their first-line antimalarial drugs for falciparum infection from chloroquine to SP. However, SP resistance was reported soon after its use as the monotherapy in these countries (Mberu et al., 2000; Sibley et al., 2001; Bwijo et al., 2003; Roper et al., 2003). In particular, in the province of KwaZulu-Natal in South Africa where SP was introduced as the first-line therapy in 1988, SP treatment failed and reached an unacceptable level (70%) by 2000. The first-line treatment was soon changed to the coartemether (artemether + lumefantrine) (Bredenkamp et al., 2001).

In the case of tuberculosis and AIDS, combination therapy delays the selection of drug resistant pathogens. For falciparum malaria, artesunate and mefloquine became the first widely used drug combination in Southeast Asia where the resistance to both drugs was prevented (Wilairatana et al., 1998; Nosten et al., 2000).

In Papua New Guinea, oral administration of 4-aminoquinoline (chloroquine or amodiaquine) was used for many years as the standard treatment for uncomplicated malaria. However, chloroquine resistant *P. falciparum* has become highly prevalent in many endemic regions in Papua New Guinea after its first emergence in 1976 (Han and Grimmond, 1976). In 2000, the combination of either one of the 4-aminoquinoline with SP was officially introduced as the first-line treatment for falciparum malaria. SP has therefore been used almost exclusively as a partner drug. Although the addition of chloroquine to SP offered lesser therapeutic advantage when compared with SP alone (Checchi et al., 2004; Talisuna et al., 2004a), it was expected that the introduction of the combination therapy could delay the activity of SP resistance (Rieckmann and Cheng, 2002).

We investigated whether the combination therapy (4-aminoquinoline + SP) will protect against the spread of resistance to SP in place where 4-aminoquinoline resistances are preexisting by determining the frequencies of *dhfr* and *dhps* haplotypes in *P. falciparum* isolates collected from Wewak, East Sepik in 2002 and 2003. We also investigated the microsatellite markers flanking the *dhfr* locus to assess the evolutionary origins of *dhfr* mutant alleles.

2. Materials and methods

2.1. Study site and patients

The study included patients attending Town and Wiryi clinics in Wewak, East Sepik Province (Fig. 1) in November 2002 and 2003, respectively. In this province, the average temperature is 27 (min 24; max 30) with an annual rainfall of about 2000 mm. Malaria is hyper-endemic with minor monthly variation, transmitted mainly by *Anopheles farauti* and *An. koliensis*. *P. falciparum* is the predominant parasite species in the province. Together with the previously reported sporozoite rate of Anopheles vectors (0.7%) in East Sepik (Benet et al., 2004), the annual entomological inoculation rate in this area was estimated to be 51 infective bites per person per year. Although the combination of either one of the 4-aminoquinoline with SP was officially adopted as the first-line treatment for falciparum malaria since 2000 in Papua New Guinea, this policy amendment was not widely implemented in our study site till 2001. Antifolate drug is exclusively used for the malaria infection. Patients of all age groups with clinical symptoms of uncomplicated malaria, e.g. fever, diarrhea, and vomiting were microscopically examined for the presence of parasites using thick and thin smears stained with Giemsa. *P. falciparum* infected patients who met the following criteria were recruited for this study: (1) asexual parasitemia from 1000 to 80,000 μL^{-1}, (2) no intake of antimalarials in the preceding 4 weeks, and (3) informed consent from patient or parent. The malaria-infected patients were treated with the combination of either one of the

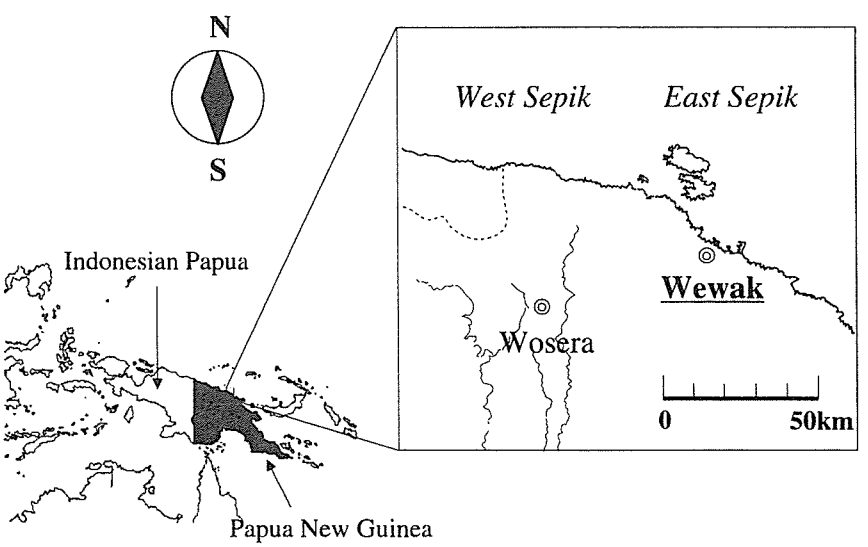

Fig. 1. Location of Wewak region in Papua New Guinea.

4-aminoquinoline and SP according to the official treatment policy.

2.2. Dhfr and dhps alleles

A finger prick blood sample (75 μL) was collected on chromatography filter paper ET31CHR (Whatman Limited, Kent, UK) before treatment. *P. falciparum* DNA was extracted using a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) with some modifications as described elsewhere (Sakihama et al., 2001). *Dhfr* and *dhps* were amplified by polymerase chain reaction (PCR) and amplified products were subjected to direct sequencing using primer pairs with BigDye terminator v1.1 cycle sequencing kit in ABI 377 DNA Sequencer (Applied Biosystems, Warrington, Cheshire, UK) as described by Reeder et al. (1996).

2.3. Microsatellite haplotyping

Nucleotide length variations (determined by the number of TA repeats) of microsatellite markers were determined to infer the origin of these alleles. We measured three microsatellite markers located at 0.1 kb and 3.87 kb upstream and 1.48 kb downstream of the *dhfr* locus as described by Nair et al. (2003). In brief, semi-nested PCR was performed using fluorescent end-labeled primers. Size variations of the amplified products were determined by electrophoresis on an ABI 377 and analyzed with Genescan software (Applied Biosystems). In the event of two or more polymorphisms being detected, we considered these isolates as mixed infections.

