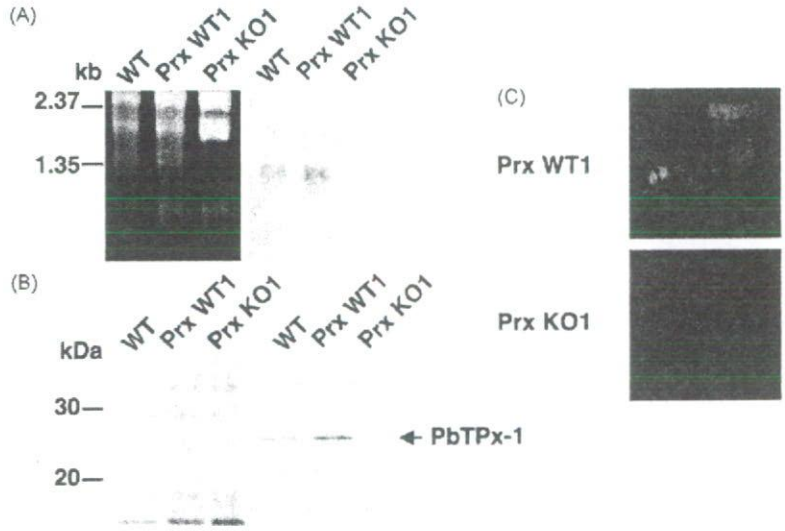

Fig. 2. Verification of the PbTPx-1 null phenotype in gene-disrupted *P. berghei*. Parasite populations, including the parent parasite (WT), Prx WT1 (*dhfr-ts/mt* at the DHFR-TS locus and intact *pbtpx-1*), and Prx KO1 (*pbtpx-1* disruptant) were inoculated into mice, and parasite-infected erythrocytes, total protein, and total RNA of the parasite cells were prepared. (A) The absence of TPx-1-specific mRNA expression in Prx KO was examined by Northern blot analysis. Total RNA samples (20 μg) were separated on 1.5% agarose-formaldehyde gel (left panel), transferred to nylon membranes, and hybridized with probe (right panel). Ethidium bromide-stained gel showed equal loading. Molecular size markers in kb are indicated on the left. (B) The absence of TPx-1 protein in Prx KO was examined by Western blot analysis. Total protein samples (20 μg) were separated by SDS-PAGE (left panel) and probed with anti-TPx-1 antibody (right panel). Coomassie brilliant blue-stained gel showed equal loading. Protein size markers in kDa are indicated on the left. (C) The absence of TPx-1 protein in Prx KO was confirmed by indirect immunofluorescence assay. TPx-1 protein is stained in green, and parasite nuclei are stained in blue.

parasitemia reached approximately 4% (4–5 days after infection). Levels of 8-OHdG (ng/mg DNA) are expressed as the mean of triplicate measurements.

2.7. Statistical analysis

Differences were evaluated with Student's *t*-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Targeted disruption of the PbTPx-1 gene

Merozoites in segmented schizonts were transfected with the targeting construct by electroporation and were subsequently inoculated into naïve rats. Integration of the construct into the PbTPx-1 locus by homologous recombination resulted in disruption of the single-copy gene and insertion of a selectable marker, the DHFR-TS gene with a pyrimethamine-resistance mutation (*dhfr-ts/mt*), at the locus (Fig. 1A). Parasites with *pbtpx-1* disruption were selected by treatment with pyrimethamine. PCR and Southern blot analyses showed that parasites selected with pyrimethamine were a mixture of wild-type parasites and *pbtpx-1* disruptants (data not shown). Parasite populations were separated into two groups by limiting dilution and subsequent inoculation into 18–22 rats. In two independent electroporation experiments, six disruptant populations (Prx KO) were obtained and cloned. In the first experiment, three Prx KO populations (Prx KO1–3) and two wild-type parasite populations (Prx WT1 and 2) were obtained (Fig. 1B). In the second experiment, two Prx KO populations (Prx KO4 and 5) were obtained (data not shown). The *dhfr-ts* locus of Prx WT parasites was amplified by PCR and sequenced, and replacement with the pyrimethamine-resistance mutation was confirmed (data not shown). Northern blotting, Western blotting, and immunofluorescence assay confirmed the TPx-1-null phenotype of Prx KO1 (Fig. 2). This phenotype was also confirmed for all Prx KO populations (data not shown).

3.2. PbTPx-1 gene disruption does not affect asexual development

Prx KO1–3 and Prx WT1 and 2 were used to infect Balb/c mice, and the course of parasite development within erythrocytes was compared to that of the parent strain (WT) (Fig. 3A). Two Prx WT populations showed equal development and multiplication within erythrocytes, similar to WT, and they showed a high level of parasitemia (>5%) 5 days after infection. The courses of parasitemia observed in Prx KO1-infected mice were also similar to that in WT-infected mice. This phenotype was confirmed in Prx KO2 and 3 and in Prx KO4 and 5, which were obtained from an independent electroporation experiment (Fig. 3B). There was no difference in the morphology of parasite cells between five Prx KO, two Prx WT, and WT populations (data not shown). These eight parasite populations did not kill mice until day 8 of observation. Prx KO1, Prx WT2, and WT populations each killed one of three mice, respectively, on day 9 of observation. It is known that Balb/c mice in some laboratories are resistant to the development of cerebral malaria (CM) induced by the *P. berghei* ANKA strain and do not develop lethal infection [25]. To determine the phenotype of *pbtpx-1* disruption in strains of mice with differing susceptibility to the *P. berghei*

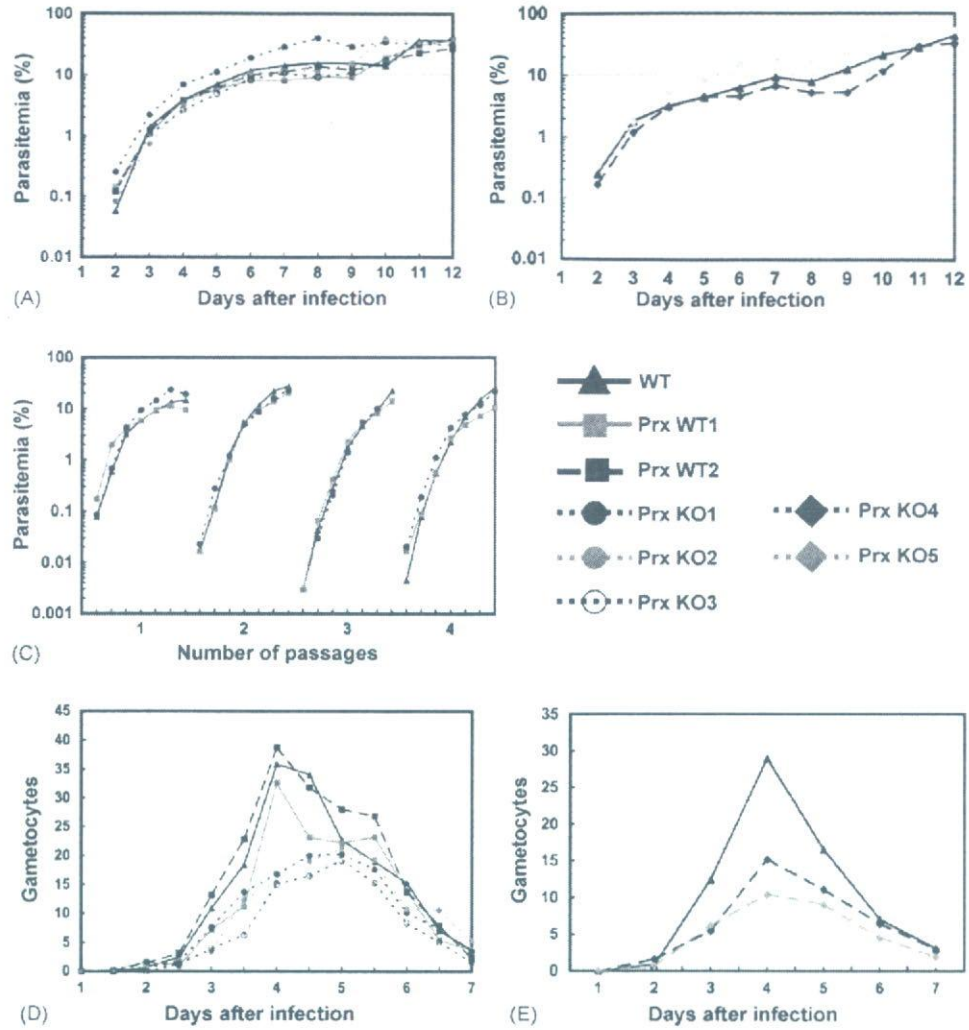

Fig. 3. Infection of mice with TPx-1 gene-disrupted *P. berghei*. Parasite populations, including the parent parasite (WT), two Prx WT (*dhfr-ts/mt* at the DHFR-TS locus and intact *pbtpx-1*) and five Prx KO (*pbtpx-1* disruptant), were inoculated into mice, and the courses of parasite development in erythrocytes were observed. (A and B) Changes in parasitemia 12 days after infection (three mice per group: six groups in (A) WT, Prx WT1 and 2, and Prx KO1–3; three groups in (B) WT and Prx KO4 and 5). (C) Changes in the course of parasitemia during four blood passages (two mice per group: three groups, WT, Prx WT1, and Prx KO1). (D and E) Changes in gametocytemia 7 days after infection (three mice per group: six groups in (D) WT, Prx WT1 and 2, and Prx KO1–3; three groups in (E) WT and Prx KO4 and 5). Data are mean values of parasitemia percentage (A–C) and gametocyte number (gametocytes/10^4 erythrocytes) (D and E) in each group.

