CURRENT STATUS OF MALARIA INFECTION IN A SOUTHEASTERN PROVINCE OF LAO PDR

Jun Kobayashi[1,4], Bouakham Vannachone[2], Yoshiya Sato[4,6], Masaki Sinjo[5], Simone Nambanya[2], Khemphavanh Manivang[3] and Souliya Inthakone[2]

[1]Japan International Cooperation Agency (JICA), PHC Project Office, Vientiane and Thakhek, Lao PDR; [2]Institute of Malariology, Parasitology and Entomology (IMPE), Ministry of Health, Vientiane, Lao PDR; [3]Malaria Station, Khammouane Provincial Health Office, Khammouane, Lao PDR; [4]Department of Parasitology, [5]Department of Preventive Medicine, and [6]Research Center of Comprehensive Medicine, School of Medicine, University of the Ryukyus, Okinawa, Japan

Abstract. In Khammouane, a southeastern province in Lao PDR, active case detection (ACD) surveys for malaria infection on the villagers in three villages were conducted four times from December, 1995 to August, 1996. The malaria infection was demonstrated in 5.3-10.5% of the villagers throughout the year in 2 villages located in mountainous forest, but the infection was rarely observed in the other village located in the plain along the Mekong River, in which only 4 malaria cases were detected in August, 1996. Although the positive rate of malaria parasites among the villagers was not so significantly different in the rainy season compared with the dry season, the intensity of infections (*ie* parasitemia) was significantly higher in the rainy season. More than 90% of the positive villagers were children under 15 years old. Most infections were due to *Plasmodium falciparum*.

INTRODUCTION

Malaria is one of the most serious health problems facing humanity in subtropical and tropical regions, because it infects approximately 300 million people in the world and kills 1.5 to 3 million people annually. Lao PDR is known as high endemic area of malaria (Pholsena, 1992). The exact prevalence of malaria in Laos, however, is not known because the recorded cases were passively detected patients admitted to hospital. The Lao government has organized a nationwide antimalaria network consisting of Malaria Stations in each province and a Malaria Center in each district, however, the condition for malaria examination is sometimes inadequate to obtain exact diagnosis in some facilities.

In Khammouane Province, a southeastern province of Lao PDR, renovation of facilities for malaria examination and training of microscopists in these facilities were implemented under the support of the Japan International Cooperation Agency (JICA), the Primary Health Care (PHC) Project cooperated with the Institute of Malariology, Parasitology and Entomology (IMPE), Vientiane. Quality control of malaria diagnosis performed by recheck system of blood smear, was also established. Under the improved conditions for malaria examination, ACD surveys were carried out in the end of 1995 and in 1996 to obtain exact data on malaria prevalence in this province.

Correspondence: Jun Kobayashi, Department of Parasitology, School of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa, 903-0125, Japan.

MATERIALS AND METHODS

Study areas

Khammouane Province occupies the center of the country at about 350 km southeast from the capital, Vientiane (Fig 1). The province surface is 16,315 km^2, with an estimated population in 1995 of 270,000. These are 9 districts, 76 communes and 882 villages. The capital city is Thakhek.

For the ACD survey in the present study, three villages (Hinboon Neua and Namdik Villages in Hinboon District and Nakham Village in Mahaxay District) were selected (Fig 1). Nakham Village is located in a shallow valley about 30 km east from Thakhek and Namdik Village located in forest at about 30 km north west from Thakhek. There are many streams in the forest and paddy fields around the two villages; the number of streams increases markedly in rainy season. The population is 402 in

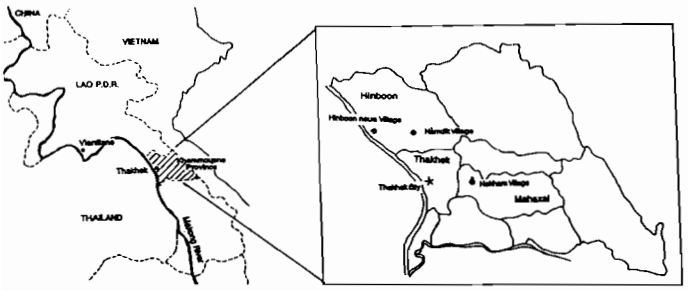

Fig 1–Three villages where malaria survey were carried out in Khammouane Province, Lao PDR.