2.4. Statistical analysis

We calculated expected heterozygosity (h) at each microsatellite locus as $h = [n/(n-1)]\ [1 - \sum pi^2]$, where n is the number of infections sampled and pi is the frequency of the ith allele. We estimated the variance of h using a Taylor's series expansion. Fisher's exact probability test was used to assess statistical associations of allele frequencies between 2002 and 2003. $P < 0.05$ was considered statistically significant.

3. Result

3.1. Dhfr and dhps alleles

A total of 266 symptomatic patients were screened for eligibility in 2002 and 2003, and 173 were found to have *P. falciparum* mono-infections. Out of the total infected patients, 113 patients satisfied the inclusion criteria and were recruited for the study. *Dhfr* and *dhps* sequences were successfully determined in all the 113 blood samples (Table 1). Two of the seven loci in *dhfr* were found to be polymorphic corresponding to amino acid positions 59 and 108. The combination of the two mutations (ACNRNVI) was the predominant allele yielding prevalences of 83% and 96% for 2002 and 2003, respectively. The prevalences of wild *dhfr* alleles was reduced from 15% (2002) to 3% (2003) ($P = 0.02$). These prevalences of the wild allele were considerably lower than the prevalence (59%) reported in 1990–1993 in the Wosera region, an area adjacent to East Sepik (Reeder et al., 1996).

Table 1
Combinations of *dhfr* and *dhps* alleles in *Plasmodium falciparum* isolates from Wewak, Papua New Guinea in 2002 and 2003

Dhfr/dhps haplotype	No. of samples	
Dhfr + *dhps*	2002 ($n = 40$)	2003 ($n = 73$)
ACNCSVI[a] + SAKAA[b]	6 (15.0%)	2 (2.7%)
ACNCNVI + SAKAA	1 (2.5%)	1 (1.4%)
ACNRNVI + SAKAA	33 (82.5%)	63 (86.3%)
ACNRNVI + CAKAA	0	1 (1.4%)
ACNRNVI + SGEAA	0	6 (8.2%)

[a] Amino acid residues at positions 16, 51, 59, 108, 140, and 164 in *dhfr*.
[b] Amino acid residues at positions 436, 437, 540, 581, and 613 in *dhps*.

Three mutations were detected within the *dhps* gene; at amino acid positions 436, 437 and 540; but 100% ($n = 40$) and 90% ($n = 66$) of the isolates still harbored the wild allele derived in 2002 and 2003, respectively (Table 1). All mutations were detected in seven isolates derived in 2003. Six of them harbored double mutations at positions 436 and 437 (SGEAA) and all these six isolates also had the double mutant (ACNRNVI) allele in *dhfr*. These isolates therefore had *dhfr/dhps* quartet mutations (ACNRNVI + SGEAA).

3.2. Microsatellite polymorphisms around the dhfr locus

Associations between *dhfr* alleles and microsatellite markers flanking *dhfr* are shown in Table 2. Isolates harboring mixed microsatellite polymorphisms at any positions ($n = 14$) were excluded from this analysis. We found significant differences of h between parasites harboring wild and mutant *dhfr* (ACNRNVI) alleles in microsatellite markers located at 0.1 kb (0.75 in wild versus 0.39 in mutant, $P < 0.001$) and 3.87 kb (0.86 versus 0.41 in mutant, $P < 0.0005$) upstream of the *dhfr* locus.

Table 2
Dhfr alleles and microsatellite haplotypes around *dhfr* in 99 *P. falciparum* isolates from Wewak, Papua New Guinea in 2002 and 2003

Dhfr allele	Microsatellite haplotype[a]									
	156/192/203	156/194/203	156/198/203	156/202/205	156/204/205	172/194/203	173/206/203	176/192/203	176/194/203	178/194/203
ACNCSVI	1	1	1	–	–	2	1	2	–	–
ACNCNVI	–	–	–	–	–	–	–	–	–	2
ACNRNVI		2		64	1				20	2

[a] Size (bp) of microsatellite markers (−0.1 kb/−3.87 kb/+1.48 kb flanking *dhfr*).

Six microsatellite haplotypes were observed in eight isolates harboring wild *dhfr* allele. In contrast, among the 89 isolates harboring ACNRNVI allele, the microsatellite polymorphisms flanking *dhfr* were considerably restricted. Only two distinct haplotypes were predominant with the respective prevalences of 72% (156/202/205 haplotype) and 22% (176/194/203 haplotype). Among the remaining three haplotypes, two (156/194/203; $n = 2$ and 156/204/205; $n = 1$) were similar to the 156/202/205 haplotype, and one (178/194/203; $n = 2$) was similar to the 176/194/203 haplotype, suggesting that the three minor microsatellite haplotypes evolved from the two distinct major haplotypes (156/202/205 and 176/194/203) in the event of recombination.

Among the six isolates harboring the "*dhfr*/*dhps* quartet mutations", five isolates shared a same microsatellite haplotype but one is different, suggesting that the origins of the *dhfr*/*dhps* quartet mutant was not single in Papua New Guinea.

4. Discussion

In 2000, Papua New Guinea officially introduced the combination of SP with 4-aminoquinoline as a new treatment policy for uncomplicated malaria. Previously, SP had only been used in combination with quinine for severe malaria or after a first-line 4-aminoquinoline therapy has failed.

In the present study, a significant decrease of the *dhfr* wild allele was observed from 2002 to 2003 in the Wewak region, East Sepik province, 2–3 years after the change of the treatment policy. In addition, our observed *dhfr* wild allele prevalences (15% in 2002 and 3% in 2003) were considerably lower than that observed in the adjacent area of East Sepik province called the Wosera region (57%, 1990–1993) (Reeder et al., 1996).

There is a possibility that the observed pattern of increased SP resistance in 2003 results from the isolates come from a unique or from a little number of clusters of related malaria cases. However, the population of our study site in East Sepik is large, estimated to be several thousands of people. Records of patient's habitation demonstrated that malaria positive cases were fairly distributed over the 61 areas and the rate of related malaria cases were considerably low (5%). Thus, the possibility that the *P. falciparum* isolates in our study were mutually dependent would be excluded.

Our results suggest that reduced sensitivity to SP has rapidly developed in Papua New Guinea after the introduction of SP, even when combined with 4-aminoquinoline. In Uganda, rapid selection of *dhfr* mutations at position 59 and 108 was similarly reported after the introduction of SP in combination with previously used chloroquine (Sendagire et al., 2005).

