

Original article

The status of malaria before and after distribution of ITNs from 1999 to 2006 in two districts of Khammouanne Province, Lao P.D.R

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Abstract: In 1999, an insecticide-treated net (ITN) distribution project was started in several malaria-endemic villages in Lao P.D.R., namely Vientiane, Bolikhamxay and Khammouanne Provinces. After the completion of the project, it was found that the ITNs were effective for malaria control based on the analysis of the slide positivity rate for malaria parasites between 1999 and 2000 [6]. We conducted malariometric, entomological and KAP surveys in 2005-06 to confirm the status of malaria and to determine the effectiveness of ITNs in three different socioepidemiological areas (*near city*, *rural* and *remote*), represented by the Xebangfay and Boualapha districts, Khammouanne Province.

A marked decrease in the annual malaria incidence and slide positivity rate was noted from 1999 to 2006 in the two districts. The malaria prevalence was significantly reduced in *near city* areas (5.6%-10.7% in 1999 to 0% in 2005-06) and *rural* areas (21.4%-50.9% in 1999 to 0%-1% in 2005-06). Twelve positive cases were recorded in *remote* areas (3.4 - 7.7% in 2006).

The illiteracy rate was significantly higher among the respondents in the *remote* (83.9%) than among those in the *near city* (32.7%) and *rural* (54%) areas. In all areas, more than 50% of the villagers indicated that they were not aware of malaria. Similarly, about 60% of the respondents were found to have incorrect knowledge concerning malaria transmission. In *remote*, 40.9% of the respondents were aware of the methods to prevent malaria infection and 49.5% used mosquito nets throughout the year, rates significantly lower than the corresponding rates in *near city* and *rural*. The density of persons per net in *remote* (3.1-5.9) was considerably higher than that in *near city* (1.8-2.1) and *rural* (1.2-2.7). Malaria vector mosquitoes, such as *An. minimus* and *An. nivipes* were collected by human-baited adult collection, and cow-baited and CDC light traps. The results of the analysis suggested that the lower the number of persons per net the more effective the reduction of malaria morbidity in these areas. It is imperative that ITN distribution and health education regarding malaria be strengthened, especially in *remote*, but also in other areas.

1. INTRODUCTION

Malaria is a serious public health problem in Lao P.D.R, with approximately 300,000 confirmed and suspected cases reported every year. Among these, nearly 10% are hospitalized and 300 to 500 deaths are officially registered [8]. In 1996, the Ministry of Health of Lao P.D.R. launched the National Malaria Control Programme (NMCP), composed of four main parts; 1) early diagnosis and treatment,

2) improvement of severe-case management, 3) vector control through the distribution of insecticide-treated nets (ITNs; K-Othrine[®]) and long-lasting-insecticide-treated nets (LLITNs; Olyset[™] net), and 4) health education campaigns in all communities. The main objective of the programme was to reduce the morbidity and mortality of malaria [2].

K-Othrine[®] nets are prepared by impregnation of 2.5% deltamethrin per m² surface area of the net. The mosquito killing effect of this net persists for 12 months. However,

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the ITNs should not be washed until the next impregnation. The insecticide used in Olyset™ nets (2% permethrin) is incorporated into the netting polyethylene fibers during the manufacture of the net. The malaria-preventive effect of Olyset™ nets is designed to last for up to 5 years without any further insecticide treatment.

In 1999, the ITN distribution programme, which is the main strategy of the aforementioned NMCP, was started in several malaria -endemic villages of Vientiane, Bolikhamxay and Khammouanne Provinces, with the assistance of the Japanese Government. After the completion of the project, it was found that the ITNs were effective for malaria control, based on the analysis of the slide positivity rate for malaria parasites between 1999 and 2000 [6]. However, there was no opportunity to confirm the status of malaria or to follow-up the effectiveness of the distribution of ITNs. Therefore, we conducted malariometric, entomological and KAP surveys in 2005-06 in three different socioepidemiological areas, represented by the Xebangfay and Boualapha districts, Khammouanne Province.

