In this work we investigated the frequency of polymorphism in most of the amino-terminal region of the serine rich antigen (SERA) in Plasmodium falciparum field samples. The blood samples were collected from P. falciparum infected individuals in three areas of the Brazilian Amazon. Two fragments have been characterized by polymerase chain reaction: one of 175 bp corresponding to the repeat region with 5 octamer units and one other of 199 bp related to the 6 repeat octamer units of SERA protein. The 199 bp fragment was the predominant one in all the studied areas. The higher frequency of this fragment has not been described before and could be explained by an immunological selection of the plasmodial population in the infected individuals under study. Since repeat motifs in the amino-terminal region of SERA contain epitopes recognized by parasite-inhibitor antibodies, data reported here suggest that the analysis of the polymorphism of P. falciparum isolates in different geographical areas is a preliminary stage before the final drawing of an universal vaccine against malaria can be reached.

Key words: malaria - Plasmodium falciparum - serine rich antigen - polymorphism
93.3) 1 (6.6)   6 (11.5)

Pará (8)  5 (62.5)    3 (37.5)

Rondônia (29) 27 (93.1) 2 (6.9)

Amazonas (15) 14 (93.3) 1 (6.6)

Total (52) 46 (88.5) 6 (11.5)

**TABLE**

Frequency of polymerase chain reaction (PCR) fragments of the repetitive region of serine rich antigen gene

<table>
<thead>
<tr>
<th>Samples (n)</th>
<th>199 bp (%)</th>
<th>175 bp (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rondônia (29)</td>
<td>27 (93.1)</td>
<td>2 (6.9)</td>
</tr>
<tr>
<td>Pará (8)</td>
<td>5 (62.5)</td>
<td>3 (37.5)</td>
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</tr>
</tbody>
</table>

higher frequency of this fragment could be explained by an immunological selection of the plasmodial population in the infected individuals under study (Daubersies et al. 1994). We should emphasize that mixed infections have not been observed in any of the isolates studied. In conclusion, if the here reported sequence polymorphism affects the immune recognition of SERA, the present re-

Fig. 2: analysis of polymerase chain reaction (PCR) products of DNA from *Plasmodium falciparum* infecting individuals living in three endemic Brazilian states. For PCR procedures, 1 ml of blood samples was suspended in 10 ml in a buffer solution (BPS), 100 µl EDTA (0.5M pH 8.0), and 100 µl saponin (15%). After centrifugation (300 x g – 10 min) the pellet was suspended in 300 µl NET buffer pH 7.5 (0.15 M NaCl, 0.01 M EDTA, 0.05 M Tris), 3 µl protease K (20 mg/ml), and 3 µl sarcosyl (10%). After incubation at 42°C for 24 h, DNA was extracted once with phenol/chloroform/isoamyl alcohol (25:24:1) and then with chloroform/isoamyl alcohol (24:1). The DNA was precipitated by the addition of 3M sodium acetate (1/10), 2 volumes ETOH (–20°C). After incubation –20°C for 16 h the DNA was centrifuged (30 min – 14,000 g) and the pellet was suspended in 1 ml cold ethanol (70% v/v). Before use centrifuged pellet was suspended in 100 µl TE buffer. For PCR, we used 5 µl of DNA extracted from blood samples. The sequences of primers used in the nested PCR were the following: A: 5’ AAT GAA GTC ATA TAT TTC CTT G 3’; B: 5’ CAA TGT TGT TCT TAA TTC GAT A 3’; C: 5’ GTG TTA TAT TTA ACA AAA ATG 3’; D: 5’ CTT ACA GGA TTG CTT GGT TCG 3’. DNA samples were amplified by double or nested PCR (Wataya et al. 1993). We firstly used the set of oligonucleotides A and B and for the second round C and D. A program of 35 cycles was used, in that each cycle corresponds to 1 min for 94°C, 1 min for 47°C and 2 min for 72°C. Distilled water was used as negative control. Electrophoresis was carried out through a 2% agarose gel in 0.5% Tris-Borate-EDTA (TBE) buffer. DNA bands were visualized by staining with ethidium bromide (0.5 µg/ml) and photographed. Oligonucleosomal fragments appeared as ladders of bands whose molecular sizes are approximate of 175 and 199 bp. Molecular size marker of 100 bp is shown on the left (M). Lanes 3 and 5 represents the fragments of 175 bp and lines 1, 2, 4, and 6 the fragments of 199 bp.
results indicate that the analysis of the polymorphism of *P. falciparum* isolates is a fundamental stage before the final drawing of an universal vaccine against malaria can be reached.

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**REFERENCES**