The fact that the decrease of the *dhfr* wild allele was observed 2 and 3 years after the adoption of the new drug policy in 2000 is interesting. We could probably explain this phenomenon as follows: first, although the drug policy was officially changed in 2000, this policy amendment was not fully implemented till 2001 in our study site. Second, some patients tended to use self-medication (commonly 4-aminoquinoline alone) when they had a fever because of the difficult accessibility to the clinics. Third, the isolates harboring a mutant ACNRNVI allele has shown low to moderate levels of in vitro resistance to pyrimethamine (Peterson et al., 1988), which appears to be associated with a slower selection of this allele in the presence of SP.

The effect of SP as a monotherapy has previously been reported from several endemic regions. In Killifi, Kenya, significant decrease of in vitro IC_{50} values to pyrimethamine was observed in *P. falciparum* field isolates several years after the introduction of SP monotherapy (Mberu et al., 2000). In KwaZulu-Natal province, South Africa, the prevalences of the resistant allele, *dhfr* triple mutant (ACIRNVI), increased from 22% to 38% in 7 and 11 years after SP monotherapy became a first-line treatment (Roper et al., 2003). Our data suggests that a combination therapy in Papua New Guinea did not prevent the development of SP resistance. We believe that the result was mainly because of the already compromised efficacy of this 4-aminoquinoline partner drug. In the study area, in vitro resistance of *P. falciparum* isolates to chloroquine is high (82%), with 93% prevalence of *dhrf* K76T mutation (Mita et al., in press). The in vitro resistance to amodiaquine is similarly high (100%) (data not shown).

The microsatellite variation flanking *dhfr* was restricted in isolates harboring *dhfr* double mutant from Papua New Guinea, producing two distinct microsatellite haplotypes. Reduced microsatellite diversity around *dhfr* indicates strong selection of resistant isolates in the presence of SP (Pearce et al., 2005). The increased prevalence of the *dhfr* double mutant would thus be a result of the selection of this allele rather than frequent mutations in the parasite populations in the presence of pyrimethamine drug. This is consistent with the previous findings in South America (Cortese et al., 2002), Southeast Asia (Nair et al., 2003), and South Africa (Roper et al., 2003).

We also obtained evidence for the emergence of a mutant *dhps* SGEAA allele in 2003, which has not been reported before in Papua New Guinea. In a previous study from Madang and Maprik Provinces in 2000 and 2001, all the 187 isolates harbored wild *dhps* allele (Casey et al., 2004). High prevalences of the mutant *dhps* allele have been reported in other geographic areas like in Thailand (78%) (Ngo et al., 2003) and Vietnam (100%) (Biswas et al., 2000) where a high level of SP resistance has been documented. Also in South Africa, the escalation of SP resistance in endemic regions has been reported to occur concomitantly with the emergence of *dhps* SGEAA allele (Roper et al., 2003). In our study, all six isolates with *dhps* SGEAA mutant allele also harbored *dhfr* ACNRNVI allele, i.e., *dhfr*/*dhps* 'quartet' mutant haplotype. The *dhfr*/*dhps* 'quartet' mutant isolates have been associated with high rates of RII/RIII resistance to SP, e.g. 83% in Indonesian Papua as compared to 0% in *dhfr*/*dhps* 'double' mutant (ACNRNVI + - SAKAA) (Nagesha et al., 2001). Similarly, in Uganda, two mutations at positions 59 and 108 in *dhfr* combined with one at position 540 in *dhps* significantly increased the risk for SP parasitological failure (Talisuna et al., 2004b).

In conclusion, we observed a significant decrease in the prevalence of wild *dhfr* allele in Papua New Guinea with the introduction of SP combined with the 4-aminoquinoline in 2000. We also found the higher resistant genotype in 2003, *dhfr*/

dhps 'quartet' mutant, which has not been reported in Papua New Guinea before the drug policy change. Thus, we argue that the combination therapy introduced to prevent the selection of *dhfr*/*dhps* resistant alleles in 2000 was not as effective as the underlying drug policy had anticipated.

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

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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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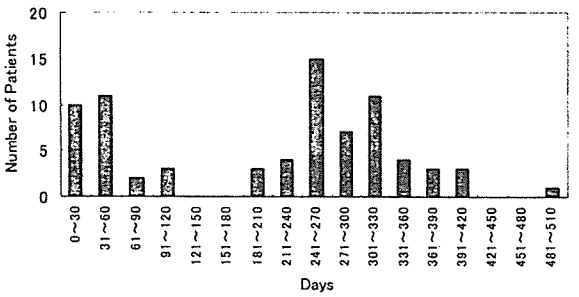

Fig. 1 The distribution of the latent period of the re-emergence of malaria in 77 patients in South Korea. Derived from Oh *et al.* (2001).

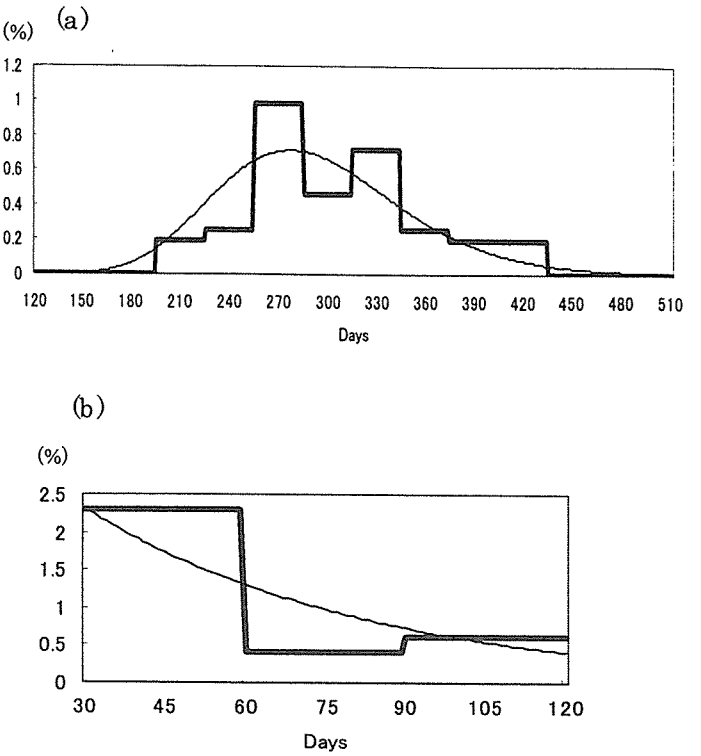

Fig. 2 The distribution of long incubation period (a) and short incubation period (b). The log-normal distribution (thin curve) (a) and the exponential distribution (thin curve) (b) compared with the surveillance data (thick line).