2. MATERIALS AND METHODS

Study area

Khammouanne Province is located about 350 km southeast of Vientiane, the capital of Lao P.D.R (Fig 1). The province, with an area of 16,315 km², is composed of 9 districts and 882 villages, with an estimated population in 2005 of 336,935. The provincial capital is Thakek. The rainy season in the province extends from May to October, and the dry season from November to April. The temperature varies from 15 °C-35 °C throughout the year. For the

malariometric survey, we chose ten villages from three different socioepidemiological areas; *near city* (Thamlay and Bounghouana tai in Xebangfay district, surrounded by paddy fields; situated 25 km from Thakek), *rural* (Napong, Nalouang, Kouanboun and Thaphachon in Boualapha district, surrounded by paddy fields; situated 130 km northeast of Thakek) and *remote* (Houayhead, Thangbeang, Namchalad and Wat in Boualapha district, surrounded by mountains and forests; situated 10-25 km from the center of Boualapha district). Two villages in *near city* were located close to the provincial and district hospital, respectively. Four villages in *rural* were located close to the district hospital. No health facilities were available in *remote*. Access to *remote* was impossible in the rainy season, thus, the surveys in *remote* were conducted only during the dry season. The people surveyed in all of these areas lived in stilted and thatched houses, constructed at a height of 1 to 2 meters from the ground. This study was conducted with the approval of the Center of Malariology, Parasitology and Entomology (CMPE), and the Research Committee of the Ministry of Public Health (MPH), Lao P.D.R.

Passive case detection

The epidemiological surveillance was mainly based on blood slide examinations and rapid diagnostic tests of symptomatic individuals at the district and provincial health facilities. These data were reported monthly to the CMPE [1]. We obtained the following parameters from the CMPE for re-analyzing the passive case detection (PCD) data in the Xebangfay and Boualapha districts from 1999 to 2006: population, examination, slide positivity rate, and the annual parasite incidence.