Nakham Village and 449 in Namdik Village. Among the villagers, the children under 15 years old constitute more than 50% in both villages. The villagers are "Lao theung" (minority) in Nakham Village and "Lao lum" in Namdik Village. The main occupations of the villagers are farming and hunting animals in the forest. Their houses are made of wood and bamboo, being constructed at a height of 1 or 2 meters from the ground to avoid floods in the rainy season.

On the other hand, the geological and social conditions of Hinboon Neua Village are quite different from the above two villages. This village is located in the plain along the Mekong River and surrounded by paddy fields and rivers. The population is 439, with 43% children under 15 years old. Their occupations are farming and fishing in the ponds and rivers. Their traditional houses are the same style as mentioned above, but European style houses are increasing recently. The villagers are "Lao lum".

Active case detection

The mass examination of blood smears collected from the villagers of the above three villages was carried out in the Provincial Malaria Station. The mass surveys were conducted four times in December, 1995, March, June and August, 1996. All of the positive smears and 10% of negative smears were further re-examined by well-trained staff of IMPE to avoid false-positive and false-negative results. The all positive cases were treated by chloroquine (10 mg/kg × 1 day and 5 mg/kg × 2 days).

Examination of blood smear and estimation of parasitemia

Microscopical diagnosis for malaria was carried out by examination of thick and thin blood smears stained by Giemsa. The fixation and staining were carried out within 24 hours after preparation of the blood smear. Species of malaria parasites were identified based on their morphological features. Blood parasitemia was calculated on positive smears collected in March and June. It was based on the number of parasites per μl of blood in a thick film by using 8,000 leukocytes as the standard. Number of parasites was recorded per 500 leukocytes.

Statistics

Statistical analysis for seasonal change of parasite rate was carried out by analysis of variance and multiple comparison (Bonferroni method), using computer statistical software (SPSS). The statistic difference of parasitemia was determined by Student's *t*-test using the same software. The probability (p value) less than 0.05 was considered to be statistically significant.

RESULTS

The results of mass survey of three villages are summarized in Table 1. In two villages, Nakham and Namdik, in mountainous forest, 5.3 to 10.5% of positive rates of malaria parasites were obtained throughout the survey period, while in Hinboon Neua Village in the plain along the Mekong River, no positive case was detected from December, 1995 to June in the next year. Only 4 villagers (2.2%) were found infected in the village on August, 1996. More than 90% of the positive villagers were children under 15 years old, including 2 cases under 1 year old in Nakham Village.

The seasonal difference of parasite rate was not statistically significant, although rainy season started from April in the province in this year (1996) (Fig 2). On the other hand, the intensity of infection as calculated parasitemia was significantly higher in the smears collected in June (rainy season), as compared to those in March (dry season) (Fig 3).

The species of malaria parasite identified was mostly *Plasmodium falciparum* (Table 2). In two cases in Nakham Village, mixed infections with *P. falciparum* and *P. vivax/P. malariae* were observed in December and August, respectively.

Table 1

Parasite rate among age group throughout the year in three villages, Khammouane Province, Lao PDR.