ANKA strain, C57B6 mice, which are known to develop CM and lethal infection [25], were infected with Prx KO1, and the course of parasite development within erythrocytes was compared to that of WT. Prx KO1 and WT multiplied equally and killed mice 7–8 days after infection. The morphology of parasite cells and the numbers of animals killed during 8 days of observation between the two parasite populations did not differ (data not shown).

A function of 2-Cys Prx in the prevention of damage to DNA by oxidative stress has been suggested in mammals [26]. Therefore, the cumulative effect of oxidative stress on parasite growth in vivo in the *pbtpx-1* disruptant was evaluated. For this purpose, Prx KO1, Prx WT1, and WT were continuously maintained in Balb/c mice by inoculating parasite-infected blood and comparing the course of parasitemia between passages. Blood passage was performed on the 7 day of infection and was repeated four times. The courses of parasitemia observed in Prx KO1-, Prx WT1-, and WT-infected mice from the first to the fourth passage are shown in Fig. 3C. The course of parasitemia after four passages compared to that after the first inoculation did not differ between the three parasite populations. Passage was repeated six more times, and the course of parasitemia observed for the three parasite populations at the 10th passage was similar to that observed at the fourth passage (data not shown). To determine whether *pbtpx-1* disruption enhances DNA damage in the parasite genome, the level of 8-OHdG, a marker of oxidative DNA damage, was compared between Prx KO1 and WT. The level of 8-OHdG in the parasite genome was similar between Prx KO1 (11.7 ng/mg DNA) and WT (9.1 ng/mg DNA), suggesting that *pbtpx-1* disruption does not affect the physiologic level of DNA oxidation. This result was confirmed in two independent experiments (data not shown).

3.3. PbTPx-1 gene disruption affects gametocytogenesis

To evaluate the effect of *pbtpx-1* disruption on development of sexual-stage parasites, the numbers of gametocytes in parasite-infected blood were counted and compared among Prx KO1–3, Prx WT1 and 2, and WT (Fig. 3D). In Prx WT1- and WT-infected mice, gametocytes were observed from 2 days after infection; they increased in number with parasitemia progression and peaked 4 days after infection. Gametocytemia, recorded as the number of gametocytes/10^4 erythrocytes in Prx WT1- and WT-infected mice at the day 4 of infection, was 32.5 ± 5.5 and 35.8 ± 11.6, respectively ($P = 0.34$). The course of gametocytemia between Prx WT1- and WT-infected mice did not differ. This phenotype was confirmed in two populations of Prx WT. In Prx KO1-infected mice, gametocytes were also observed from 2 days after infection and peaked 5 days after infection. The peak of gametocytemia in Prx KO1-infected mice was thus delayed 1 day from that of Prx WT1- and WT-infected mice. Gametocytemia, recorded in Prx KO1-infected mice as the number of gametocytes/10^4 erythrocytes on day 5 of infection, was 20.2 ± 0.9. The highest number of gametocytes was thus significantly lower ($P < 0.01$) in Prx KO1-infected mice than in WT-infected mice. The *pbtpx-1* disruption phenotype, in addition to the delay in gametocytemia peak, showed a significant decrease in the numbers of gametocytes ($P < 0.02$) in Prx KO1–3 and in Prx KO4 and 5, which were obtained from an independent electroporation experiment (Fig. 3E).

To determine whether *pbtpx-1* disruption affects the male/female ratio of gametocytes or exflagellation activity of male gametocytes, Prx KO1, Prx KO4, Prx WT1, and WT were used to infect mice (Table 1). The male/female ratios of gametocytes in the three parasite populations at the day of peak gametocytemia were similar: 0.44, 0.53, and 0.64 for Prx KO1-, Prx WT1-, and WT-infected mice in experiment 1; 0.62 and 0.50 for Prx KO4- and WT-infected mice in experiment 2. This phenotype was confirmed in five populations of Prx KO (data not shown). The percentages of exflagellation, calculated according to the numbers of exflagellation centers and the numbers of male gametocytes in the blood at the day of peak gametocytemia, were 70, 64, and 76% for Prx KO1-, Prx WT1-, and WT-infected mice in experiment 1; 59 and 60% for Prx KO4- and WT-infected mice in experiment 2. Thus, the majority of male gametocytes observed in blood were equally viable in terms of exflagellation activity among the three parasite populations. This finding suggests that low gametocytemia in Prx KO is attributed to impaired development of gametocytes rather than to their decreased survival.

4. Discussion

The results presented here suggest that *pbtpx-1* disruption does not affect asexual intraerythrocytic growth of parasites. The results from passage experiments and evaluation of 8-OHdG suggest that TPx-1 does not prevent parasite DNA oxidation, in contrast to mammalian Prx [26], and that it is not essential for asexual parasite growth in mouse erythrocytes. However, the fact that the parasite lacks catalase and genuine GSH peroxidase and that the major cytosolic peroxide-detoxifying capacity appears to be provided by Prx may contradict the present findings [1,2]. Microarray analysis of the disruptant and the parent parasite may provide insights with respect to redundancy in parasite antioxidant defenses and compensation for the lack of Prx. Our results may not indicate that the gene is inessential for asexual growth in *P. falciparum* because there are differences in the life cycle of the mammalian stage between *P. falciparum* and rodent malaria parasites. *P. falciparum* develops in erythrocytes sequestered in the microvasculature, where the parasite may experience more severe oxidative stress than do parasites in the blood circulation [5]. The parasite may require Prx for development under such stressful conditions. Such a possibility should be investigated in a monkey model of *P. falciparum* infection [27]. The relatively high level of 8-OHdG formation in the parasite genome compared to that in mammalian cells [24] may reflect the absence of nuclear Prx in this organism [1,2,9]. How malaria parasites deal with the accumulation of oxidative DNA damage is of interest; a hypothetical protein with putative excision DNA repair function or DNA glycosylase function (PF10835c) has been identified in the genome of the malaria parasite.

Table 1
Male/female ratios of gametocytes and exflagellation activity of male gametocytes in TPx-1 gene-disrupted *P. beghei*[a]

	Experiment 1			Experiment 2	
	WT	Prx WT1	Prx KO1	WT	Prx KO4
Peak of gametocytemia					
Gametocyte number[b]	30 ± 5.5	37 ± 5.5	18 ± 2.5	31 ± 3.2	14 ± 1.6
Days after inoculation	4.0	4.0	4.5	4.0	4.5
Male/female ratio					
Day of peak gametocytemia	0.64	0.53	0.44	0.50	0.62
Day 5.5	1.06	1.07	0.92	1.01	1.15
Exflagellation (%)[c]					
Day of peak gametocytemia	76 (52–88)	64 (51–81)	70 (53–81)	60 (54–64)	59 (55–63)
Day 5.5	16 (10–20)	13 (7–27)	24 (9–54)	14 (12–17)	4.1 (0.6–10)

[a] Values are means ± standard deviations (gametocyte number), mean (male/femal ratio), and mean with range (exflagellation, %) calculated from data obtained from four mice.
[b] Gametocytes per 10^4 erythrocytes.
[c] Exflagellation (%) = (number of exflagellation centers in 10^4 erythrocytes/number of male gametocytes in 10^4 erythrocytes) × 100.

Nonetheless, the results presented here suggest that PbTPx-1 is required for normal gametocyte development; however, it does not affect the male/female ratio of gametocytes. We could not observe the defect in gametocyte development in *P. falciparum*, since we disrupted the gene in the FCR-3 strain, which produces only a little numbers of gametocyte [16]. Gametocytes are sexual-stage parasites involved in the transition between the mammalian host and the mosquito. This stage arises during asexual cycling, and it has generally been accepted that the trophozoites of the preceding asexual generation are already committed to either develop into gametocytes or continue asexual cycling [28]. Although the mechanism by which PbTPx-1 contributes to gametocyte development is unknown, ROS and antioxidants, including Prx, are known to influence the expression of a number of genes and to influence signal transduction pathways during cell differentiation and during organismal development [29,30]. A model in which Prx regulates peroxide-mediated signaling cascades by acting as a floodgate for H_2O_2 in mammalian cells has been proposed [12,14]. Homology modeling of PbTPx-1 based on structural alignment with human homologues showed that it possesses a redox-sensitive type of peroxidatic active site structure, which enables the enzyme to act according to the floodgate model (data not shown). A model in which 2-Cys Prx regulates activation of stress-activated mitogen-activated protein kinase (MAPK) (Sty1) by forming peroxide-induced disulphide complexes in yeast has also been proposed [31]. A molecule homologous to Sty1 (MAPK1) has been identified in the *P. falciparum* genome, although its function is unknown. Gametocyte development can be induced by environmental factors such as host factors or drug treatments, and there is consistent evidence for the involvement of signal transduction pathways in this process [28]. PbTPx-1 may be involved in transducing extracellular signals for gene expression and in initiation of gametocyte development. If this is the case, gametocyte development and its sexual determination may occur independently, with PbTPx-1 participating only in the former process. Alternatively, PbTPx-1 may contribute to protein synthesis in both male and female gametocyte development as a molecular chaperone. Evidence that Prx acts as a molecular chaperone in yeast and in human cells has recently been reported [32,33]. PbTPx-1 may not take part in the process of male gamete formation, including gender-specific signaling, because the disruptant retained normal exflagellation activity [34,35]. Whether it participates in female gametogenesis will require fertilization experiments in vitro and in the mosquitos [35,36]. Although the mechanism by which PbTPx-1 contributes to gametocyte development remains unknown, the present study suggests the involvement of this moleclue in the sexual development of the malaria parasite. Further studies to elucidate the role of TPx-1 in gametocyte development will provide further insight into the involvement of this antioxidant protein in the sexual development of malaria parasites and may provide novel transmission-blocking strategies.

