

Description of a possible clonal expansion of *Plasmodium vivax* in Manaus - Amazonas - Brazil

Descrição de uma possível expansão clonal do *Plasmodium vivax* em Manaus - Amazonas - Brasil

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Abstract Atypical *P. vivax* cases reported in Manaus municipality led us to detect a genetic isolate of *P. vivax*. Variable regions of SSUrRNA were examined from the initial time of infection and in the two recrudescences/relapses from a patient exhibiting chloroquine and primaquine resistance. A unique isolate, found at all stages of infection, suggests the presence of a clonal expansion.

Key-words: *P. vivax*. Clonal expansion. Resistance.

Resumo Casos atípicos de *P. vivax* relatados no Município de Manaus nos levou a detectar um isolado genético de *P. vivax*. Regiões variáveis do SSUrRNA foram examinadas no momento inicial da infecção e nas duas recrudescências de um mesmo paciente exibindo recrudescência/recidiva à cloroquina e primaquina. Um único isolado, encontrado em todos os estágios da infecção, sugere a presença de uma expansão clonal.

Palavras-chaves: *P. vivax*. Expansão clonal. Resistência.

The nature and prevalence of *Plasmodium vivax* infection in Manaus Brazil seem to have changed over the past few years. Overall, the proportion of cases in Brazil due to *P. vivax* infection have increased from less than 50% in 1988 to 70% in 1996^{5 6 7 8} Likewise, the ratio of reportable cases of *P. vivax* versus *P. falciparum* in local clinics and hospitals around Manaus has increased, e.g., *P. vivax* accounted for 15,427 diagnoses in Manaus in 1998². Chloroquine resistant *P. vivax* in one patient in Manaus municipality (Amazonas State) was reported by

Alecrim et al¹. There is evidence of increase in primaquine resistance. In clinical examinations since 1997, we began to see evidence of severe malaria caused by *P. vivax* in patients diagnosed at FMT-AM. It appears that a potentially serious transition is taking place in the area and this may be linked, at least in part, to the changing nature of the parasite. We have been unable to relate consistent morphological features of the parasite to any *P. vivax* infection parameters. We have observed, however, a molecular event, which is worthy of note. We suggest that a rare

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subset(s) of the *P. vivax* population is disease associated and that it maintains a degree of genetic isolation.

In most malaria species, there is intra-species DNA sequence polymorphism in variable regions of the small subunit rRNA^{3 4 9}. We have used sequence variation in the rRNA to distinguish clones of *P. falciparum* in the past. Initially one may wonder why variation in this gene would be an indicator of genetic similarity between organisms. There are three different rRNA genes on three different chromosomes from which rRNA is simultaneously transcribed during asexual blood-stage development⁹. Why would all three genes be identical to each other if chromosomes are constantly independently reassorting? The data to date, using *P. falciparum* clones as a model, has consistently revealed homogeneity within the clone but not between clones. Presumably this is because mechanisms designed to maintain homogeneity among the different alleles and the extent of self-matings maintain similarity in spite of whatever chromosomal reassortment that is occurring. If ribosomal sequence identity is an indicator of genetic relatedness between *P. vivax* isolates, as it appears to be with *P. falciparum*, one could use ribosomal sequence identities to follow the spread of a particular genotype.

Working on the above assumption we compared the sequence of the initial infection, a recrudescence/relapse (35 days later), and a subsequent relapse/recrudescence (180 days after the first recrudescence) of a single patient with vivax malaria from Manaus with sequences obtained from multiple randomly selected *P. vivax* patients. The initial infection was treated with chloroquine. The first recrudescence/relapse was treated with both chloroquine and primaquine.

In a panmictic population, diversity should be found in the initial infection and this diversity should represent a subset of variation in the population. Analysis of recrudescence/relapse materials should also represent a subset of population diversity. Departure from random breeding would be indicated by only one type of rRNA being found in the blood. Here we cloned and analyzed three sequences from each the initial infection and two relapse/recrudescence. All nine sequences were identical. We compared these sequences with sequence taken from randomly chosen infections. For the purposes of displaying the extent of diversity in randomly taken sequences, we chose material both from El Salvador and nine sequences from patients in Thailand. The point of the dendrogram is to graphically show the differences in degrees of diversity from the different sources (Figure 1).

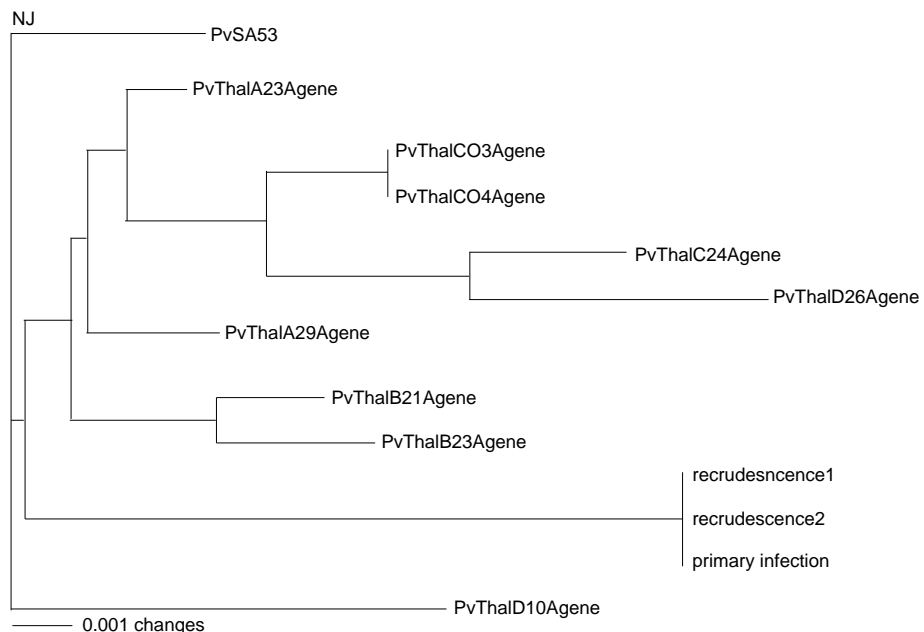


Figure 1 - Dendrogram of Plasmodium vivax from different sources.

We routinely find variation in isolates in both Manaus and villages in Thailand. The identity of sequences shown in our patient indicates a degree of isolation of this sequence from other *P. vivax* isolates. Genetic isolation is suggested by our finding that the three different genes on three different chromosomes were identical in each sample, much like the situation in clones of *P. falciparum*. This finding suggests a lack of random interbreeding. Furthermore, the source of sporozoites was unusually homogeneous because their relapses/recrudescences were the same as the initial infection. These observations indicate that the original source of parasites (infected human) yielded an apparently homogeneous blood feed with regard to parasites. Interpretation of these data seems to suggest that we are following the expansion of a clone.

The changes in *P. vivax* infection in Manaus are very striking. In part, these changes could reflect an urbanization of malaria that simply favors transmission of *P. vivax* over *P. falciparum*. However, along with this process of malaria urbanization are changes in infection severity and susceptibilities to chloroquine. Still, even these changes possible relate to extrinsic factors that alter parasite population make-up, e.g., environmental alterations and vector control measures that influence vector population structure or chemotherapeutic drug pressure on parasite populations. Regardless, a method for following the expansion of parasite "clones" and their relationship to malaria burden is important. Parasites like the one described here which carry sequences not seen before represent prime candidates for prospective study.

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