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.

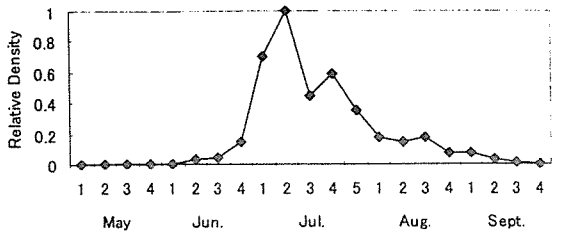

Fig. 3 The relative variation of the weekly density of female mosquitoes to the highest density in August. Derived from Lee *et al.* (2002).

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 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?

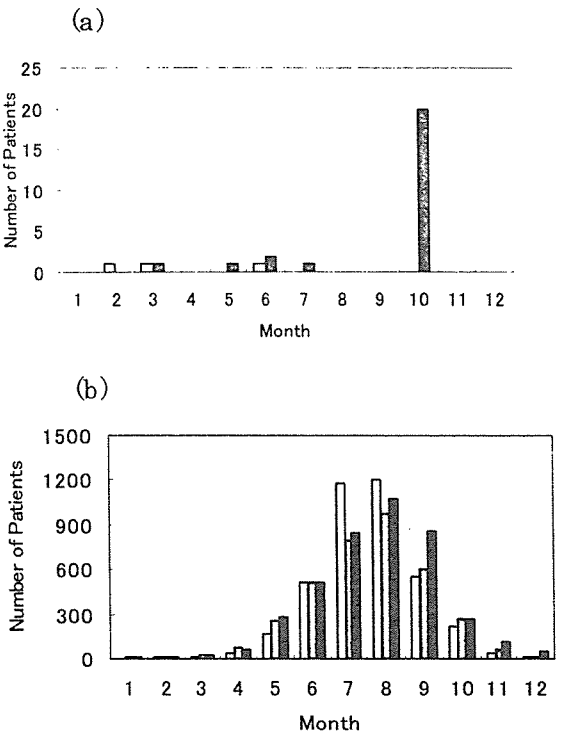

Fig. 4 The monthly incidence of *P. vivax* in South Korea (a) in 1993 (white bars) and 1994 (gray bars) and (b) in 1998 (white bars), 1999 (gray bars) and 2000 (black bars).

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).

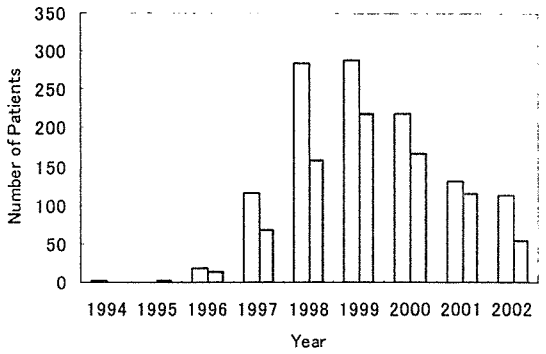

Fig. 5 Cases of malaria in civilians in Paju-shi (white bars) and Yonchon-gun (gray bars) in 1994 – 2002. The vertical axis represents the number of cases per 100,000.

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

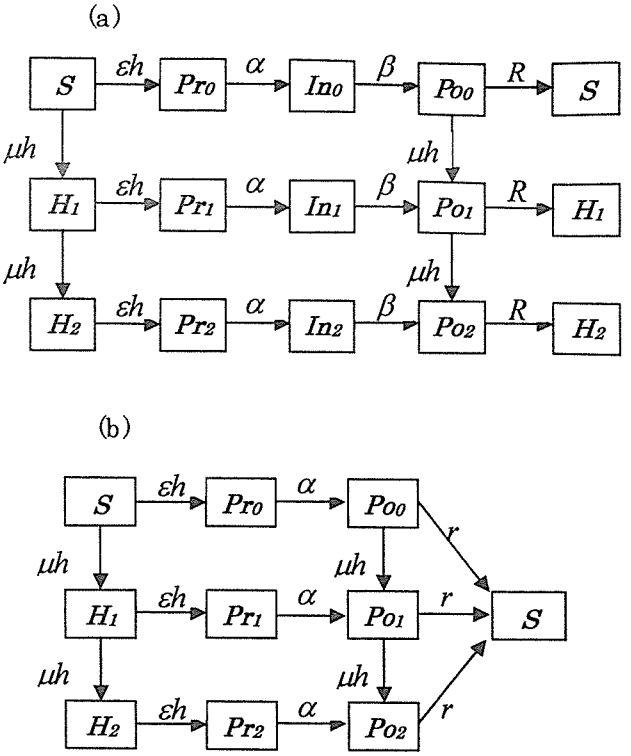

Fig. 6 The scheme of models in North Korea (a) and South Korea (b).

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.

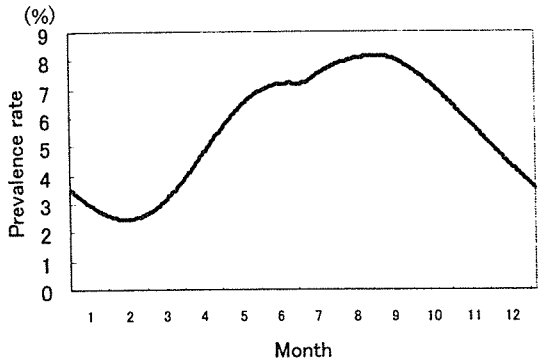

Fig. 7 The time-course of the prevalence in North Korea predicted by simulation by the model (the average rate of prevalence being 5.6%).

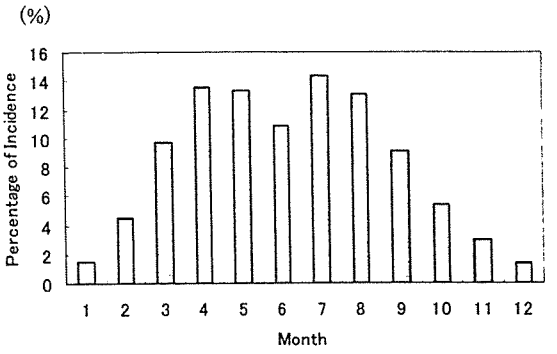

Fig. 8 The monthly incidence predicted by simulation using the model.

