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# MOLECULAR ANALYSIS OF *Plasmodium berghei* MEROZOITE SURFACE PROTEIN-1 (PBMSP-1) GENE IN ERYTHROCYTE OF MICE INFECTED WITH *Plasmodium berghei*

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## ABSTRACT

This study aimed to analyze MSP-1 gene in mice erythrocyte infected with *Plasmodium berghei* using polymerase chain reaction (PCR). The result showed the existence of 462 bp DNA band which was assumed to encode MSP-1 protein with molecular weight of 19 kDa that can only be found in erythrocyte infected with *Plasmodium berghei.* BLASTn analysis showed that PbMSP-1 obtained in this study have 100% similar identity with mRNA of PbMSP-1 partial sequence (462 bp), 93% similarity with PbMSP-1 complete sequence (5750 bp and 5376 bp), and 87% similarity with PbMSP-1 incomplete sequence (333 bp).

Key words: malaria, MSP-1 gene, PCR, *Plasmodium*, vaccine

## *ABSTRAK*

*Penelitian ini bertujuan mengkaji gen MSP-1 dari eritrosit mencit yang terinfeksi parasit Plasmodium berghei menggunakan metode polymerase chain reaction (PCR). Hasil penelitian menunjukkan kehadiran pita DNA berukuran 462 bp yang diprediksi menyandi protein MSP-1 dengan berat molekul 19 kDa yang hanya ditemukan pada sampel eritrosit terinfeksi Plasmodium berghei, dan tidak ditemukan pada sampel eritrosit yang tidak diinfeksi dengan Plasmodium berghei. Analisis BLASTn dari urutan 462 bp menunjukkan adanya kemiripan identitas dengan urutan parsial gen mRNA PbMSP-1 sebesar 100%, sekuen lengkap gen PbMSP-1 5750 bp dan 5376 bp sebesar 93%, serta urutan parsial gen PbMSP-1 333 bp sebesar 87%.*

*Kata kunci: malaria, Gen MSP-1, PCR, Plasmodium, vaksin*

## INTRODUCTION

MSP-1 (merozoite surface protein-1) gene is a gene originated from Plasmodium species that encode MSP-1 protein. MSP-1 protein is present on the surface of merozoite with molecular weight of 185-210 kDa and presence in all malaria species (Blackman, 2000; Wickramarachchi *et al*., 2007). This protein is considered essential because of its function to promote antibody production and protection against malarial infections (Helg *et al*., 2003; Sachdeva *et al*., 2005; Wan Omar *et al*., 2007). MSP-1 protein is synthesized on merozoite surface at final phase in intracellular level of erythrocyte (Leung *et al*., 2004). Shortly after differentiation of merozoite, this protein is hydrolyzed to a few small fragments that form polypeptide complex (Jennings *et al*., 1998; Leung *et al*., 2004). MSP-1 gene sequence display homologous resemblance with most of Plasmodium species, because of its same sequence and block number and typical in MSP-1 with the total of 17 blocks belongs to *P. berghei* species, so it is homologous with *P. falciparum* that infects human (Jennings *et al*., 1998; Sherman, 1998; Rodriguez *et al*., 2003). Wiser *et al.* (1997) reported that Plasmodium species in rodent undergo initial cleavage by protease enzymes that do not happen in *P. falciparum.* MSP-1 gene sequence of Plasmodium in rodent is sequences that have high amino acid and divided into four varied blocks which is VAR I-IV. These blocks show similar acid composition of amino acid up to 92-100% (Jennings *et al*., 1998).

MSP-1 protein amino acid sequence is a random bundle in all blocks, while 'conserved' regions have alfa helix or beta primer (Wiser *et al*., 1997; Jennings *et al*., 1998). These blocks reported to contain repeated tandem elements that can be detected in nucleotides levels. Conserved region forms backbone structure that prevents proteolysis of the fragments by protease enzyme (Mc Bride, 1997). Region that close to each other with varied and conserved blocks will produce various domains in merozoite differentiations (Jennings *et al*., 1998) and this domain utilized effectively to induce immune system (Cavanaugh *et al*., 2001; Mancilla *et al*., 1994; Toebe *et al*., 1997). For example, a recombinant protein that is expressed from VAR I *P. berghei* that lacks repeat tandem can protect the animal from infection of *P. berghei* (Toebe *et al*., 1997). Therefore, gene that encodes PbMSP-1 protein will be a perfect study subject as comparison and model of malarial infection in human.