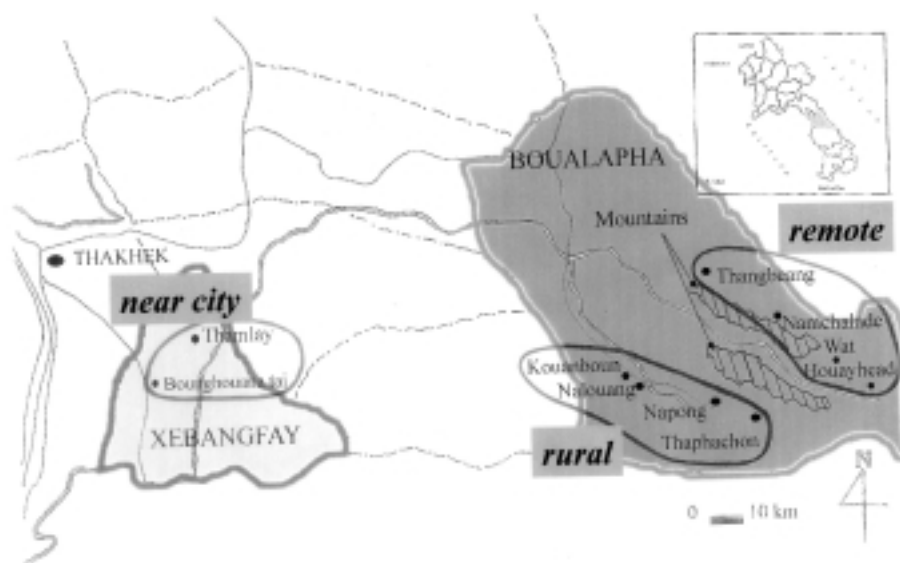


Fig 1 Study areas of two districts of Khammouanne Province, Lao P.D.R

Active case detection

We conducted active case detection (ACD) for malaria in both the rainy (July, 2005) and dry seasons (March, 2006) in a total of ten villages of *near city*, *rural* and *remote*. A total of 1941 inhabitants were examined for *Plasmodium falciparum* malaria infection by the dipstick method (Para check[®]-F test, Orchid Biomedical Systems, Australia). The results of the dipstick were re-confirmed by Lao and Japanese specialists. Microscopic confirmation was also carried out to identify the *plasmodium* species by expert microscopists from the CMPE. All positive cases were treated with Coartem (Artemether 20 mg/ Lumefantrine 120 mg) according to the treatment protocol laid down in the Malaria Control Programme propounded by the MPH, Lao P.D.R.

Knowledge and behavior regarding malaria

There were 550 households with a total of 3,113 inhabitants in the ten villages.

We visited 340 households and interviewed the key person in each household, who was also the target of the ACD after 50% randomization of the households in each village in 2006. The interview included questions on knowledge (name, cause and prevention) and health behavior (using mosquito net) related to malaria. The number, type and condition of the mosquito nets in each house were also confirmed.

Mosquito collections

An entomological survey was performed for two consecutive nights in each village of *near city* (Thamlay) and *rural* (Napoung, Kouanboun and Thaphachon) in November 2006. Human-baited adult collection was conducted by three authors (BK, HE and AT) at one point. All the mosquitoes landing on them from 18:00 to 0:00 hours were collected in a glass tube. For the cow-baited collection, two large double nets (outer net measuring 4 x 4 x 2 m) were installed and a cow was enclosed in the net from 18:00 to 0:00 hours. The mosquitoes trapped inside the outer net from 21:00 to 0:00 hours were collected. A standard miniature CDC light trap (Model 512; John W. Hock Company, Gainesville, Florida, USA) was hung 1.5-2 m above the floor in the bedroom of eight houses. The mosquitoes trapped between 18:00 and 06:00 hours were collected around 06:00. All collected mosquitoes were killed without dissection by exposure to chloroform and the female *anopheles mosquitoes* were identified morphologically.

Statistical analysis

The statistical analyses were conducted using the software SPSS version 11 and the Chi-square and two-tailed

fisher's exact proba-billing test. P-values less than 0.05 were considered to be statistically significant.

3. RESULTS

PCD

Data on the annual malaria incidence from 1999 to 2006 in the Xebangfay and Boualapha districts are shown in Table 1. In both districts, a marked annual decrease in the slide positivity rate was noted. However, this trend was more apparent in the Xebangfay (18.3% in 1999, to 2.2% in 2006) than in the Boualapha (28.7% in 1999, to 16.4 in 2006) district. Interestingly, an abrupt fall in the positivity rate from 28.2% in 2000 to 7.8% in 2001 and 8.5% in 2002 was found in Boualapha district. The number of blood samples examined microscopically was lower in the Boualapha district than in the Xebangfay district, mainly because of the lower number of medical care facilities in this district and the difficulty of access [1].

ACD

Table 2 shows the malaria prevalence after the ITN distribution in 2005 (rainy season) and 2006 (dry season) and also the results of the first malaria surveillance conducted before the ITN distribution of 1999 [5]. In 1999, the slide positivity rate in the villages of *near city* was lower (5.6 - 10.7%) than that in those of *rural* (21.4 - 50.9%). No seasonal difference in the malaria prevalence was seen in