Age group	No. of population	December, 1995 No. of sample	No. of positive	PR %	March, 1996 No. of sample	No. of positive	PR %	June, 1996 No. of sample	No. of positive	PR %	August, 1996 No. of sample	No. of positive	PR %
Nakham village													
0	17	17	0	0	15	1	0	9	0	0	14	1	7.1
1	21	10	0	0	9	1	11.1	10	0	0	17	3	17.6
2-4	48	43	4	9.3	37	0	0.0	20	5	25.0	43	4	9.3
5-9	73	57	6	10.5	44	5	2.3	11	2	18.2	42	4	9.5
10-14	49	37	5	13.5	50	8	16.0	8	1	12.5	35	3	8.6
15-	194	84	0	0	55	1	1.8	37	2	5.4	78	2	2.6
Total	402	248	15	6.0	210	16	7.6	95	10	10.5	229	17	7.4
Namdik village													
0	21	13	0	0	9	0	0	7	0	0	12	0	0
1	45	14	1	7.1	12	0	0	8	1	12.5	20	0	0
2-4	53	28	2	7.1	36	4	11.1	17	1	5.9	45	3	6.7
5-9	75	47	8	17.0	64	4	6.3	31	4	12.9	52	8	15.4
10-14	62	37	4	10.8	45	3	6.7	14	1	7.1	31	2	6.5
15-	193	73	1	1.4	61	1	1.6	42	1	2.4	84	2	2.4
Total	449	212	16	7.5	227	12	5.3	119	8	6.7	244	15	6.1
Hinboon Neua village													
0	13	7	0	0	4	0	0	2	0	0	5	0	0
1	23	14	0	0	7	0	0	7	0	0	5	0	0
2-4	38	26	0	0	27	0	0	19	0	0	25	2	8.0
5-9	63	68	0	0	62	0	0	46	0	0	41	0	0
10-14	46	36	0	0	46	0	0	21	0	0	33	0	0
15-	256	72	0	0	28	0	0	54	0	0	71	2	2.8
Total	439	223	0	0	224	0	0	149	0	0	180	4	2.2

PR: Parasite rate

DISCUSSION

Lao People's Democratic Republic (Lao PDR) is land-locked and bordered by 5 countries. It covers an area of approximately 240,000 km^2. Estimated population in 1995 was 4.6 million. A large part of the country is mountainous and hardly accessible. About 85% of the population live in these rural areas where communications particularly in rainy season are difficult. The climate is tropical and a rainy season is from May to October.

Malaria is major problem of public health as it causes high morbidity and mortality in children and severe losses in socio-economical development in this country. The Lao government put the priority on malaria control among infectious disease in 1986 and started antimalaria activities including DDT residual spraying, mass distribution of chloroquine, improvement of diagnostic facilities, proper treatment of malaria cases and other measures. However, many areas in the country are still malarious. Although almost all data on prevalence of malaria in Lao PDR based on the number of passively detected cases, a total of 307,189 malaria cases including 588 fatal cases were reported in 1994 by IMPE. Khammouane Province surveyed in the present study is known as a high endemic area; a total 1,630 cases including 24 fatal ones were reported in Khammouane Provincial Hospital in a year from October, 1995 (Fig 4) and 851 cases from 5 district hospitals; Hinboon, Mahaxay,

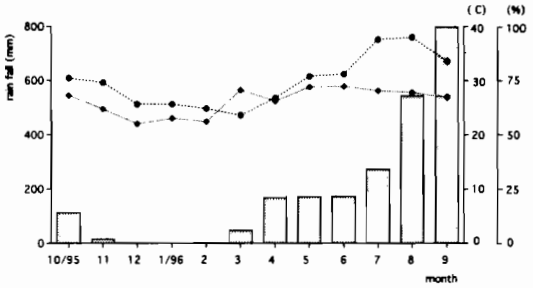

Fig 2–Rain fall, temperature and humidity in Thakhek City, Khammouane Province from October, 1995 to September, 1996.