Specific aim on studying Plasmodium parasite is to understand significant and important molecular aspects such as DNA analysis of Plasmodium parasite, especially *P. berghei* on PbMSP-1 gene in mice malaria model. The data will be useful in creating and designing anti-malarial vaccine that can eradicate malaria disease in human, so it can be used as research basis for future studies especially in endemic areas such as Indonesia.

## MATERIALS AND METHODS

A total of 15 male white mice (*Mus musculus*) strain Balb/c were used in this study. The mice aged 6-8 weeks, and weighed 20-22 g were divided into three groups namely parasite stock breeding group (Group

S), treatment group which received parasite inoculation from stock breeding group (Group A), and control group without any treatment (Group B). Mice were observed in plastic container in vivarium at Biology Department, Faculty of Math and Science (FMIPA), Unsyiah and given pellets and water *ad libitum*.

**Inoculation of Mice with *Plasmodium berghei***

*P. berghei* from cryopreservation stock was unfrozen using water heater at 37° C for about 10 minutes. Then, the solution contained parasite was mixed with physiologic NaCl with ratio of 1:1, centrifuged for 10 minutes with speed of 650x g, and the parasite infected erythrocyte was counted in $1x10^6$ concentrations. Pellet contained parasite then injected into 5 mice intraperitoneally and grouped as parasite stocks. When parasitemia reach 30%, the parasite inoculum was injected into mice in treatment group. Parasitemia were counted by hemocytometer and levels of parasitemia were observed using glass that was previously smeared with a drop of mice blood from each treatment and control group, the results then compared (Field and Shute, 1955).

***Plasmodium berghei (P. berghei )* Inoculation**

*P. berghei* from cryopreservation stock was unfrozen using water heater at 37° C for about 10 minutes. Then, the solution contained parasite was mixed with physiologic NaCl with ratio of 1:1, centrifuged for 10 minutes with speed of 650 x g, and the parasite infected erythrocyte was counted in $1x10^6$ concentrations. Pellet contained parasite then injected into 5 mice intraperitoneally and grouped as parasite stocks. When parasitemia reach 30%, the parasite inoculum was injected into mice in treatment group (Group A). Parasitemia were counted by hemocytometer and levels of parasitemia were observed using glass that was previously smeared with a drop of mice blood from each treatment and control group, the results then compared (Field and Shute, 1955).

***P. berghei* DNA Extraction**

The extraction of *P. berghei* DNA from blood of mice in treatment group was conducted using DNA extraction kit. Pellets produced were diluted in 150 ml buffer cold SET (20% sucrose, 50 mM Tris-HCl pH 8.0 and 50mM EDTA) and homogenized. Afterward, 350 µL lytic alkalized solution (0.2 M NaOH, 1% SDS) was added into the tube, gently turned four to six times until the solution became clear before putting into the ice for 5 minutes. Further, 275 µL 3M NaOAc cold solution with pH 4.8 was added and gently shaken for four to five times. The tube was centrifuged using cold centrifuge (4° C) at 10,000x g for 10 minutes. Supernatants obtained were put into micro centrifuge tube then added 600 µL cold isopropanol, homogenized and centrifuged at 10,000x g in 4° C for about 30 minutes. Pellets produced then added with 1 mL methanol 75% and centrifuged again at the same speed. Pellets were dried and suspended in 20 µL distilled water with RNAase (10 mg/mL). After centrifuged in 37° C for 30 minutes, DNA pellet from extraction were stored in freezer for electrophoresis analysis.

**Agarose Gel Electrophoresis**

*P. berghei* DNA from extraction were separated by agarose gel electrophoresis to prove the presence of *P. berghei* DNA bands. Prior to agarose gel addition, DNA sample mixed with sample buffer (50% glycerol, 0.01% bromophenol blue, 0.01% xylene cyanol and 50% TBE (x2). The 1 kb marker was used to measure bands size separation. After putting sample inside agarose gel, electrophoresis was run with speed of 70 mV in 1x TAE until the sample reach ¾ of the whole gel. DNA fragments that were separated inside agarose gel were observed under UV transilluminator.