Table 1. Annual malaria incidence from 1999 to 2006 in Xebangfay and Boualapha districts, Khammouanne Province, Lao P.D.R

| Year | Total population ^A | Examination ^B | Slide positive rate | Annual parasite incidence ^C |
|------------------|-------------------------------|--------------------------|---------------------|--|
| Xebangfay | | | | |
| 1999 | 21857 | 3953 | 18.3 | 33.1 |
| 2000 | 21875 | 3877 | 14.0 | 24.9 |
| 2001 | 22425 | 1592 | 9.7 | 6.9 |
| 2002 | 23346 | 2227 | 10.1 | 9.7 |
| 2003 | 23657 | 5165 | 2.2 | 4.9 |
| 2004 | 24684 | 2080 | 3.9 | 3.3 |
| 2005 | 25548 | 2187 | 4.8 | 4.1 |
| 2006 | 25561 | 2156 | 2.2 | 1.9 |
| Boualapha | | | | |
| 1999 | 22121 | 113 | 28.7 | 14.7 |
| 2000 | 22714 | 400 | 28.2 | 4.9 |
| 2001 | 23019 | 203 | 7.8 | 0.6 |
| 2002 | 23895 | 550 | 8.5 | 1.9 |
| 2003 | 24318 | 416 | 15.8 | 2.7 |
| 2004 | 25001 | 537 | 11.1 | 2.4 |
| 2005 | 25009 | 329 | 14.5 | 1.9 |
| 2006 | 25721 | 463 | 16.4 | 2.9 |

^A Data from population census data

^B Number of blood samples microscopically examined

^C Positive/1000 population

Table 2. Malaria prevalence before and after ITNs distribution in areas of *near city*, *rural* and *remote*, Khammouanne Province, Lao P.D.R

| | before ITNs distribution | | | | after ITNs distribution | | | |
|--------------------|---------------------------------|-------|------------------------------|-------|--------------------------------|----|-------------------------------|------|
| | rainy season (1999) (n=1304) | | dry season (1999) (n=956) | | rainy season (2005) (n=877) | | dry season (2006) (n=1064) | |
| Xebangfay District | | | | | | | | |
| <i>near city</i> | | | | | | | | |
| Bounghouana tai | 18/320 | 5.6% | ND | ND | 0/200 | 0% | 0/145 | 0% |
| Thamlay | 35/338 | 10.3% | 34/317 | 10.7% | 0/251 | 0% | 0/149 | 0% |
| Boualapha District | | | | | | | | |
| <i>rural</i> | | | | | | | | |
| Napong | 62/289 | 21.4% | 65/258 | 25.2% | 0/245 | 0% | 0/183 | 0% |
| Nalouang | 35/83 | 42.0% | 46/91 | 50.5% | 0/80 | 0% | 1/102 | 1% |
| Kouanboun | 55/171 | 32.0% | 83/163 | 50.9% | 0/101 | 0% | 0/103 | 0% |
| Taphachon | 30/103 | 29.0% | 26/127 | 21.4% | ND | ND | 0/126 | 0% |
| <i>remote</i> | | | | | | | | |
| Houayhead | ^A ND | ND | ND | ND | ND | ND | 2/58 | 3.4% |
| Thangbeang | ND | ND | ND | ND | ND | ND | 4/71 | 5.6% |
| Namchalad | ND | ND | ND | ND | ND | ND | 0/49 | 0% |
| Wat | ND | ND | ND | ND | ND | ND | 6/78 | 7.7% |

^A ND: Not determined

either area. In 2005 and 2006, we could not find any malaria cases among the 1,685 subjects surveyed in *near city* and *rural*, except for one case of falciparum infection detected in Nalouang village. In contrast, twelve malaria-positive cases (10 caused by *P. falciparum*, 1 caused by *P. vivax* and 1 caused by *P. malariae*) were documented in three villages (including 5 cases over ten years of age and 7 cases under 10 years of age) in *remote* (3.4 - 7.7%).