Acknowledgments

We are very grateful to Dr. A.P. Waters of Leiden University (Leiden, Netherlands) for providing targeting vector pMD204 to the Malaria Research and Reference Reagent Resource Center; MR4/ATCC and to Dr. M. Yuda of Mie University (Mie, Japan) for kind advice regarding gene targeting. We are also grateful to Dr. H. Oku of Gunma University (Isezaki, Japan) for comments regarding protein structure. This work was supported by a Grant-in-Aid for Scientific Research (C) (16590351 to S.I.K.) from the Japan Society for the Promotion of Science; Grants-in-Aid for Scientific Research (15406015 and 16390126 to M.T.) and Scientific Research on Priority Areas (2) (16017318 to S.I.K. and 16017273 to T.T.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; and a grant for Precursory Research for Embryonic Science and Technology from the Japan Science and Technology Agency (to S.I.K.). Sequence data for *P. berghei* chromosomes were obtained from the *Plasmodium* Genome Resource (PlasmoDB; http://plasmodb.org/).

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Molecular & Biochemical Parasitology 150 (2006) 353–358
MOLECULAR & BIOCHEMICAL PARASITOLOGY

Short communication

Plasmodium vivax serine repeat antigen (SERA) multigene family exhibits similar expression patterns in independent infections☆

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Received 14 June 2006; received in revised form 13 July 2006; accepted 14 July 2006
Available online 11 August 2006

Keywords: *Plasmodium vivax*; Serine repeat antigen (SERA); Transcription; Polymorphism; Malaria

Plasmodium vivax is the most prevalent form of human malaria accounting annually for 70–80 million cases in the tropics and subtropics [1]. Unfortunately, generally regarded as benign and sympatric with *Plasmodium falciparum*, research on *P. vivax* has lagged disproportionately. However, re-emergence, increased transmission and drug resistance [2] has led to renewed pressure for the development of an effective vaccine to control vivax malaria. Efforts are, currently, hampered by the lack of continuous *in vitro* culture and the scarcity of available information regarding the transcriptome or proteome [3].

The N-terminal 47-kDa domain (SE47′) of *P. falciparum* serine repeat antigen 5 (PfSERA5) has been exploited as a potential vaccine candidate. Recombinant SE47′ elicits specific antibodies that inhibit *in vitro* the parasite intraerythrocytic proliferation [4–6] and confers protective immunity in vaccinated *Aotus* and squirrel monkeys against challenge infection [7,8]. In a malaria-hyperendemic region in Uganda, naturally induced antibody response to the N-terminal domain positively correlated with increased protective immunity in adults; and higher levels of IgG3 anti-SE47′ and IgG anti-SE36 (the recombinant construct without the serine repeat region) were associated, respectively, with the absence of fever and lower parasitemia in the peripheral blood of children aged under 15 [9] and with protection against severe malaria in children under 5 years old [10]. This blood stage antigen is now on Phase I clinical trials in Japan.

PfSERA5 belongs to a large multigene family [11]. All *P. falciparum SERA* (*PfSERA1–9*) are transcribed most actively at trophozoite and schizont stages [12,13]; with PfSERA3, -4, -5 and -6 proteins found to be co-expressed in every single parasite cell [12]. The dominant expression of *PfSERA5* along with *PfSERA6* is consistent with the failure to disrupt these genes during blood stage growth [13]. These observations have led to the premise that only a subset of the family is essential for normal erythrocytic development.

In silico analysis of available *P. vivax* sequence from GenBank (AAKM01000018) have identified six more *SERA* homologues downstream of the five *V-SERA* genes found by Kiefer et al. [14] and flanking one *SERPHvivax* gene identified by Gor et al. [15] (Arisue et al., submitted for publication). In contrast to the co-expression of each *PfSERA* family member in the blood stages, previous studies using RT-PCR and an erythrocytic stage *P. vivax* cDNA library indicate that only a single SERA gene was transcribed from among previously annotated

Abbreviations: SERA, serine repeat antigen; MSP1, merozoite surface protein 1; SNP, single nucleotide polymorphism

☆ The nucleotide sequence data reported in this paper are available in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers AB260077–AB260114.

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doi:10.1016/j.molbiopara.2006.07.006

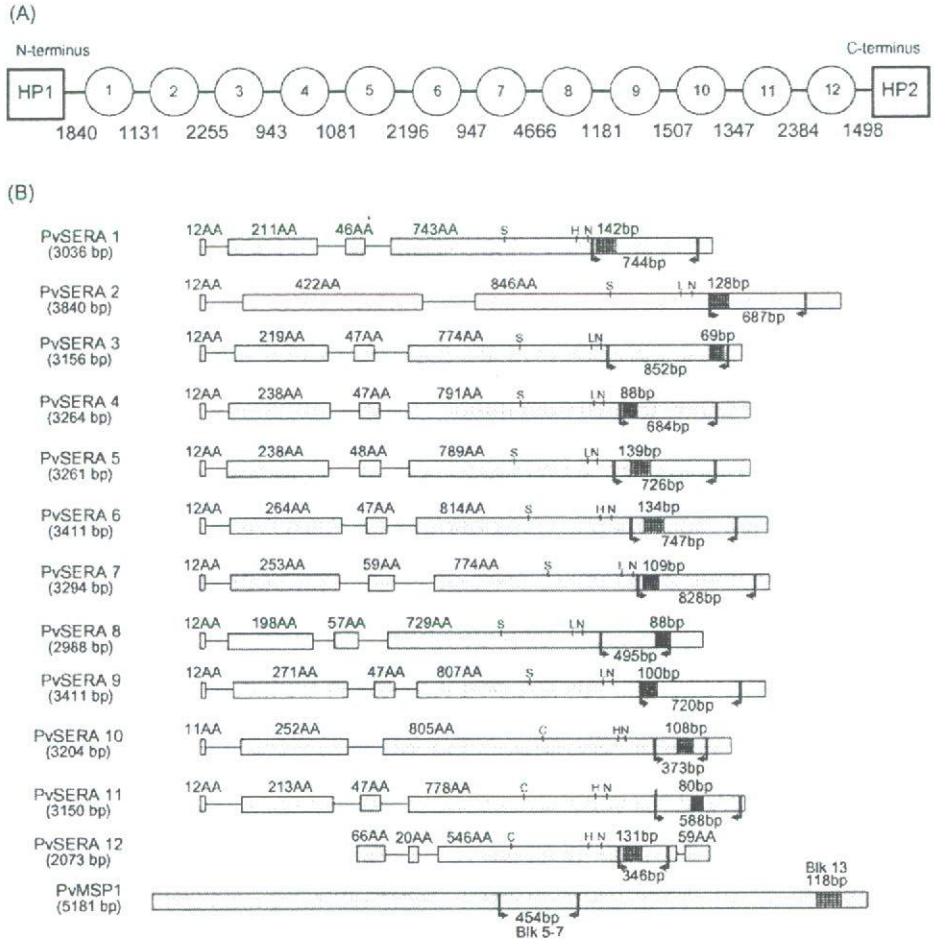
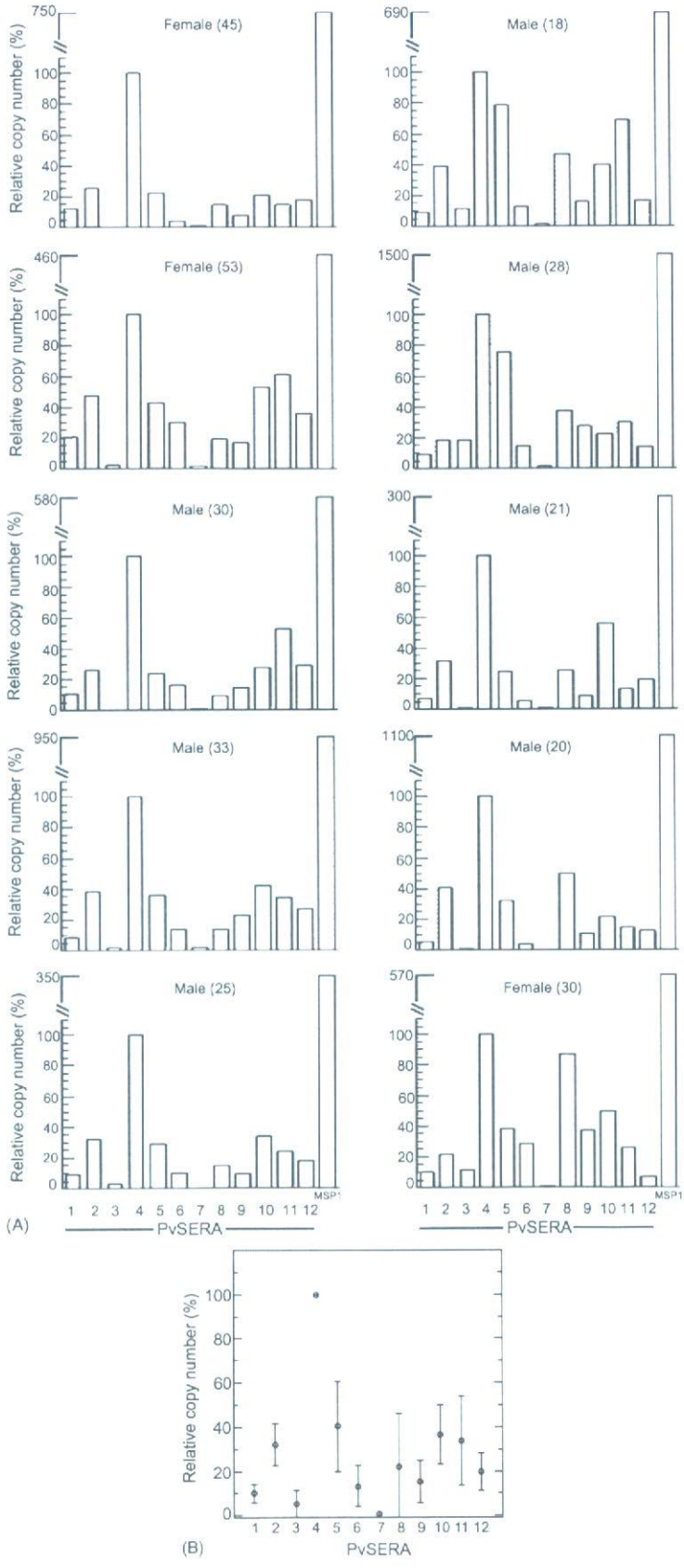