**DNA Amplification**

Extracted DNA was amplified with polymerase chain reaction (PCR) using Sambrook and Russel method (2001). This method used because its ability to amplify DNA copy inside sample so that it is easy to observe. Amplification DNA consists of three cycle that is denaturation, attachment, and DNA elongation.

PCR were performed using basic mixture of 100 ng DNA sample (as template), 100 ng on each 5‘ and 3’ terminal primer of PbMSP-1 (5’-CTTTAATATTTATTTTTACACAAATTAG-3’) and PbMSP-1 R (5’-TAATCCAGTTGTCATGTCTTCTTTAATTGTTGA-3’), 200 µM dNTP, 2U Taq DNA polymerase (GIBCO-BRL), 1x penimbal PCR (GIBCO-BRL), 5% dimethyl sulfoxide (DMSO) and distilled water so that end volume reach 25 µl. The mixture then placed in 0.5 ml Eppendorf tube and put inside ‘DNA thermal cycler’ (Perkin Elmer) and the program was set on denaturation (94° C, 1 minute), attachment (55° C, 1 minute), elongation (70° C, 3 minutes) and with a total of 30 cycles. After PCR processing finished, 5-10 µL sample was analyzed of its presence of specific DNA by using agarose gel electrophoresis.

**DNA Sequencing**

We used automatic sequencing machine ABI PRISM 377 from Perkin Elmer Corporation, USA to sequence DNA. Clones were sorted as ‘*antisense*’ and ‘*sense*’ with its matched primer. Data sorted were edited manually to discard vector sequence from 5’ and 3’ terminal by using Chromas program. Edited sequencing results were analyzed with BLASTn program (Altschul *et al*., 1990) from http://www.ncbi.nlm.gov/.

## RESULTS AND DISCUSSION

DNA genomic extraction results from mice infected with *P. berghei* (Group A), uninfected mice (Group B), as well as its PCR analysis can be seen on Figure 1 and 2. Agarose gel electrophoresis analysis on genomic DNA extraction results showed the presence of DNA bands sized around 7 kb on both samples. Large band

size shows that extracted DNA is in form of DNA total that contain various gene. Concentration and quality of DNA extraction results are determined by spectrophotometer with absorbance of A260/A280 (Zhong *et al*., 1999). Qualified DNA was stored and used in next PCR for observation on PbMSP-1 gene.

PCR analysis by using primer PbMSP-1 detected DNA band sized 426 bp. This band is fit with PbMSP-1 gene band reported by Zhong *et al*. (1999) by using cDNA λgt11 that corresponds with block 3 of conserved region and block 4 of variable region from *P. falciparum*. Sequence with size of 426 bp that encode MSP-1 from *P. berghei* species have similarity with MSP-1 sequence from other rodent Plasmodium species, also it has similarity with Plasmodium that infects human (Del Portillo *et al*., 1991; Toebe *et al*., 1997). MSP-1 gene sequence from rodent parasite is reported to have identical high amino acid that consisted of few variations such as VAR I-IV. All four variables showed small inter-species homologous, even so they have identical amino acid composition and predominant with residue of serine, threonine, glycine, alanine, and proline and tend to consist of repeated tandem on nucleotide level. Region of conserved N and C terminal from glycosylphosphatidylinositol is also presence in PbMSP-1 gene sequence (Jennings *et al*., 1998).

**DNA Sequencing**

PCR result of PbMSP-1 gene completely sequenced and compared with nucleotide sequence and amino acid in gene database by using BLASTn program (Altschul

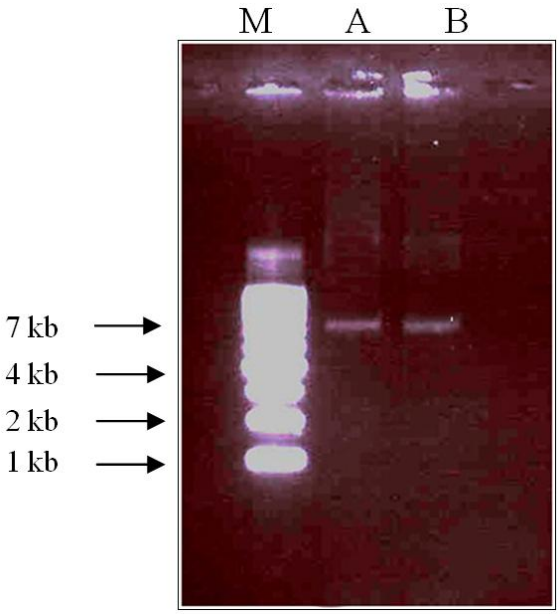


**Figure 1**. Genomic DNA electrophoresis on erythrocyte sample of mice. A= Mice infected with *Plasmodium berghei*, B= Uninfected mice, M= Marker