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

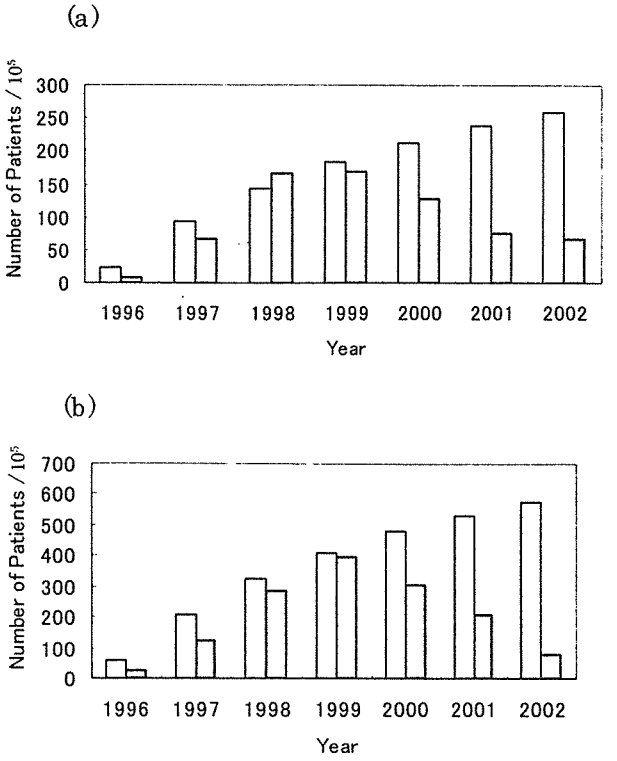

Fig. 9 The comparison of the prevalence between actual surveillance and the results of simulation in Paju-shi (a), Yonchon-gun (b). The time-courses of malaria cases per 100,000 obtained from surveillance and from the results of simulation are shown as gray bars and white bars, respectively.

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.

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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,

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1999). Subsequently, malaria cases rapidly decreased, with the implementation of the National Malaria Eradication Service, which was established jointly by the South Korean government and the World Health Organization (WHO) in 1959 (Ministry of Health and Social Affairs, Republic of Korea, 1966). Consequently, after the 1970s, indigenous malaria cases were almost unheard of, though 2 such cases were reported in 1984 (Soh et al., 1985). During the same period a substantial number of imported malaria cases was reported (Chai, 2002).

However, indigenous vivax malaria reemerged in 1993; a South Korean soldier working at the western edge of the demilitarized zone (DMZ; the border between South and North Korea) in Kyonggi-do (Province), was confirmed to have contracted *P. vivax* malaria (Chai et al., 1994). Thereafter, the number of malaria cases increased exponentially year by year, peaking in 2000 (Feighner et al., 1998; Chai, 1999; Lee et al., 2002; Park et al., 2003). North Korea, which stated that it was free of malaria from the 1970s, also started reporting cases in 1998 from the northern part of the DMZ bordering South Korea (Chol et al., 2005). Since then, the number of malaria cases in North Korea has increased dramatically and reached around 300,000 in 2001. Therefore, in 1999, the North Korean government developed a national malaria control program in cooperation with WHO, to reduce the malaria burden (Chol et al., 2005).

In South Korea, during the period 1993-1996, the outbreak area was confined to the northern part of Kyonggi-do and northwestern Kangwon-do, near the DMZ (Chai, 1999). However, after 1997, the outbreak area extended in an easterly direction to the northeastern region of Kangwon-do and in a southerly direction in Kyonggi-do (Lee et al., 2002; Park et al., 2003), and it was feared that this southward trend would continue. To cope with this risk, the present national malaria control program was launched in 1997 (Korea Center for Disease Control and Prevention, Republic of Korea, 2002). This program includes early case detection and treatment, chemoprophylaxis of soldiers, vector control, personal protection, and financial aids to North Korea for malaria control.

In addition to control activities, meteorological (temperature and rainfall) and entomological factors (mosquito density) may have significant impacts on malaria transmission. For instance, the incidences of malaria were related to local climatic variables in China (Bi et al., 2003) and Rwanda (Loevinsohn, 1994). However, in South Korea, no published data is available concerning the relations between temperature, rainfall, the population density of the vector mosquitoes, and the incidence of malaria.

The aim of the present paper is to briefly summarize vivax malaria outbreaks over the period 1993 to 2005 in South Korea, and to analyze the efficacies of the control activities implemented since 1997, and the impacts of meteorological and entomological factors on disease occurrence.

MATERIALS AND METHODS

Malaria is designated an important communicable disease and case details must be reported immediately to the Ministry of Health and Welfare in South Korea. In the present study, all cases reported since the first reemergence of indigenous vivax malaria case in 1993 were subjected to analysis. Patients' occupations, i.e., civilians, soldiers on duty, and retired soldiers, were obtained from the Communicable Diseases Information System (http://dis.cdc.go.kr) and from the Communicable Diseases Monthly Reports issued during the study period by the Korea Center for Disease Control and Prevention (KCDC), Ministry of Health and Welfare, South Korea. Information about malaria prevalence in North Korea and financial support for malaria control was obtained from the KCDC, World Health Organization (WHO) (http://www.who.int) and the United Nations Office for the Co-ordination of Humanitarian Affairs (OCHA), Pyongyang, Democratic Peoples' Republic (DPR) of Korea (http://www.humanitarianinfo.org/dprk) and from the United Nations (UN) (http://www.reliefweb.int).

The annual geographic distributions of malaria cases in South Korea over 12 years were determined by grouping cases by city and province where patients were located when a diagnosis of malaria was

made. Information about the time required to make a diagnosis of malaria after the onset of symptoms, were obtained from the reports of patients admitted to local health centers and hospitals in Kangwon-do, South Korea.

Meteorological data, i.e., mean temperature and rainfall for the main transmission period (the 6 mo period from May to October), recorded at local weather stations in Cheolwon-gun, Kangwon-do, a malaria endemic area near the DMZ, were obtained from the Korean Meteorological Administration, South Korea.