Fig. 1. The *P. vivax SERA* multigene family. (A) The tandem gene cluster arrangement of *SERA* family members in a region between two hypothetical proteins (HP) (Arisue et al., submitted for publication). Individual members are represented by ovals numbered 1–12. *PvSERA*1–9 unshaded ovals possess an active site serine and *PvSERA*10–12 shaded ovals possess an active site cysteine in the central proteinase domain. The length, in base pair, between each member is indicated. (B) SERA gene structure and relative positions of the primers used in the study. Numbers in parenthesis indicates the total length of the exons, in base pair (bp). Exons are denoted by grey boxes and lines linking boxes represent introns. Length of each exon is shown in amino acids (AA). The critical amino acid residues involved in cysteine proteinase activity are indicated. Positions of the primers for real-time PCR and the expected fragment sizes are in cross-striped bars superimposed on the grey exons. Primer positions for genotyping and the expected amplified lengths are symbolized as lined-arrows in the C-terminal portions of *PvSERA* and in the central region in *PvMSP1*. Blk, variable block numbers in *PvMSP1* as described [23].

members [14]. Whether the disparity in expression reflects interspecies difference or dissimilarity of expression patterns *in vivo* and *in vitro* needs to be addressed. Furthermore, because malaria antigens generally exhibit remarkable polymorphisms and their allelic forms differ in the ability to abrogate recognition of the host immune response, the extent of sequence variation is undoubtedly important for malaria vaccine development as well as enabling antigenic diversity-generating mechanisms to become elucidated [16]. Sequence polymorphism of PfSERA5 was virtually restricted to a region in the 47 kDa domain (amino

Fig. 2. Relative transcription of the *PvSERA* genes as assessed by real-time PCR. For quantification purposes, standard curves were generated using serially diluted (1.25 ng–1.25 pg) linearized plasmid DNA for each *PvSERA* gene. Standard curves associating the threshold cycles (C_T) against log amount of starting plasmid were created over a concentration range of standards within which the C_T value showed reproducible linearity [average correlation coefficients, $R^2 = 0.99638$; range = 0.98547–0.9999; $n = 78$; S.D. = 0.00321]. Averages of the slopes and *y*-intercepts for each gene standard were imported in the formula: $C_T = sn + y$; where s stands for the slope, *n* for log copy number, and *y* for *y*-intercept; to estimate relative copy number based on C_T values of the sample. As reference gene, transcription of *P. vivax* merozoite surface protein 1 (MSP1) was measured in each run. (A) Bar graphs show the copy numbers of individual *PvSERA* genes, relative to the copy number of *PvSERA4*, as assayed in different patient samples (gender and age are indicated). Patient samples were obtained during May 2002 and February 2005. In samples containing predominantly ring-stage parasites, *ex vivo* cultures were performed for 30–36 or 18–24 h when the parasites were at ring or at amoeboid stages, respectively; and processed (as modified from Chotivanich et al. [24]). *P. vivax*-infected erythrocytes were enriched by percoll-gradient centrifugation, frozen and stored at −80 °C until analysis. Enriched fractions contained trophozoite/schizont stage parasites (76–93%; mean = 83%) and gametocytes (7–24%; mean = 17%). Representative results of two independent assays are shown. (B) Mean transcription, expressed as relative copy number, of each *SERA* family member ($n = 10$); error bars depict standard deviation.


Female (45)
Male (18)
Female (53)
Male (28)
Male (30)
Male (21)
Male (33)
Male (20)
Male (25)
Female (30)
Relative copy number (%)
MSP1
1 2 3 4 5 6 7 8 9 10 11 12
PvSERA
(A)
(B)

acids 23–382), due mostly to deletion/insertion events in the octomer repeat units and in the stretch of serine residues; thereby, suggesting that a localized segment is under immune-mediated selection [17]. Virtually, nothing is known for the genetic diversity of the *SERA* genes of *P. vivax* (*PvSERA*). In this study, we investigate the relative transcription levels and the genetic diversity of *PvSERA* family members in field isolates from Thailand.

Blood samples were collected from *P. vivax*-infected uncomplicated adult (≥16 years old) patients consulting the Mae Sot Malaria Clinic, Northwestern Thailand. *P. vivax* infection incidence was 7.06/1000 population based on the 2004 Annual Report of the Rural Vector Borne Diseases (Department of Disease Control, Thailand). Informed consent, under the guidelines of the Ethical Review Committee of Mahidol University, Thai Ministry of Public Health and U.S. Army, was obtained from selected volunteers that have not received or are taking antimalarial drugs at the time of blood collection. Diagnosis for *P. vivax* infection was confirmed using Giemsa-stained blood smears and by real-time PCR using 18S rRNA species-specific primers [18].

Parasite total RNA was isolated using RNeasy Mini column (Qiagen) and the RNase-Free DNase Set (Qiagen). First-strand cDNA was synthesized with Qiagen Omniscript Reverse Transcriptase (RT) in 20 μl reaction volumes of 1 μl total RNA, 0.2 mM each dNTP, 1 μM oligo-dT primer, 10 units RNase inhibitor, and 4 units RT.

Real-time PCR was performed by using Qiagen QuantiTect SYBR Green PCR kit and an ABI PRISM 7900 detection system. The programmed protocol was 95 °C, 15 min; (94 °C, 20 s; 60 °C, 60 s; 57 °C, 15 s) × 40 cycles. Reaction volumes of 20 μl included 1 μl cDNA, 0.2 μM each primer, 10 μl of 2× QuantiTect SYBR Green PCR Master Mix and 0.03 units Pfu polymerase (Promega). All cDNA samples were assayed in duplicate using primers targeting the C-terminal region of each *PvSERA* gene family member (Fig. 1B and Supplementary Table 1). Fidelity of the reactions was confirmed by sequencing. A standard PCR assay using *P. falciparum* (3D7) genomic DNA as template yielded no amplified product, confirming the species specificity of the primers used. RT negative and no cDNA template controls were included in each real-time PCR run.

Genomic DNA was isolated using either DNAzol Reagent (Invitrogen) or QIAamp DNA Blood Mini Kit (Qiagen). Primers for genotyping amplified short C-terminal fragments of 500–800 bp (Fig. 1B and Supplementary Table 1). A 25 μl PCR mixture contained 1 μl template, 0.2 mM each dNTP, 0.2 μM of each primer and 0.6 units Pfu polymerase. The thermal profile was 95 °C, 5 min; (91 °C, 30 s; 53 °C, 30 s; 61 °C, 3 min) × 40 cycles. Amplified products were cloned into pCR-Blunt II-TOPO (Invitrogen) and sequenced in an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). At least two independent amplifications for each gene locus were used. When singleton mutation was found, bi-directional sequencing was repeated from the original template for verification. Nucleotide and deduced amino acid sequence alignments were performed using GENETYX Ver. 7.0.3 and ClustalW Ver. 1.83. Haplotype diversity or expected heterozygosity was calculated as described [19].

Genes arranged in tandem cluster between two hypothetical proteins are referred to in this paper as *PvSERA1–12*, with *PvSERA1–5*, being the previously described *V-SERA 2* (AAB41485.1), *V-SERA 4* (AAB41486.1), *V-SERA 3* (AAB41487.1), *V-SERA 1* (AAB41488.1), and *V-SERA 5* (AAB41489.1), respectively [14]; and *PvSERA10* corresponds to previously identified *SERPHvivax* (AF052747.1) [15]. Deduced amino acid sequences reveal a highly similar exon/intron organization with *PvSERA1*, *-3* to *-9* and *-11* possessing the characteristic four-exon structure of the family (Fig. 1A and B). *PvSERA2* and *-10* are atypical, due to the fusion of exons III and IV. *PvSERA12* bears a different structural organization, with a truncated N-terminal domain, similar to *PfSERA8* and all other identified *Plasmodium SERA* sequences lying upstream to the second hypothetical protein (Arisue et al., submitted for publication). The canonical Cys His Asn triad of cysteine proteinases is replaced by Ser Leu Asn in seven members: *PvSERA2* to *-5* and *-7* to *-9* (Fig. 1B). In contrast to the *P. falciparum SERA* serine cluster, which was further subdivided into three groups according to the presence of additional mutations to the active site histidine (His to Met; His to Leu; no mutations) [20], only one mutation was observed in *P. vivax* (His to Leu).