Knowledge and behavior regarding malaria

As shown in Table 3, valid responses to the questionnaires were obtained from 340 subjects (148 males and 192 females). The age distribution of respondents was similar among the three areas (*near city*, *rural* and *remote*). Nearly all the respondents were farmers by occupation (98%). Most of the respondents from *remote* (78/93; 83.9%) were illiterate, the rate of illiteracy being significantly higher than that in *near city* (36/110; 32.7%; $P < 0.0001$) and *rural* (74/137; 54%; $P < 0.0001$). In contrast, there was a significantly higher percentage of respondents with more than 7 years of education in *near city* (10/110; 9.1%) than in either *rural* (2/137; 1.5%) ($P = 0.026$) or *remote* (0/93; 0%) ($P = 0.009$).

The level of knowledge and behavior regarding malaria are shown in Table 4. In all areas, more than 50% of the respondents indicated that they had no awareness about malaria. In addition, about 60% of the respondents had incorrect knowledge regarding malaria transmission. In *remote*, 40.9% of the respondents were aware of the methods to prevent malaria infection, which was significantly lower

Table 3. Distribution of sociodemographic characteristics of respondents of *near city*, *rural* and *remote* (n=340)

| | <i>near city</i> (n=110) | <i>rural</i> (n=137) | <i>remote</i> (n=93) |
|-------------|-----------------------------|-------------------------|-------------------------|
| Gender | | | |
| Male | 57 | 53 | 38 |
| Females | 53 | 84 | 55 |
| Age | | | |
| 10-19 years | 1 | 6 | 0 |
| 20-29 years | 21 | 37 | 27 |
| 30-39 years | 32 | 37 | 28 |
| 40-49 years | 24 | 29 | 21 |
| ≥ 50 years | 32 | 28 | 17 |
| Occupation | | | |
| Farmer | 106 | 134 | 93 |
| Others | 4 | 3 | 0 |
| Education | | | |
| Illiterate | 36 | 74 | 78 |
| 1-3 years | 20 | 28 | 9 |
| 4-6 years | 44 | 33 | 6 |
| ≥ 7 years | 10 | 2 | 0 |

than the corresponding percentage in *near city* (68.2%; $P < 0.0001$) and *rural* (69.3%; $P < 0.0001$). Regardless of the awareness about malaria, more than 90% of the respondents used mosquito nets all year round in *near city* and *rural*. In contrast, 49.5% used mosquito nets throughout the year in *remote*, which was significantly lower than the corresponding figure in *near city* and *rural* ($P < 0.0001$). Out of 93 respondents in *remote*, 17 indicated that they did not have ITNs. The number, type, condition and density of persons

Table 4. Knowledge and behavior regarding malaria in areas of *near city*, *rural* and *remote* in 2006

| | <i>near city</i> (n=110) | | <i>rural</i> (n=137) | | <i>remote</i> (n=93) | |
|--|-----------------------------|-------|-------------------------|-------|-------------------------|-------|
| Knowledge | | | | | | |
| Do you know what malaria is ? | | | | | | |
| Yes | 50 | 45.5% | 60 | 43.8% | 46 | 49.5% |
| No | 60 | 54.5% | 77 | 56.2% | 47 | 50.5% |
| Do you know how malaria is infected ? | | | | | | |
| Mosquito bite | 42 | 38.2% | 49 | 35.8% | 34 | 36.6% |
| Drinking un-boiled water | 7 | 6.4% | 1 | 0.7% | 0 | 0.0% |
| Others | 3 | 2.7% | 1 | 0.7% | 2 | 2.2% |
| Unknown | 58 | 52.7% | 86 | 62.8% | 57 | 61.3% |
| Do you know how to prevent malaria infection ? | | | | | | |
| Yes | 75 | 68.2% | 95 | 69.3% | 38 | 40.9% |
| No | 35 | 31.8% | 42 | 30.7% | 55 | 59.1% |
| Behavior | | | | | | |
| Using mosquito net all the year | 103 | 93.6% | 133 | 97% | 46 | 49.5% |