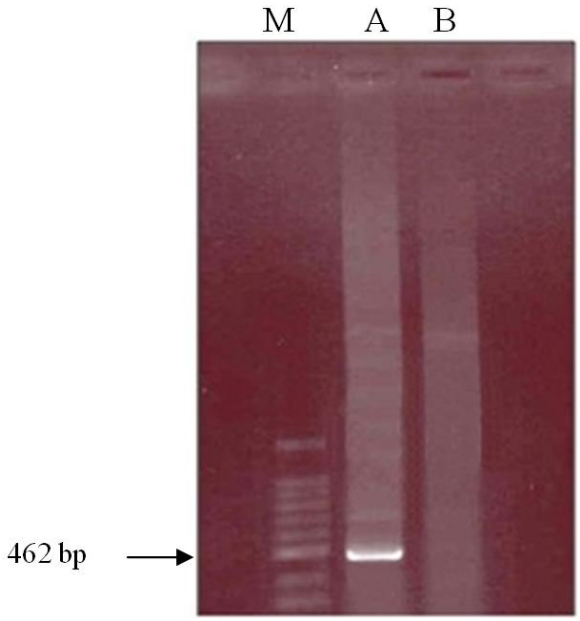


**Figure 2**. Electrophoresis PCR product analysis on erythrocyte genomic DNA of mice. A= Mice infected with *Plasmodium berghei*, B= Uninfected mice (column B), M= Marker

```
1   atgaaagtaaatatagtaaaaaaaaaataatatatcaagctatgtataatttatatttta
    M  K  V  N  I  V  K  K  K  -  Y  I  K  L  C  I  I  Y  I  L

61  acaaaaagcaattagctgaaatacaaaaggttattgaaaccttagaaaaacgagtttctg
    T  K  S  N  -  L  K  Y  K  R  L  L  K  P  -  K  N  E  F  L

121 cattaaagaaaaatgatgtcataaagccattattgcaacaaattgaagatatcaaggctg
    H  -  R  K  M  M  S  -  S  H  Y  C  N  K  L  K  I  S  R  L

181 cccctgttactaccgaaggacaaataactacatcagggcaatctagtacagaacccgcta
    P  L  L  L  P  K  D  K  -  L  H  Q  G  N  L  V  Q  N  P  L

241 gtacaggaacacctagttcaggtgaagttagtacaggaactagtacaggaggagctagtg
    V  Q  E  H  L  V  Q  V  K  L  V  Q  E  L  V  Q  E  E  L  V

301 caggcgtcactaatacaggagcagctactacaggaactactggtacaggagcagctacta
    Q  A  S  L  I  Q  E  Q  L  L  Q  E  L  L  V  Q  E  Q  L  L

361 caggaactactggtgcagaagcagttactacaggaaatactggtgcagaagcagctacta
    Q  E  L  L  V  Q  K  Q  L  L  Q  E  I  L  V  Q  K  Q  L  L

421 caggaaatactaatacagaagtaactcaagtgcaaaccgtat
    Q  E  I  L  I  Q  K  -  L  K  C  K  P  Y
```

**Figure 3**. Partial nucleotide sequence of PbMSP-1 gene and amino acid that encode MSP-1 protein

**Table 1**. Analysis of PbMSP-1 gene using BLASTn program

| Gene | Identity number | Identity (%) | Positive (%) | E-Value | Sequence length |
|---|---|---|---|---|---|
| PbMSP-1 mRNA, partial sequence | L21712.1 | 99 | 100 | 7e-46 | 462 bp |
| PbMSP-1, complete sequence | U43521.1 | 93 | 93 | 3e-06 | 5750 bp |
| PbMSP-1, complete sequence | AF187232 | 93 | 93 | 3e-06 | 5376 bp |
| PbMSP-1, partial sequence | gi1762645 | 87 | 87 | 1e-04 | 333 bp |