Real-time PCR analysis of infected erythrocytes from all patient samples show almost similar transcription profiles, though transcription levels varied to some extent depending on the individual isolates as observed for *PfSERA* family members [12,13]. *PvSERA4* was always more strongly transcribed than other *SERA* genes; *PvSERA3* and *-7*, the least transcribed members (Fig. 2). Relatively high transcription was also noted for *PvSERA2*, *-5*, *-10*, and *-11* (Fig. 2B). The asynchronous parasite populations and the differences in parasitemias likely affected transcription levels as expected when using isolates from natural infections. Attempts to correlate differential expression of *PvSERA* family members with gametocyte levels failed, due to relatively constant proportion of gametocytes in the samples. Although we cannot rule out the possibility that some gene family members might be strongly expressed in the gametocyte stage, the 12 *PvSERA* family members do not appear to be differentially expressed in 10 patient samples collected at various times.

The highest transcription of *PvSERA4* in all isolates parallels that of *PfSERA5*, the predominantly expressed gene member in *P. falciparum*. This contrasts an earlier report that only a single *SERA* (*PvSERA5*) gene was transcribed in *P. vivax* blood stages using a cDNA from Sal-1 infected *Aotus vociferans* [14]. Differences in detection technique and primer locations/sensitivities might explain this discrepancy. The expression profile is clearly different from that observed for the *P. vivax vir* gene family in different patients [21], suggesting that *SERA* does not function as a gene resource for antigenic variation.

In assessing *PvSERA* diversity, C-terminal sequences of *PvSERA4*, *-5*, and *-8*, with *PvMSP1* as control, were used for analyses. These gene family members were arbitrarily chosen based on preliminary screening that showed *PvSERA1*, *-2* and *-12* polymorphisms due mainly to single nucleotide polymorphisms (SNPs); *PvSERA3* to *-11* due to dele-

Table 1
Polymorphisms of *PvSERA4*, *-5*, *-8* and *PvMSP 1* from Thai field isolates

Gene locus	Allele distribution			Haplotype diversity[a]
	Allele	n^b	Frequency	
PvSERA4	4A-1	1	0.111	0.944 ± 0.070
	4A-2	1	0.111	
	4B-1	2	0.222	
	4B-2	2	0.222	
	4B-3	1	0.111	
	4C	1	0.111	
	4R	1	0.111	
PvSERA5	5A-1	8	0.364	0.784 ± 0.063
	5A-2	1	0.045	
	5A-3	1	0.045	
	5R	1	0.045	
	5B-a1	2	0.091	
	5B-a2	1	0.045	
	5B-b1	7	0.318	
	5B-b2	1	0.045	
PvSERA8	8A-1	5	0.312	0.900 ± 0.062
	8A-2	1	0.062	
	8A-3	1	0.062	
	8A-4	2	0.125	
	8A-5	1	0.062	
	8A-6	1	0.062	
	8B-1	2	0.125	
	8B-2	1	0.062	
	8B-3	1	0.062	
	8B-4	1	0.062	
PvMSP1	S1	8	0.267	0.892 ± 0.036
	S2	2	0.067	
	S3	1	0.033	
	S4	2	0.067	
	S5	4	0.133	
	S6	1	0.033	
	S7	5	0.167	
	S8	1	0.033	
	S9	1	0.033	
	S10	1	0.033	
	S11	2	0.067	
	S12	1	0.033	
	B1	1	0.033	

[a] Haplotype diversity index (h) and Student's t-test were calculated as described [19].
[b] n = number of isolates (patient samples) in which the allele was observed.

tion/insertion events compared to Sal-1 sequence type. Table 1 shows alleles at the three *PvSERA* and *PvMSP1* loci. The heterogeneity in the C-termini region of *PvSERA* was in sharp contrast to the complete sequence conservation in the C-termini of *PfSERA5* [5]. At each locus of *PvSERA*s, variable regions can be grouped into 2–3 major allelic forms and each allele in turn shows polymorphism resulting from point mutations or deletions (Supplementary Fig. 1).

PvSERA4 allele groups showed 41–67% pairwise identity with deletions and insertions as well as differing numbers of QG(P/S)(P/S) dispersed repeats. One recombinant type (Allele 4R) was identified. *PvSERA5* alleles had 50–80% pairwise sequence identity between basic sequence types characterized by having either glycine imperfect repeats (Alleles 5A) or GVGVA(P/T) repeats (Alleles 5B). One recombinant type (5R) was also identified. *PvSERA8* alleles had 63–72% pairwise identity, with dimorphic SNPs in one group (Alleles 8A) and another group having a number of substitutions relative to Sal-1 type (Alleles 8B). *PvMSP1* polymorphism was also essentially dimorphic in the region sequenced. Majority of the parasites are of the Sal-1 sequence type. Eight of the observed SNPs were previously reported nucleotide polymorphisms and seven are novel substitutions.

Haplotype diversity of *PvSERA4*, *-5*, and *-8* was substantially high, ranging from 0.78 to 0.94. The haplotype diversity in this three loci did not differ significantly from each other whether the gene family member was highly expressed or not; and their diversity levels were comparable to *PvMSP1*. All infections were complex, having a mean of 2–3 genotypes per infection based on *PvSERA* and *PvMSP1*, respectively. The prevalence of diverse *PvSERA* alleles and multiplicity of genotype infections may well be the source of the recombinant types observed in *PvSERA4* and *-5* from different isolates and sampling periods. Thus, we observed a high level of heterozygosity of *PvSERA* alleles in a *P. vivax* population from Thailand where malaria transmission is low.

In this first analysis of *PvSERA* transcription and genetic diversity in natural infections, we observed that all SERA gene members are transcribed in the blood stage and that the expression profile of the gene family is similar in different patient samples. The significantly dominant transcription of *PvSERA4* parallels the expression profile of PfSERA5, a blood stage vaccine candidate for falciparum malaria. That the SERA multigene family is unique to *Plasmodium* provides a glimpse of the molecules role in parasite survival yet fails to explain the presence of so many homologues, especially in *P. vivax*. Further studies are necessary to determine whether the number of family members is related to the unique aspects of vivax malaria with regard to the invasion of host reticulocytes and/or the presence of hypnozoites; or, similar to *PfSERA*, other members may be substantially upregulated or differentially expressed in another life cycle [22, Arisue et al., submitted for publication]. The sequence diversity of *PvSERA4* is clearly distinct from *PfSERA5*, which shows virtually no diversity in the C-terminal part [5]. This study revealed remarkably high haplotype diversity of *PvSERA* family, a level comparable to that of *PvMSP1*, one of the most polymorphic *P. vivax* antigen genes so far known. In general, the genetic diversity of *P. falciparum* is higher in high transmission areas than in low transmission areas. Here, we observed a high genetic diversity of *P. vivax* in a local area of Thailand with low transmission intensity, indicating a unique feature of antigen diversity of *P. vivax*. The nature and extent of polymorphisms in the C-terminal regions might have important implications for estimating potential positive selection operating at the *PvSERA* gene family.

Acknowledgements

Researchers and funding agencies of The Institute for Genomic Research (http://www.tigr.org) are thanked for the sequence data of *P. vivax* (Salvador 1). Sequencing of *P. vivax* was accomplished with support from the National Institute of

Allergy and Infectious Diseases (NIAID), the U.S. Department of Defense (DoD), and the Burroughs Wellcome Fund. We also thank K. Tai for technical assistance and E. Palacpac for figure preparation. This work was supported by grants from The 21st Century COE Program (Combined Program on Microbiology and Immunology), the CLUSTER, and Grant-in-Aid for Scientific Research (A) (13357002) from the Japanese Ministry of Education, Science, Sports, Culture and Technology to T.H.; from Mahidol University to R.U. and the U.S. Military Infectious Disease Research Program to J.S.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molbiopara.2006.07.006.

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Modeling of re-emerging *Plasmodium vivax* in the northern area of the Republic of Korea based on a mathematical model

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Journal of
The Faculty of Environmental Science and Technology
Okayama University
Volume 11, Number 1, March 2006

Journal of the Faculty of Environmental Science and Technology,
Okayama University
Vol.11, No.1, pp.1-7, March 2006

Modeling of re-emerging *Plasmodium vivax* in the Northern Area of the Republic of Korea Based on a Mathematical Model

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(Received November 30, 2005)

Plasmodium vivax re-emerged in 1993 near the demilitarized zone (DMZ) in South Korea, although *P. vivax* malaria disappeared in South Korea in 1979. The re-emergence of malaria in South Korea is believed to have originated from infection by mosquitoes from North Korea across the DMZ. The principal vector of *P. vivax* in the Korean Peninsula is *Anopheles sinensis*. The density of *An. sinensis* has a peak during the second week of July. The North Korean strain of *P. vivax* has 2 characteristics: a wide distribution of the terms of relapse and a high rate of relapse. Therefore, we may well wonder why the incidence of malaria is concentrated in summer, especially in August. Mathematical models in North Korea and South Korea were constructed, in which the South Korean model was affected unidirectionally by the North Korean model. We carried out simulations of the model for the Paju-shi and Yonchon-gun situations near the DMZ region. The simulation results followed the time-course of the re-emergence of *P. vivax* there, and revealed the mechanism of the elevation of the incidence of *P. vivax* in summer.