The population densities of adult anopheline mosquitoes, over 90% of which is *Anopheles sinensis*, the main vector mosquito for vivax malaria in the Republic of Korea (Chai, 1999), were determined during the transmission period at one location in Cheolwon-gun, Kangwon-do, from 1993 to 2004 by two (WS Seok and YS Kim) of the authors. Adult anopheline mosquitoes emerged from the first week of May (1-10 mosquitoes/trap/night) and disappeared from the last week of October (0-14 mosquitoes/trap/night). A black light trap (Nozawa type, Shinyoung Korea Co., Seoul, Korea) was hung from a fence about 1.5 m above the ground in shed housing one cow. Black light traps were operated without additional attractants from 18:00 to 06:00 hr twice a week during the study period. All captured mosquitoes were transported the following morning to the Kangwon Institute of Health and Environment, where they were identified, separated, and the number of anopheline mosquitoes was counted.

Mass chemoprophylaxis (1 chloroquine tablet; 300 mg base) has been administered by the Ministry of Defense to a total of 985,282 soldiers working around outbreak areas (northern parts of Kyonggi-do and Kangwon-do) weekly from 1997 to 2005. Chemoprophylaxis was also prescribed to a total of 12,189 US soldiers in South Korea during the period 1997-2000. Retiring Korean soldiers were advised to take primaquine 15 mg base daily for 14 days for chemoprophylaxis against the liver stage parasite at the time of their retirement.

Spearman's correlation analysis was used to examine correlations between the number of new malaria cases, year, climatic factors, i.e. annual mean temperature (°C) and rainfall (mm), and the annual mean number of mosquitoes trapped during May to October. The monthly mean number of anopheline mosquitoes, and the mean number of mosquitoes trapped weekly and annual totals were calculated from mean monthly numbers trapped during the 6 month transmission period. P values of < 0.05 were regarded as statistically significant.

RESULTS

During the past 13 years (1993-2005), at least 937,634 indigenous vivax malaria cases have been reported in the Korean peninsula (South Korea and North Korea) (Table 1). Based on available data between 1999 and 2004 in South and North Korea, the number of cases reported peaked in 2001 with 298,058 cases in the Korean peninsula. In South Korea, during the period 1993-2005, a total of 21,419 indigenous vivax malaria and 488 imported malaria cases were confirmed (Table 1). The indigenous malaria patients included 8,353 (39.0%) civilians and 13,066 (61.0%) soldiers, including 5,626 retired soldiers (26.3%) who had retired from military service for less than one year at disease onset. The number of reported cases peaked in 2000 with 8.9 cases per 100,000 of the South Korean population. Thereafter, the number of reported cases declined sharply by approximately 26-40% per annum to 1.8-2.9 cases per 100,000 of the population in 2004-2005 (Table 1).

The annual incidence rate (including retired soldiers discharged < 1 year prior to onset and soldiers on duty) peaked at 457.3 cases per 100,000 soldiers in 2000. The incidence decreased by more than 84% between 2000 and 2004, but then increased by 35% in 2005 (Table 1). The same trend, i.e., peak in 2000 followed by a sharp decline until 2004 and a rise in 2005, was observed both among serving and retired soldiers. Among civilians, the annual incidence rate peaked at 3.3-3.4 cases per 100,000 in 1999-2000, and then decreased to 0.9 in 2004, but increased again to 1.9 in 2005 (Table 1).

In 1999, total 95,960 malaria cases were reported in

Table 1. Vivax malaria cases reported annually among civilians and soldiers in South Korea and North Korea

Group	Number of reported cases (Annual cumulative incidence per 100,000 population)													
	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	Total
South Korea														
Civilians	0	2	7	46	361	1,148	1,541	1,580	1,047	864	542	413	802	8,353
	(0.0)	(0.0)	(0.0)	(0.1)	(0.8)	(2.5)	(3.3)	(3.4)	(2.3)	(1.9)	(1.2)	(0.9)	(1.9)	
Soldiers														
Retired[a]	0	1	12	25	207	1,127	996	1,273	756	472	279	159	319	5,626
On duty	1	18	88	287	1,155	1,655	1,085	1,288	685	430	282	236	230	7,440
Subtotal	1	19	100	312	1,362	2,782	2,081	2,561	1,441	902	561	395	549	13,066
	(0.2)	(3.4)	(17.9)	(55.7)	(243.2)	(496.8)	(371.6)	(457.3)	(257.3)	(161.1)	(102.0)	(71.8)	(96.7)	
Total	1	21	107	358	1,723	3,930	3,622	4,141	2,488	1,766	1,103	808	1,351	21,419
	(0.0)	(0.0)	(0.2)	(0.7)	(3.6)	(8.3)	(7.7)	(8.9)	(5.4)	(3.9)	(2.4)	(1.8)	(2.9)	
North Korea[b]	ND[e]	ND	ND	ND	ND	ND	95,960	204,428	295,570	240,339	46,251	33,677	ND	916,225
							(432.3)	(920.8)	(1,331.4)	(1,082.6)	(208.3)	(151.7)		
Total, indigenous cases							99,582	208,569	298,058	242,105	47,354	34,485		937,634
US Army soldiers[c]	0	1	0	14	34	47	53	42	29	41	23	15	ND	299
Imported malaria[d]	ND	6	30	41	40	63	53	41	43	44	61	37	29	488

[a]Retired soldiers, who were infected during military service in risk areas and developed febrile illness at home after discharge from the service.

[b]Data were obtained from webpages of World Health Organization (http://www.who.int), the United Nations Office for the Co-ordination of Humanitarian Affairs, Pyongyang, Democratic Peoples' Republic of Korea (http://www.humanitarianinfo.org/dprk), and from the United Nations (http://www.reliefweb.int).

[c]United States Army cases were diagnosed either in South Korea or after return to the United States.

[d]Imported malaria cases in South Korea, who were infected in Southeast Asia, Africa, Oceania, and in Central and South Americas.

[e]ND = no available data.

North Korea, but this increased explosively 3-folds between 1999 and 2001 (1,331.4 per 100,000 North Korean population), and after 2002 decreased sharply to 208.2 and 151.7 per 100,000 population in 2003 and 2004, respectively (Table 1).

