failure will be presented during the meeting.

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MICROSATELLITE ANALYSIS REVEALS DIFFERENT TRANSMISSION PATTERNS IN THE PERUVIAN AMAZON

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Population genetics studies provides critical information about where and when the infection took place, so imported cases as result of migration can be separated from endogenous cases that indicate the efficacy of the control programs. To address the hypothesis that endogenous transmission is the main mechanism that maintain malaria in rural communities in Peruvian Amazon, 1031 *Plasmodium vivax* positive samples were identified from a population-based cohort by PCR performed every three months from March 2013 to March 2014 in two communities: Cahuide (CAH), community with high mobility by road; and Lupuna (LUP), riverine and isolated community. 390 samples were chosen for microsatellite (MS) genotyping with 9 previously reported MS and 7 new MS. High genetic diversity was observed among communities ($H_e 0.622 \pm 0.045$) but it was low (0.38 ± 0.043) in LUP in December 2013. Higher proportion of polyclonal infections was found in CAH (19%-36%) in comparison to LUP (5%-24%). AMOVA analysis showed that most of the variance occurs within population (58%) and among communities (37%), suggesting strong population structure within and between communities. Genetic differentiation was high between communities, but low between months in each community (Pairwise F_{ST} 0.039), except for LUP in March 2013 which showed moderate differentiation with respect to other months in LUP (0.25), but low differentiation in comparison to CAH (0.09). Analysis of population structure revealed the presence of 4 clusters or subpopulation within these communities (Cluster A, B1, B2 and C). Cluster A was mainly present in March and June 2013 in CAH and only in March 2013 in LUP. Cluster C was predominantly along the follow-up in LUP, except in March 2013. Neighbor joining and burst analysis showed a clonal expansion of these two clusters. Regarding clusters B1 and B2, they are polyphyletic groups that did not maintain fixed over time. In conclusion, results are consistent with an outbreak in Cahuide caused by a clonal expansion of cluster A. In contrast, Lupuna showed endogenous stationary transmission pattern caused by Cluster C that prevails along the seasons.