Key words: *DMZ, Korea, model, Plasmodium vivax, re-emergence*

1. Introduction

Plasmodium vivax malaria was endemic in the Korean Peninsula for many centuries. Infection by *P. vivax* was common after the Korean War. Later, the National Malaria Eradication Service (NMES) was established in collaboration with the World Health Organization, and the WHO declared in 1979 that indigenous malaria had disappeared in South Korea (Paik *et al.*, 1987). In 1993, the first occurrence of the re-emergence of *P. vivax* was reported in Paju-shi of Kyonggi-do near the demilitarized zone (DMZ). Thereafter, the incidence of malaria increased exponentially, and in 2000, more than 4,000 cases of *P. vivax* infection were diagnosed. After 2000, the incidence of malaria decreased due to malaria control measures. It was suggested that the re-emerging malaria in South Korea originated from infection by mosquitoes from North Korea (Kho *et al.*, 1999). The North Korean strain of *P. vivax* has 2 characteristics: the terms of relapse after infection are widely distributed from several months to 1 or 2 years (Kim, 2001; Oh *et al.*, 2001; Bray *et al.*, 1982), and the rate of relapse is relatively high compared with that of other strains of *P. vivax* (Kim, 2001; Oh *et al.*, 2001; Bray *et al.*, 1982). Most cases of malaria in South Korea are observed in June-September, with especially high incidence in August.

There are 7 *Anopheles* species in Korea, and only 2 species, that is, *An. sinensis* and *An. yatsushiroensis*, are capable of acting as vectors for the transmission of *P. vivax* (Ree *et al.*, 1967). The principal vector of *P. vivax* is *An. sinensis*, and the density of *An. sinensis* has a peak during the second week of July (Lee *et al.*, 2002).

Mathematical models are useful for forecasting the prevalence of infections diseases and for evaluating control strategies. Such a model for *Plasmodium falciparum*, the DMT model, was constructed by Dietz *et al.* (1974). In the present study, a mathematical model of the re-emergence of malaria in South Korea was constructed based on the DMT model and using the characteristics of the North Korean strain of *P. vivax*, which include a wide distribution of the terms of relapse and a high rate of relapse. The model consisted of models in North Korea

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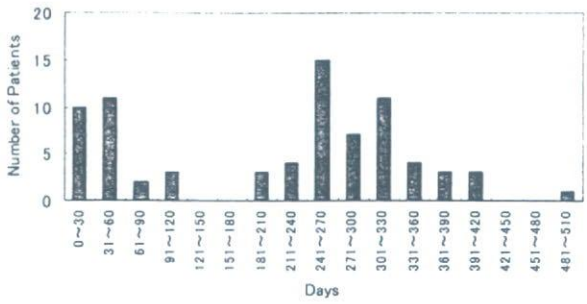

Fig. 1 The distribution of the latent period of the re-emergence of malaria in 77 patients in South Korea. Derived from Oh *et al.* (2001).

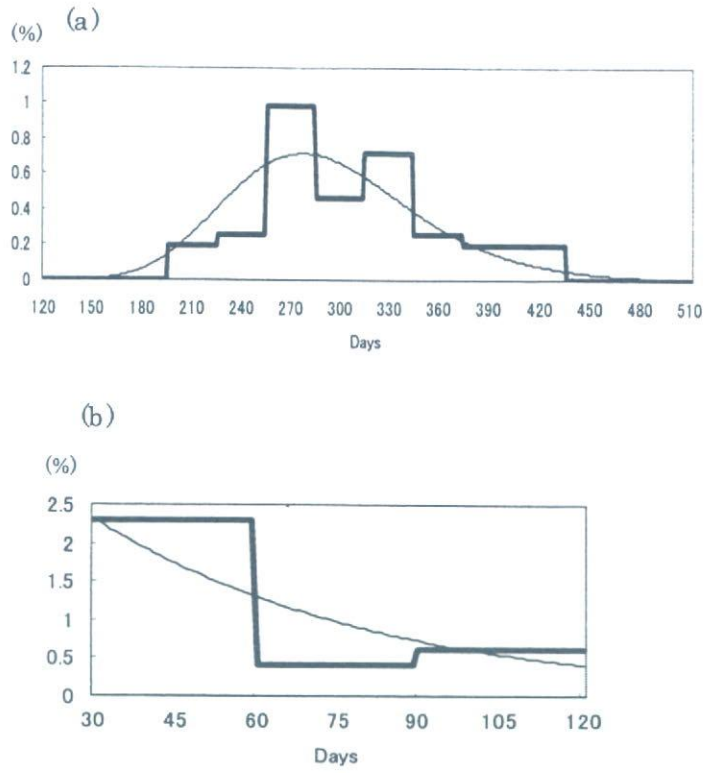

Fig. 2 The distribution of long incubation period (a) and short incubation period (b). The log-normal distribution (thin curve) (a) and the exponential distribution (thin curve) (b) compared with the surveillance data (thick line).

and South Korea. In the North Korean model, the population of individuals was divided into 5 epidemiological classes. On the other hand, in the South Korean model, the population of individuals was divided into 4 classes due to the different public health circumstances. The South Korean model was affected unidirectionally by the North Korean model.

The simulation was carried out for the situations of Paju-shi and Yonchon-gun in Kyonggi-do with the initial condition that there were no infected individuals. The results of simulations in Paju-shi and Yonchon-gun followed the time-course of the re-emergence of *P. vivax*. They also revealed the mechanism by which the incidence of *P. vivax* is highest in the summer considering the above 2 characteristics of the North Korean strain of *P. vivax*.

In the near future, *Anopheles* mosquito vectors capable of malaria transmission may invade countries that are free from malaria because of the effect of global warming, and malaria may re-emerge. Our method will be useful for the prediction of the prevalence of malaria in countries in which malaria re-emerges.

2. Materials and Methods

2-1 Relapses

The North Korean strain of *P. vivax* has the characteristics that the terms of relapse after infection are widely distributed from several months to 1 or 2 years (Kim, 2001; Oh *et al.*, 2001; Bray *et al.*, 1982). The surveillance of the incubation periods of relapse for 73 veterans receiving prophylaxis of chloroquine and 4 civilians who were diagnosed at 3 university hospitals, Seoul National University Hospital, Chungbuk National University Hospital and Chonnam National University Hospital (January 1, 1996 - December 31, 1999) is shown in Fig. 1 (Oh *et al.*, 2001). It was reported that there were no cases of relapse 4-6 months after infection and that the outbreak of relapse cases had 2 peaks at 1-2 months and 8-9 months after infection. Therefore, the distribution of the terms leading to relapse is grouped into 2 parts: 1 - 4 months after infection and more than 6 months after infection, which are called "short incubation period" and "long incubation period", respectively, while the cases who develop parasitemia within 1 month after infection are regarded as "primary infection". In this study, it was assumed that prophylaxis of chloroquine would not prevent relapses, while it prevents primary infections. In order to establish modeling of the relapse-distribution, the distribution of short incubation period would be applied to the exponential distribution for the regression curve (mean = 0.019 1/days), (Fig. 2-(b)), and the distribution of long incubation period would be applied to the log-normal distribution (mean = 288 days, mean $\pm$ S. D. =288-52, 288+64 days) (Fig. 2-(a)). A χ^2-value 13.37 (15.51, *d. f.* = 8) was accepted as statistically significant ($P < 0.05$) by the χ^2-fitness test.

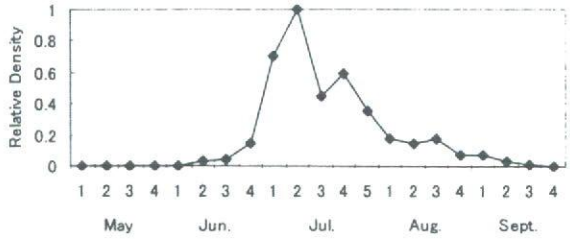

Fig. 3 The relative variation of the weekly density of female mosquitoes to the highest density in August. Derived from Lee *et al.* (2002).

2-2 The ratio of primary infection to relapse

The North Korean strain of *P. vivax* has the characteristics that the rate of relapse is relatively high compared with that of other strains of *P. vivax*. Kim (2001) observed that only 4 cases (8%) were primary infection while 52 cases (92%) were relapse. Moreover, Park *et al.* (2003) reported that the number of people infected with malaria in the military was 393 during 1993 - 2000 and that 56 cases had been in the non-endemic region of *P. vivax*. Therefore, it is speculated that the rate of primary infection is at least 14% of the total cases. Oh *et al.* (2001) also indicated that the numbers of primary infection and relapse were 10 (13%) and 67 (87%), respectively. We adopted 87% as the average rate of relapse based on the above 3 reports.

2-3 The seasonal fluctuation of *Anopheles sinensis*

It is known that there are 7 species of *Anopheles* in Korea, and that only 2 species, that is, *An. sinensis* and *An. yatsushiroensis,* are capable of acting as vectors for the transmission of *P. vivax*. (Ree *et al.,* 1967). *An. sinensis,* the principal malaria vector, comprises more than 80% of total malaria vectors (Cho *et al.,* 2002). Therefore, it is assumed that malaria in Korea would be transmitted only by *An. sinensis* species. The population of *An. sinensis* rises steeply from the second week of June, reaches a peak during the second week of July, and then gradually decreases through the fourth week of September (Lee *et al.,* 2002). The seasonal fluctuation of relative density compared with the peak week (the second week of July) is shown in Fig. 3.