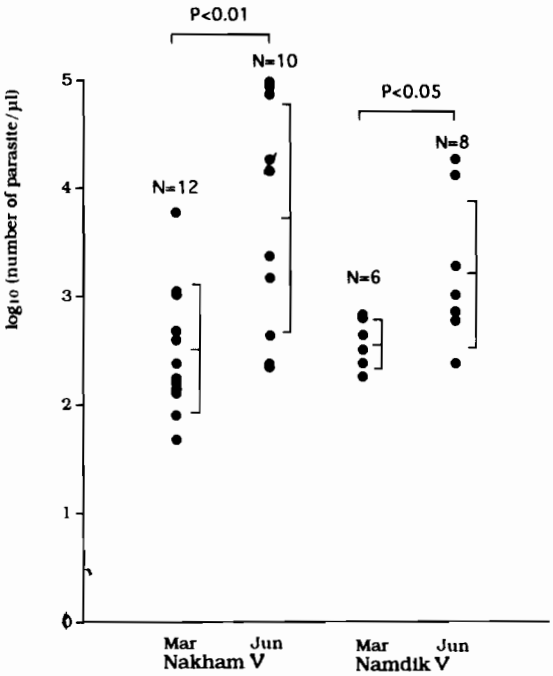

Fig 3–Comparison of number of malaria parasite among positive smear within dry season (March, 1996) and rainy season (June, 1996).

Nhommarath, Sebanphay and Nongbok, and Namdong hospital in Thakhek District were reported in half year from April, 1996 (Fig 5) by Khammouane Malaria Station.

On the other hand, there have been few ACD surveys to estimate pre-patent or latent infection in

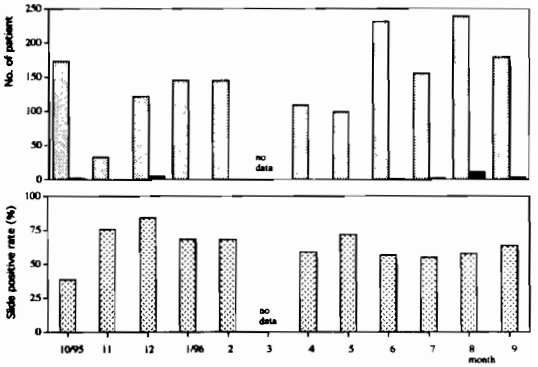

Fig 4–Number of malaria patient and fatal case, and slide positive rate in Khammouane Provincial Hospital from October, 1995 to September, 1996.

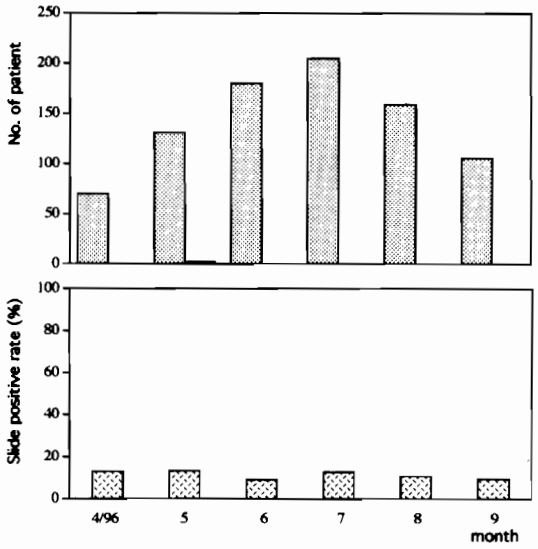

Fig 5–Number of malaria patient and fatal case, and slide positive rate in five district hospitals and one health post from April to September, 1996.

this country. In a past ACD survey in the northern area (Keodom District) of Vientiane Province, a total of 1,015 villagers, accounting for 70% of the total population, were actively examined for malaria infection, and parasite positive rate was reported to be 2.4% of the villagers (Giboda *et al*, 1992).

Table 2

Malaria species and gametocytes among malaria positive samples.

Village	Month of collection	No. of sample	No. of positive	No. of case of malaria species			Gametocytes	
				Pf	*Pf* and *Pv*	*Pf* and *Pm*	No. of positive	Positive rate (%)
Nakham	December	248	15	14	1	0	-	-
	March	210	16	16	0	0	2	12.5
	June	95	10	10	0	0	2	20.0
	August	229	17	16	0	1	3	5.9
Namdik	December	212	16	16	0	0	-	-
	March	227	12	12	0	0	0	0.0
	June	119	8	8	0	0	3	37.5
	August	244	15	15	0	0	5	33.3
Hinboon Neua	December	223	0	0	0	0	-	-
	March	224	0	0	0	0	0	0
	June	0	0	0	0	0	0	0
	August	180	4	4	0	0	2	50.0