The annual numbers of malaria cases reported by nationwide administrative districts (provinces and large cities) are given in Fig. 1, as sums of 2 years, from 1994-1995 to 2004-2005. Of the total 21,419 cases registered during the 12 year period, most (85.4%) developed febrile illness in northern provinces and cities near the DMZ (the highest risk areas), including 10,411 cases (48.6%) in Kyonggi-do, 3,083 (14.4%) in Kangwon-do, 2,710 (12.7%) in suburban Seoul, and 2,089 (9.8%) in suburban Incheon (Fig. 1). In Kyonggi-do, the most serious outbreak area, the peak incidence occurred in 1998 with 2,197 cases, and decreased gradually afterwards. However, in Kangwon-do, the second most serious outbreak area, the peak incidence of 825 new cases, occurred in 2000. Small numbers of cases were reported from various Provinces and Cities countrywide through 12 years, although these cases were predominantly among retired soldiers who had served in northern parts of Kyonggi-do or Kangwon-do about a year previously, thus indicating a long incubation period. The numbers of patients reported in other Provinces and Cities are shown in Fig. 1.

Meteorological data, i.e., annual mean temperature and rainfall, and mean mosquito population densities, during 1993-2004, were analyzed in terms of their relationships with the annual total numbers of malaria cases reported in Kangwon-do, South Korea (Table 2). Spearman's correlation analysis showed that the occurrence of malaria in high risk areas was correlat-

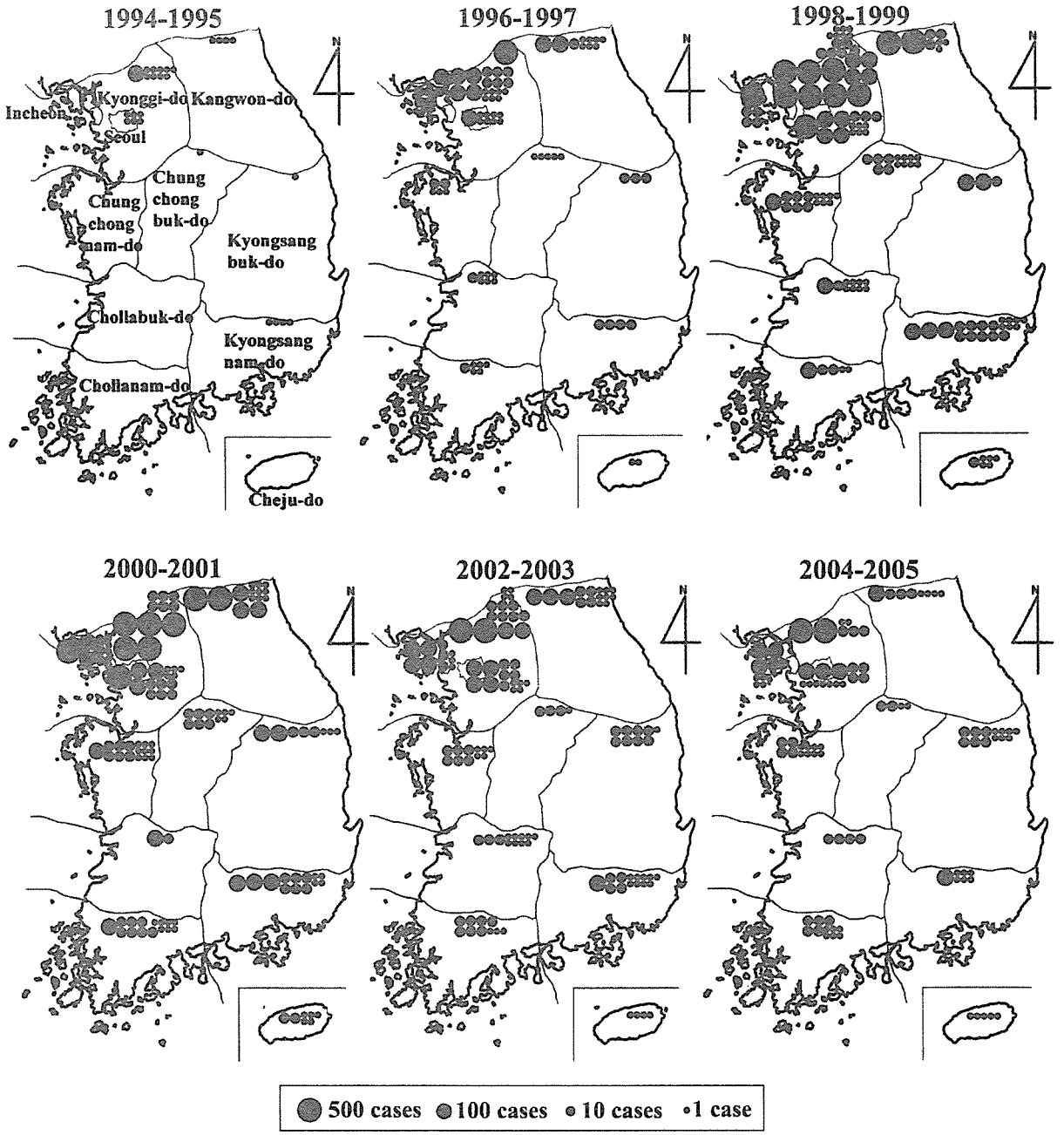

Fig. 1. Maps of South Korea, showing the numbers of indigenous vivax malaria cases reported by administrative districts (Provinces and Cities, including Incheon and Seoul) from 1994-1995 to 2004-2005. The figure represents the number of patients who developed febrile illness and were diagnosed in the district, but does not necessarily mean actual contraction of malaria in each district.

ed with the mosquito population, only with low significance (P = 0.048), and no positive association was observed with temperature or rainfall (Table 2).

The time required for a diagnosis of malaria from the onset of febrile paroxysm has reduced year by year in most outbreak areas of Kangwon-do. For

Table 2. Mean annual temperatures, rainfalls, and anopheline mosquito population densities compared to annual malaria incidence rate in Kangwon-do, South Korea, from 1993 to 2004

Item	Mean annual variables												*P*-value[a]
	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	
Mean temperature (°C)[b] (May - Oct.)	18.0	19.6	18.5	18.8	18.8	19.8	19.6	19.5	19.9	18.3	18.3	18.7	0.096
Mean rainfall (mm) (May - Oct.)	130.0	165.8	229.3	196.3	162.9	225.6	255.4	162.9	195.9	165.9	243.4	162.9	0.593
Mean number of mosquitoes[c] (May - Oct.)	604	799	779	290	550	1,293	1,801	1,574	890	1,194	1,282	1,299	0.048
Annual number of patients (Malaria incidence; I)[d]	0 (0.0)	0 (0.0)	4 (0.3)	40 (2.6)	177 (11.5)	519 (33.4)	514 (33.0)	825 (53.1)	544 (35.0)	216 (14.0)	132 (8.6)	43 (2.8)	-

[a]*P*-value: between the total number of patients and climatic variances (mean temperature and mean rainfall) and mean number of mosquitoes.
[b]Main transmission season in each year.
[c]Mean number of anopheline mosquitoes/cow/trap/night. Over 90% were *Anopheles sinensis*, the main vector mosquito.
[d]Incidence (I) per 100,000 population in Kangwon-do, South Korea. The correlation coefficient between I and mean temperature was 0.605, between I and rainfall 0.514, and between I and mosquito density 0.591.

example, 66 (44.0%) of 150 patients were diagnosed and treated within 6 days of symptom onset in 1999, but this increased to 61.7-73.6% during 2000-2002 (Table 3).