2-4 The seasonal pattern of the incidence of *P. vivax*

The monthly time-course of malaria cases in 1993-1994 and 1998-2000 is shown in Fig. 4 (National

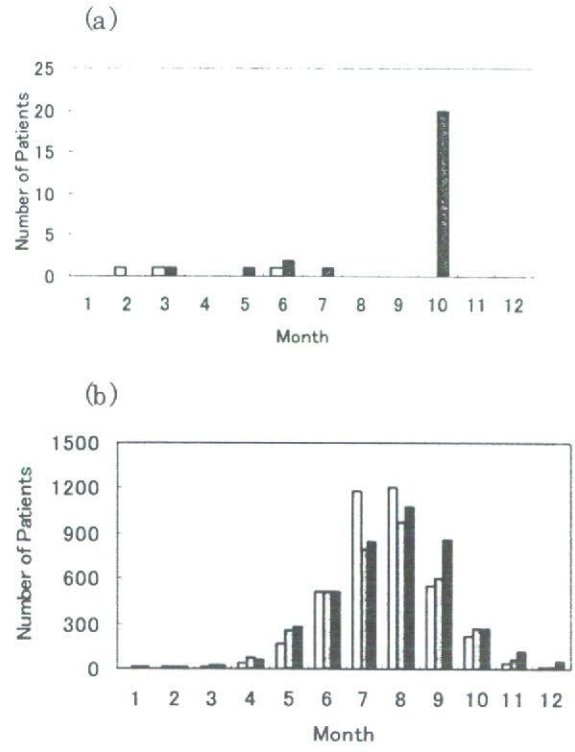

Fig. 4 The monthly incidence of *P. vivax* in South Korea (a) in 1993 (white bars) and 1994 (gray bars) and (b) in 1998 (white bars), 1999 (gray bars) and 2000 (black bars).

Institute of Health, Korea, 2003). Most cases of malaria were observed in June-September, with an especially high incidence in August from 1995 to 2003, whereas an elevated incidence of malaria cases was not observed in the summer in 1993-1994. The seasonal pattern of the incidence was also noted in an old report (Hasegawa, 1913) stating that the incidence of *P. vivax* was highest in June - October. Given these 2 characteristics, that is, the wide distribution of terms to relapse (1-16 months) and the high relapse rate (87%), why is the incidence of malaria concentrated in the summer, especially in August?

2-5 Malaria cases in the northern part of South Korea

In 2 regions near the DMZ, Paju-shi, where the first case of the re-emergence of malaria was discovered, and Yonchon-gun, where most of the cases of malaria were observed in the beginning of re-emergence of malaria, the incidence of *P. vivax* increased until 1999, but it subsequently decreased thereafter (CDMR, 2003). Fig. 5 shows the civilian cases of malaria in Paju-shi (1994-2002) and Yonchon-gun (1995-2002), where the surveillance data was derived from CDMR (2003); Moon (2001); Lee (1998); Park (2003).

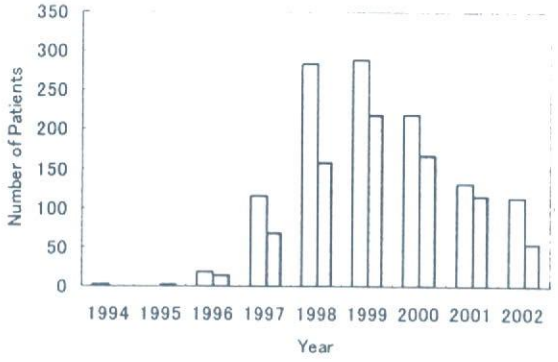

Fig. 5 Cases of malaria in civilians in Paju-shi (white bars) and Yonchon-gun (gray bars) in 1994 – 2002. The vertical axis represents the number of cases per 100,000.

2-6 Re-emergence of *P. vivax*

In 1993, *P. vivax* malaria re-emerged in South Korea, although WHO declared in 1979 that indigenous malaria had disappeared. The first possibility regarding this re-emergence was that in the beginning of the 1990's, immigrant workers from countries with endemic malaria provided a nidus for re-establishment of the epidemic of *P. vivax*. The second possibility was that mosquitoes infected with *P. vivax* came to South Korea across the DMZ. The facts that most of the cases in the beginning of the re-emergence occurred in military personnel who served near the DMZ, and that the cases of malaria spread from the DMZ toward the south supported the second possibility (Kho *et al.*, 1999). In this study, it was assumed that the infected mosquitoes will come across the DMZ at a constant rate based on the second possibility.

2-7 Mathematical model

The mathematical model in South Korea and North Korea was established on the basis of the DMT model (Dietz *et al.*, 1974). The South Korean model is affected unidirectionally by the North Korean model, because the infected mosquitoes will come to South Korea across the DMZ.

North Korean model

In the North Korean model, the population of individuals is divided into 5 epidemiological classes: susceptible (S), dormant hypnozoites without parasitemia (H), latent for primary infection (Pr), positive with gametocytes (In) and positive without gametocytes (Po). Moreover, H, Pr, In, Po classes are subdivided into 3 subclasses which are denoted by the suffix i (i=0, 1, 2) according to the number of hypnozoites in their livers. We

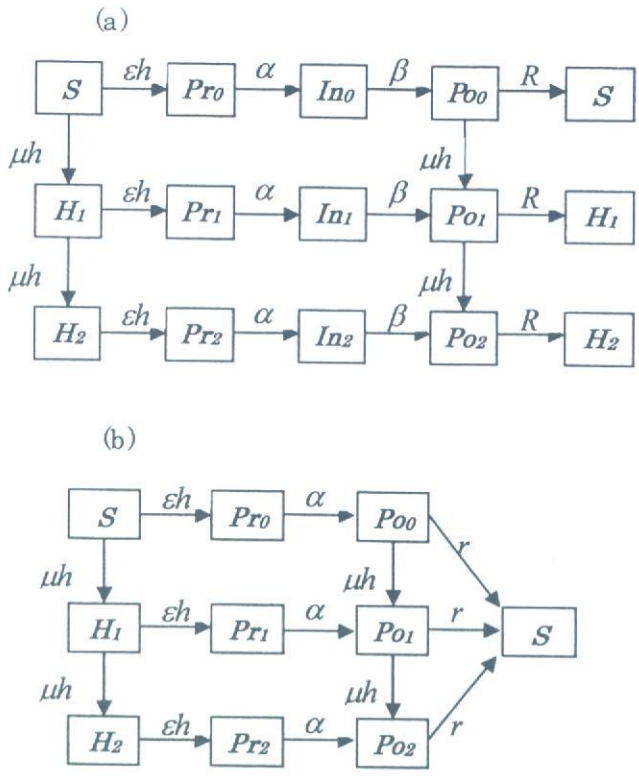

Fig. 6 The scheme of models in North Korea (a) and South Korea (b).

introduce the epidemiological parameters: inoculation rate (h), the onset rate of symptoms with infection (α), the rate of loss of infection (β) and the rate of recovery taking account of superinfection (R). Moreover, ε and μ are the ratio of primary infection and that of relapse, respectively ($\varepsilon+\mu=1$). The classes H_i, Pr_i and In_i, (i=1, 2) transfer into the class In_{i-1} when the individuals of these classes relapse into parasitemia. Moreover, the class Po_i (i=1, 2) transfers into the class Po_{i-1} when the individuals of this class relapse into parasitemia. The scheme of the model in North Korea is illustrated in Fig. 6-(a).

South Korean model

Although the North Korean strain of *P. vivax* prevails in both North Korea and South Korea, the model for transmission in South Korea is modified compared to that for North Korea due to the different public health circumstances. In South Korea, cases of malaria usually recover in about 1 week upon treatment with chloroquine, and therefore the positive with gametocytes class (In) is combined with the positive without gametocytes class (Po). Since the radical treatment results in the clearance of hypnozoites in the malaria patients, it is assumed that the class (Po) transfers to the class (S) on recovery.

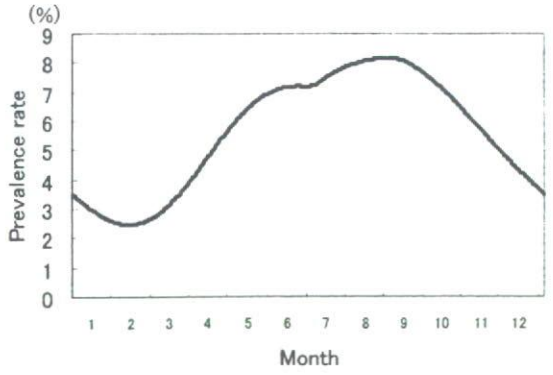

Fig. 7 The time-course of the prevalence in North Korea predicted by simulation by the model (the average rate of prevalence being 5.6%).

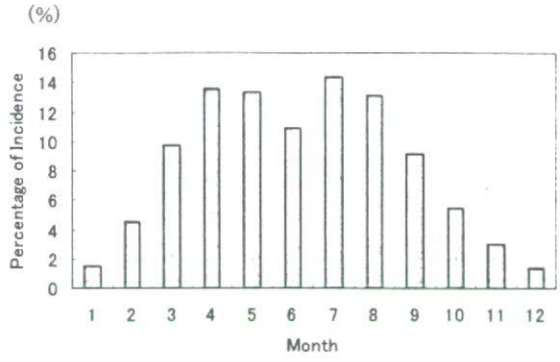

Fig. 8 The monthly incidence predicted by simulation using the model.