*et al*., 1990). BLASTn analysis showed that PbMSP-1 obtained in this study have high similar identity sequence with mRNA of PbMSP-1 partial sequence sized 462 bp (100%), PbMSP-1 complete sequence sized 5750 bp (93%), PbMSP-1 complete sequence sized 5376 bp (93%), and PbMSP-1 partial sequence sized 333 bp (87%). Jennings *et al*. (1998) states that amino acid identity between PbMSP-1 and PyMSP-1 is around 76%, while identity between PbMSP-1 and PcMSP-1 is around 68% and all these sequence is interrupted by four varied blocks. Mancilla *et al*. (1994) and Cavanaugh *et al.* (2001) studying about antigenicity of MSP-1 and stated that varied blocks in this gene have good immunogenicity. This statement is supported by Toebe *et al*. (1997) that studied the protein recombinant from tandem region of PbMSP-1 that can protect these mice infected with *P. berghei.* Figure 3 shows PbMSP-1 gene sequence and amino acid that encode PbMSP-1 protein.

BLASTn analysis showed that PbMSP-1 gene obtained have high nucleotide sequence similarity with partial sequence of PbMSP-1 mRNA sized 462 bp (100%) and complete sequence of PbMSP-1 sized 5750 bp (93%). PbMSP-1 gene obtained encodes rPbMSP-1 protein that consisted of 154 amino acid and molecular weight of 19 kDa. ClustalW analysis was used to observe the comparison of genes that encode PbMSP-1 protein with PbMSP-1 gene sequence inside data bank. Through the analysis, plasmid recombinant sequence size 462 bp was compared with PbMSP-1 mRNA partial sequence (L21712) and PbMSP-1 complete sequence (U43521.1) from data bank. The results showed that gene that encode PbMSP-1 protein in this study have similar identity (100%) with PbMSP-1 mRNA gene sequence (L21712), while analysis of PbMSP-1 complete sequence (U43521.1) showed a sequence with size of 426 bp was a PbMSP-1 gene partial sequence. This partial sequence was consisted of 154 amino acid and assumed to encode PbMSP-1 protein weighed 19 kDa.

## CONCLUSION

The PbMSP-1 genes found in mice which were infected with *Plasmodium berghei* in this study have 100%, 93%, and 87% sequence similarity with mRNA PbMSP-1 partial sequence at 462 bp, PbMSP-1 complete sequence at 5750 bp and 5376 bp, and PbMSP-1 partial sequence at 333 bp, respectively

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## REFERENCES

Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. **J. Mol. Biol**. 215(3):403-410.

Blackman, M.J. 2000. Proteases involved in erythrocyte invasion by the malaria parasite: Function and potential as Chemateurapeutic targets. **Current Drug Targets**. 1(1):59-83.

Blackman, M.J., H. Whittle, and A.A. Holder. 1991. Processing of the *Plasmodium falciparum* major merozoite surface protein-1: Identification of a 33-kilodalton secondary processing product which is shed prior to erythrocyte invasion. **Mol. Biochem. Parasitol**. 49:35-44.

Cavanaugh, D.R., C. Dobano, I. Elhassan, M. March, K. Elhassan, A.A. Hviid., Khalil., E.A.T.G. Theander., D.E Arnot, and J.S. McBride. 2001. Differential patterns of human immunoglobulin G subclass responses to distinct regions of a single protein, the merozoite surface protein 1 of *Plasmodium falciparum*. **Infect. Immun**. 69(2):1207-1211.

Del Portillo, A.H., M. Lanzer, S.M. Rodriguez, F. Zavala, and B.C. Fernandez. 2004. Variant genes and the spleen in *Plasmodium vivax* malaria. **Int. J. Parasitol**. 34:1547-1554.

Field, J.W. and P.G. Shute. 1955. The Microscopy Diagnosis of Human Malaria: II-A Morphological Study of the Erythrocytic Parasites. In **Studies from The Institute for Medical Research Federation of Malaya**. Gaur, D. (Ed.). The Government Press, Kuala Lumpur.