Pf: Plasmodium falciparum, Pv: Plasmodium vivax, Pm: Plasmodium malariae
- :no data

In the present study in Khammouane Province, it was also demonstrated that malaria is seriously prevalent throughout the year mainly in the area in mountainous forest. In the areas, infection with malaria was found in 5% to 10% of the subjects examined. On the other hand, malaria infection was rarely observed in the village in an open field along the Mekong River. Although main vector for transmission of malaria is not yet identified in Lao PDR, *Anopheles minimus* is suspected as a principal vector on the basis of results in north Thailand. In the same areas, the authors have also conducted surveys on Anopheline mosquitos and reported that adult *An. minimus* were collected in Nakham and Namdik village, and a considerable number of immature *An. minimus* were also collected in the stream margins in hilly regions around Nakham village (Kobayashi *et al*, 1997). On the other hand, there was no slow running stream suitable for breeding of *An. minimus* around Hinboon Neua village where few malaria cases were detected, and *An. minimus* was not collected in this village. These results indicate that *An. minimus* is a suspected vector in this country, as in Thailand. Additionally, *An. dirus*, which is known as another vector in Thailand, were collected in Hinboon Neua village by human bait collection in August, 1996 when 4 malaria positive cases were temporarily detected in the village. *An. dirus* is known to breed in small, fresh temporary pools during rainy season in Thailand. Thus, *An. dirus* was also considered to be possible vector in the area. However, *An. minimus* and *An. dirus* could not be collected in the village where malaria parasites were detected from 25% of villagers in our latest survey. It is necessary to carry out further surveys to determine the principal vector in Khammouane, Lao PDR.

On the basis of results in the past PCD surveys, it has been indicated that malaria transmission is perennial with its peak in rainy season in this area between July and August. The present ACD surveys, however, did not show a significant seasonal difference in malaria endemicity in the population of the study areas. On the other hand, the number

of cases passively detected in hospital significantly increased in the rainy season. The number of cases showed increasing tendency in rainy season from April to September. Especially on June and August, more than 200 cases were reported monthly in provincial hospital. Seventeen (70%) out of 24 fatal cases were also observed in rainy season from June to September. This was interpreted as an increase of clinical cases with high parasitemia in the rainy season, rather than increase of parasite positive rate. Actually, the authors confirmed that the intensity of infection increased significantly in the rainy season in the present surveys. *P. falciparum* has been regarded as the dominant species in Lao PDR. There was also a predominace of *P. falciparum* with the proportion reaching 90% in the present study. In this study, two cases were found to be mixed infection with *P. falciparum and P. vivax* or *P. malariae*. Recently it was reported that low intensity of malarial infection was able to be detected by PCR methods (Snounou *et al*, 1993). The authors have carried out further analysis of malaria prevalence in Khammouane by this method for detection of actual prevalence of mixed infections.

ACKNOWLEDGEMENTS

This study was performed under Lao/Japan medical cooperation project on primary health care supported by Japan International Cooperation Agency (JICA). The authors would like to thank all staff members in IMPE and JICA PHC Office, Vientiane, for their helpful support and advice in this study. We would also like to thank staff members in Provincial Health Office in Khammouane Province for their sample collection, examination and implementation of the surveys.

REFERENCES

Giboda M, Pholsena K, Hongvanthong B, *et al.* Malariometric survey in Keoudom District, Laos: Sensitivity of *Plasmodium falciparum* to anti-malarials and automedication with chloroquine. *Southeast Asian J Trop Med Public Health* 1992; 23 : 383-8.

Kobayashi J, Nambanya S, Miyagi I, *et al.* Mosquitoes collection in the three villages in Khammounae, Lao PDR. *Southeast Asian J Trop Med Public Health* 1997; 28 : 615-20.

Pholsena K. The malaria situation and antimalaria program in Laos. *Southeast Asian J Trop Med Public Health* 1992; 23 (suppl 4) : 39-42.

Snounou G, Viriyakosol S, Zhu XP, *et al.* High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. *Mol Biochem Parasitol* 1993; 61 : 315-20.