It is assumed that there are no infected individuals at the initial time in South Korea and that the infected mosquitoes come across the DMZ from North Korea, the number of which depends on the distance from the DMZ. The scheme of the model for South Korea is illustrated in Fig. 6-(b).

3. Results

North Korean model

The average prevalence rate is estimated as 5.6% by the simulation based on the transmission model of *P. vivax* in North Korea (Fig. 7).

Seasonal change of incidence

The seasonal change of incidence in South Korea is obtained through the simulation, where we take account of the distribution of relapse terms, the rate of relapse, the seasonal fluctuation of *An. sinensis* and the average prevalence rate in North Korea (Fig. 8). The simulation indicates that the prevalence is maintained at a high level from March to September, and has 2 peaks in April – May and July – August. In spite of the wide distribution of relapse terms and high rate of relapse, the simulation succeeded in modeling the peak incidence of malaria cases in the summer.

Re-emergence of malaria in Paju-shi and Yonchon-gun

A comparison of the surveillance data of cases of malaria in Paju-shi and Yonchon-gun in 1996 – 2002 and the prediction of the simulations is shown in Fig. 9-(a) and (b). The results of simulations followed the time-course of the re-emergence of *P. vivax* in 1996-1999 for both regions. On the other hand, the time-courses of the actual prevalence in Paju-shi and Yonchon-gun deviated from the predictions of the model simulation after 2000, because the model did not take into account any malaria control measures.

4. Discussion

In this study, we assumed that the southward movement of infected mosquitoes across the DMZ caused the re-emergence of *P. vivax* in South Korea. Mosquitoes that had been released 21 days before in Kyonggi-do were recaptured at rates of 37.1%, 29.4%, 21.1%, 10.3% and 2.1% at 1, 3, 6, 9 and 12 km from the release point, respectively, namely, about 90% of the mosquitoes were recaptured within 6 km from the release point (Cho *et al.*, 2002). Therefore, it is reasonable for mosquitoes to fly across the DMZ because the DMZ is about 2 km wide.

The 2 mathematical models in North Korea and South Korea could be constructed with 4 epidemiological parameters. It was difficult to decide the rate of prevalence in North Korea in the beginning of the 1990's, because there was little and uncertain information about the cases of malaria in North Korea. In the parasite survey implemented in South Korea in 1960, the year after the Korean War, 212 blood smears were positive among 18,697 collected blood smears (the average parasite rate being 1.1%), and the highest prevalence detected was 5% (Paik *et al.*, 1987). Therefore, we presume the prevalence in North Korea to be 5%. The transmission model for South Korea is modified from that for North Korea due to the different public health circumstances. We succeeded in evoking the re-emergence of *P. vivax* in Paju-shi and Yonchon-gun near the DMZ in South Korea, through 2 cooperative models in which the South Korean model was unidirectionally affected by the North Korean model.

In South Korea, *P. vivax* malaria has re-emerged since

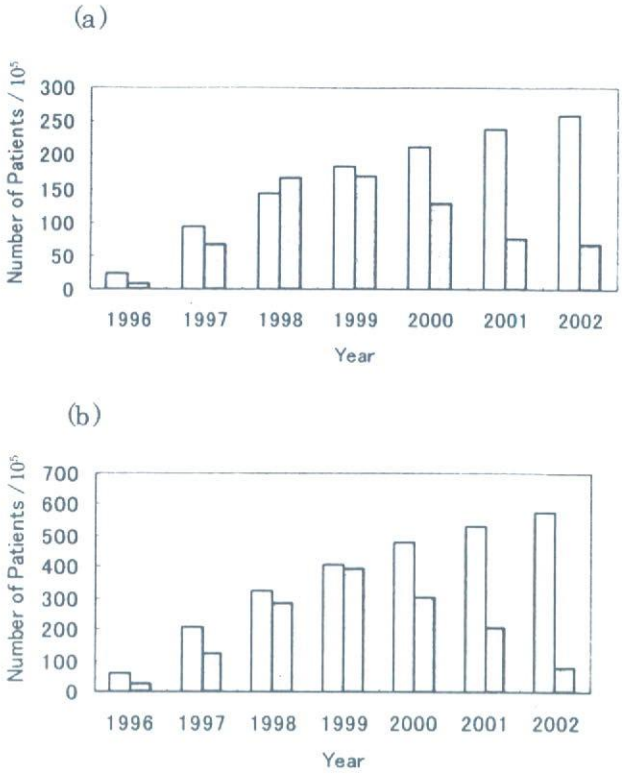

Fig. 9 The comparison of the prevalence between actual surveillance and the results of simulation in Paju-shi (a), Yonchon-gun (b). The time-courses of malaria cases per 100,000 obtained from surveillance and from the results of simulation are shown as gray bars and white bars, respectively.

1993 and the prevalence increased yearly until 2000. The incidence of *P. vivax* was highest in summer (June - September) in spite of the 2 characteristics of North Korean strain of *P. vivax* described in the text, that is, the high relapse rate and the wide distribution of relapse terms (Kim, 2001; Park *et al.*, 2003; Oh *et al.*, 2001; Bray *et al.*, 1982). The incidence in Korea had 2 peaks (April – May, July - August) in the simulation using our model. The simulation results revealed the mechanism by which the incidence of *P. vivax* was highest in summer, assuming the above 2 characteristics of *P. vivax*. The seasonal pattern of incidence would be affected by the incubation period. The first peak (April - May) and the second peak (July - August) would be related to the long incubation period and the short incubation period, respectively. If the average of the long incubation period would be prolonged from 288 days to 1 year, the incidence of *P. vivax* would be concentrated in summer and have a peak in August. However, the reason for the divergence of the peaks of the incidence of *P. vivax* in South Korea between the surveillance and the simulation is unknown.

The long incubation period (6 – 16 months) would be applied to the log-normal distribution, where the χ^2-value 13.37 was accepted as statistically significant (15.51, $d.f. = 8$, $P < 0.05$) by the χ^2-fitness test. The log-normal distribution was selected because of the long incubation period. The skewness (G) and the kurtosis (H) of the logarithmic transformation of 50 surveillance data are estimated as G=0.257 (0.533, P<0.05) and H=0.127 (0.99, P<0.05), respectively, which lead to fitting the distribution of the long incubation period to the log-normal distribution.

The number of civilian malaria cases increased until 1999 in Paju-shi and Yonchon-gun, where the malaria cases in these 2 regions accounted for 60-70% of the total malaria cases in Korea from 1993 to 1997. We succeeded in modeling the re-emergence of *P. vivax*. The time-courses of the true prevalence in Paju-shi and Yonchon-gun diverged from the time-course predicted by the model simulation after 2000, because the model took no account of any malaria control measures. The rate of contact with infected mosquitoes may be reduced by public health education and the use of window screens, while chemoprophylaxis prevents malaria infection. Early diagnosis and treatment may reduce the prevalence. In order to accurately predict the prevalence of *P. vivax* in South Korea, it would be necessary to incorporate the effect of malaria control measures in the South Korean model.

In the coming years, *Anopheles* mosquito vectors capable of malaria transmission may invade countries that are free from malaria because of the effect of global warming, and may cause the re-emergence of malaria. Our method will be useful for prediction of the prevalence in countries in which malaria re-emerges.

ACKNOWLEDGEMENTS: This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (Grant No. 16540105) and by grants from the Ministry of Health, Labour and Welfare, Japan for "Research for emerging and re-emerging infections" (Principal investigator: Dr H. Watanabe).

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Korean Journal of Parasitology
Vol. 44, No. 4: 285-294, December 2006

Reemerging vivax malaria: changing patterns of annual incidence and control programs in the Republic of Korea

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Abstract: Changing patterns of the reemerging *Plasmodium vivax* malaria in the Republic of Korea (South Korea) during the period 1993 to 2005 are briefly analyzed with emphasis on the control measures used and the effects of meteorological and entomological factors. Data were obtained from the Communicable Diseases Monthly Reports published by the Korea Center for Disease Control and Prevention, and webpages of World Health Organization and United Nations. Meteorological data of Kangwon-do (Province) were obtained from local weather stations. After its first reemergence in 1993, the prevalence of malaria increased exponentially, peaking in 2000, and then decreased. In total, 21,419 cases were reported between 1993 and 2005 in South Korea. In North Korea, a total of 916,225 cases were reported between 1999 and 2004. The occurrence of malaria in high risk areas of South Korea was significantly ($P < 0.05$) correlated with the mosquito population but not with temperature and rainfall. Control programs, including early case detection and treatment, mass chemoprophylaxis of soldiers, and international financial aids to North Korea for malaria control have been instituted. The situation of the reemerging vivax malaria in the Republic of Korea is remarkably improving during the recent years, at least in part, due to the control activities undertaken in South and North Korea.

Key words: *Plasmodium vivax*, vivax malaria, reemerging malaria, incidence, geographical distribution, seasonality, South Korea, North Korea

INTRODUCTION

Vivax malaria due to *Plasmodium vivax*, the only naturally occurring human malaria species in the Republic of Korea (hereafter referred to as South Korea), was endemic in South Korea until the late 1970s, when the country became malaria free (Paik et al., 1988; Chai, 1999). In particular, during the Korean War (1950-1953), 15,000 South Korean soldiers and more than 3,000 U.S. soldiers were reported to have contracted vivax malaria (Jones et al., 1953; Chai,

• Received 13 October 2006, accepted after revision 10 November 2006.
• This study was supported by a Korea Research Foundation Grant (KRF-2003-003-E00043), Ministry of Education, Republic of Korea.
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