In 2001, the South and North Korean governments started to provide budgetary supports for facilitating malaria control programs in both countries. In the case of South Korea, 2 northern provinces (Kyonggi-do and Kangwon-do) and one city (Incheon), received budgetary supports for mosquito control from the KCDC, and this was followed by fiscal support from provincial and city health bureaus from 2001 to 2004. The total expenditures over this 4 year period in South Korea was 5,154,700 USD (Table 5). In North Korea, during the period 2001-2004, international supports for malaria control have been provided by WHO (for education and assistance for technician training), the International Federation of Red Cross and Red Crescent (IFRC) (anti-malarial drugs) and South Korea. The total amount of anti-malarial aid given to North Korea over this 4 year period was 3,150,650 USD (Table 5). In South and North Korea, during the same period (2001-2004), 8,305,350 USD were spent on malaria control. Items of supports provided by South Korea to North Korea included anti-malarial drugs (chloroquine and primaquine), mosquito control related materials and equipments (insecticide impregnated-bednets, personal protection fabrics, insecticides, and insecticide spraying equipment) and laboratory supplies for prompt diagnosis (microscopes and staining reagents) in local health centers and hospitals, and small amount of cash for the education of health personnel (Table 5).

DISCUSSION

Our study demonstrated that the number of reemerging vivax malaria cases in South Korea increased exponentially during the years 1993-2000, but then decreased steadily until 2004 with a slight increase in 2005. This post 2000 decrease in malaria incidence was observed countrywide, and included high risk areas near the DMZ. Control programs were operated, including mass chemoprophylaxis, vector control, and financial aids to North Korea for malaria control, and are believed, at least in part, to have contributed to the reduction of malaria incidence.

Malaria transmission requires the combined presence of the *Plasmodium* parasite, the anopheline mosquito vector, and the human host. Both parasites and vectors are strongly affected by climate, for example, temperature determines parasite and vector develop-

ment, and rainfall provides the water required for vector breeding. In Rwanda and China, monthly mean temperature was found to play an important role in malaria transmission (Loevinsohn, 1994; Bi et al., 2003). However, with regard to rainfall levels, reports are contradictory; some studies have reported that rainfall is a key factor (Lindblade et al., 1999; Bi et al., 2003), whereas others have reported negative effects (Singh and Sharma, 2002). In a previous South Korean study, increases in temperature and precipitation were found to be correlated with seasonal vector mosquito population densities, and with the subsequent seasonal incidence of malaria (Lee et al., 2002). In this previous study, 2 climatic factors were compared with averaged data collected over a 30-year period, though no statistical analysis was performed (Lee et al., 2002).

In our study, low grade statistical significance (P = 0.048) was observed in the correlations between anopheline mosquito densities and the annual malaria incidence during the 1993-2004 period in Kangwon-do, but none between the climatic variables and malaria incidence. Although mosquito densities during 1998-2001 could not be clearly correlated with malaria incidences, mosquito densities during these years were significantly ($P < 0.05$) higher than those before 1998 when malaria incidence was comparatively low (Table 2). Nevertheless, detailed ecological and epidemiological studies are needed to assess the true impact of climatic variables on malaria outbreaks in South Korea.

Regardless of the control strategy adopted, the early diagnosis and proper treatment of those infected is essential (Lee et al., 2003). In South Korea, the average duration between the onset of malarial fever and diagnosis at a health center or a hospital was 23.6 days in 1995, 9.5 days in 1997, and 8.0 days in 2000 (Park et al., 2003). Since 2000 about two-thirds of malaria patients have been more quickly diagnosed and treated, within 6 days, for example, in Kangwon-do (Table 3). Moreover, in 2004, the average detection time became as short as 3-4 days in highly endemic areas in Kangwon-do (data not shown), and it is speculated that early case detection substantially reduced the malaria transmission from patients to mosquitoes.

Table 3. Days required for confirmation of malaria diagnosis after the onset of symptoms among civilians and veterans in risk areas of Kangwon-do, South Korea, from 1999 to 2002

Year	Number of cases (%)				
	Days until diagnosis after the onset of febrile paroxysm				
	0-6	7-15	16-25	> 26	Total number of cases
1999	66 (44.0)	53 (35.3)	18 (12.0)	13 (8.7)	150
2000	209 (73.6)	66 (23.2)	6 (2.1)	3 (1.1)	284
2001	138 (66.7)	51 (24.6)	12 (5.8)	6 (2.9)	207
2002	58 (61.7)	21 (22.3)	11 (11.7)	4 (4.3)	94

Mass chemoprophylaxis is another major contributor to the observed recent reduction in malaria cases. Before 1997, more than 80% of malaria cases occurred in northern parts of Kyonggi-do and Kangwon-do, the major outbreak areas (Chai, 1999; Park et al., 2003), and most patients were soldiers stationed near the DMZ. Therefore, mass chemoprophylaxis was administered to soldiers located in these endemic areas in 1997 and has continued ever since (Table 4). From 1997 to 2005, a total of 985,282 soldiers received chloroquine and primaquine prophylaxis. As a consequence, malaria incidence among soldiers on duty and retired soldiers decreased rapidly during 2001-2005. This prophylaxis program must have been largely responsible of the observed reduction in the malaria incidence.

However, it should be noted that the proportion of civilian cases among all malaria cases has increased from 38.2% in 2000 to 50.6% in 2004. This increase in the proportion of civilian cases suggests an increase in local transmission away from the DMZ (civilians usually live some distance from the DMZ). This increase in local transmission is also suggested by the fact that outbreak areas have expanded in southerly and easterly directions since 1998 (Chai, 1999; Park et al., 2003; Yeom et al., 2005).

It is also of note that a substantial number of cases