Mayer, D.C. and L.H. Miller. 2004. Parasite ligand-host receptor interactions during invasion of erythrocytes by Plasmodium merozoites. **Int. J. Parasitol**. 34:1413-1429.

Helg, A., M.S. Mueller, A. Joss, P.F. Frank, F. Stuart, J.A. Robinson, and G. Pluschke. 2003. Comparison of analytical methods for the evaluation of antibody responses against epitopes of polymorphic protein antigens. **J. Immunol. Methods**. 276:19-31.

Jennings, G.J., S.T. Carole., A. van Belkum, and M.F. Wiser. 1998. The complete sequence of *Plasmodium* berghei merozoit surface protein-1 and its inter and itra species variability. **Mol. Biochem. Parasitol**. 93:43-55.

Leung, W.H., Z.Q. Meng, G. Hui, and W.K.K. Ho. 2004. Expression of an immunologically reactive merozoite surface protein (MSP-142) in *E. coli*. **Biochimica et Biophysica Acta.** 1675:62-70.

Mancilla, L.I., G. Levitus, K. Kirchgatter, F. Mertens, S. Herrera, and H.A. Del-Portillo, 1994. *Plasmodium vivax*: Dimorphic DNA sequences from the MSP-1 gene code for regions that are immunogenic in natural infections. **Exp. Parasitol**. 79:148-158.

Mc Bride, J.S. 1997. Antigenicity of recombinant proteins derived from *Plasmodium falciparum* merozoit surface protein-1. **Mol. Biochem. Parasitol**. 85:197-211.

Rodriguez, M.H., M.G. Cunha, R.L. Machado, O.C. Ferreira, Jr., M.M. Rodrigues, and I.S. Soares. 2003. Serological detection of *Plasmodium vivax* malaria using recombinant protins corresponding to the 19-kDa C-terminal region of the merozoit surface protein-1. **Malar J.**. 2: 39.

Sachdeva, S., A. Mohammed, P.V.N. Dasaradhi, B.S. Crabb, A. Katyal, P. Malhotra, and V.S. Chauhan. 2005. Immunogenicity and protective efficacy of *Eschericia coli* expressed *Plasmodium falciparum* merozoite surface protein-$1_{42}$ using human compatible adjuvants. **Vaccine**. 24(120):2007-2016.

Sambrook, J. and D.W. Russel. 2001. **Molecular Cloning**. A laboratory Manual. Cold Spring Harbor Laboratory Press, New York.

Sherman, I.W. 1998. **Malaria. Parasite Biology, Pathogenesis, and Protection**. American Society for Microbiology, Washington DC. USA.

Toebe, C.S., J.D. Clements, L. Cardenas, G.J. Jennings, and M.F. Wiser. 1997. Evaluation of immunogeniciti of an oral Salmonella vaccine expressing recombinant *Plasmodium berghei* merozoit surface protein-1. **Am. J. Trop. Med. Hyg**. 56:192-199.

Wan Omar. A., A.M. Roslaini, Z.U. Ngah., A.A. Azahari, M. Zahedi, and O. Baharuddin. 2007. A recombinant 19 kDa *Plasmodium berghei* merozoite surface protein 1 in formulated with alum induces protective immune response in mice. **Trop. Biomed**. 24:119-126.

Wickramarachchi, T., R.J. Illeperuma, L. Perera, S. Bandara, I. Holm, S. Longacre, S.M. Handunnetti, and P.V. Udagama-Randeniya. 2007. Comparison of naturally acquired antibody responses against the C-terminal processing products of *Plasmodium vivax* Merozoite Surface Protein-1 under low transmission and unstable malaria conditions in Sri Lanka. **Int. J. Parasitol**. 37:199-208.

Wiser, M.F., C.S. Toebe, and G.J. Jennings. 1997. An additional primary proteolytic processing site in merozoit surface protein-1 of *Plasmodium berghei*. **Mol. Biochem. Parasitol**. 85:125-129.

Zhong, H., Y.J. Fan, S. Yang, and E.A. Davidson. 1999. Cloning and characterization of the merozoit surface antigen-1 of *Plasmodium berghei*. **Am. Journal. Trop. Med. Hyg**. 60(6):994-999.