Table 5. Number, type, condition and density of person per net in areas of *near city*, *rural* and *remote* in 2006

| | No. of nets | Type (%) | | | Condition (%) ^c | | | Density (persons/net) |
|---------------------------|-------------|-----------------|------------------|--------------------|----------------------------|--------|------|--------------------------|
| | | Non-insecticide | ITN ^a | LLITN ^b | Excellent | Normal | Poor | |
| Xebangfay District | | | | | | | | |
| <i>near city</i> | | | | | | | | |
| Bounghouana tai | 158 | 7.6 | 89.2 | 3.2 | 44.3 | 27.8 | 27.8 | 2.1 |
| Thamlay | 137 | 9.5 | 90.5 | 0 | 37.2 | 30.7 | 32.1 | 1.8 |
| Boualapha District | | | | | | | | |
| <i>rural</i> | | | | | | | | |
| Naping | 193 | 5.7 | 42.5 | 51.8 | 8.8 | 85 | 6.2 | 1.7 |
| Nalouang | 63 | 7.9 | 81 | 11.1 | 3.2 | 93.7 | 3.2 | 2.7 |
| Kouanboun | 94 | 12.8 | 69.1 | 18.1 | 10.6 | 78.7 | 10.6 | 1.2 |
| Taphachon | 97 | 15.5 | 58.8 | 25.8 | 17.5 | 70.1 | 12.4 | 1.8 |
| <i>remote</i> | | | | | | | | |
| Houayhead | 33 | 30.3 | 69.7 | 0 | 54.5 | 27.3 | 18.2 | 5.9 |
| Thangbeang | 31 | 25.8 | 74.2 | 0 | 29 | 45.2 | 25.8 | 4.8 |
| Namchalad | 33 | 51.5 | 48.5 | 0 | 57.6 | 27.3 | 15.2 | 3.1 |
| Wat | 41 | 4.9 | 92.7 | 2.4 | 41.5 | 41.5 | 17.1 | 3.2 |

^a ITN: insecticide- treated net.

^b LLITN: long-lasting-insecticide-treated net.

^c Excellent: without holes; Normal: with holes covering less than 20% of the net area; Poor: with holes covering more than 20% of the net area

per net are summarized in Table 5. In all areas, the predominantly used net type was ITNs. In *remote*, the use of non-insecticide-treated mosquito nets was also common. More than 70% of the nets were found to be in excellent (without holes) or normal (with holes covering less than 20% of the net area) condition in all areas. However, nearly 30% of mosquito nets in *near city* were in poor condition (with holes covering more than 20% of the net area).

The density of persons per net was 3.1-5.9 in *remote*, which was considerably higher than that in *near city* (1.8-2.1) and *rural* (1.2-2.7).

Entomological survey

A total of 15 species of *Anopheles* were caught by the human-baited adult collection, cow-baited and CDC light trap collection in *near city* and *rural* (Table 6). In *near city*, *An. nivipes* (35.8%) and *An. philippinensis* (28.4%) were predominant. In contrast, *An. minimus* (37.1%) and *An. philippinensis* (27.1%) were predominant in *rural*. *An. minimus* accounted for more than 70% of the human-baited adult collection. *An. philippinensis* accounted for more than 50%, followed in frequency by *An. nivipes* and *An. minimus* in the cow-baited collection. *An. minimus* accounted for more than 85% of the CDC light trap collection.

Table 6. Anopheline mosquitoes collected in areas of *near city* and *rural* in November 2006

| Species | <i>near city</i> | | <i>rural</i> | | Total | |
|-------------------------|------------------|-------|--------------|-------|-------|-------|
| (Cellia) | | | | | | |
| <i>cullicifacies</i> | | | 2 | 0.8% | 2 | 0.6% |
| <i>aconitus</i> | 7 | 6.4% | 1 | 0.4% | 8 | 2.2% |
| <i>maculatus</i> | | | 6 | 2.4% | 6 | 1.7% |
| <i>minimus</i> | 6 | 5.6% | 93 | 37.1% | 99 | 27.5% |
| <i>nivipes</i> | 39 | 35.8% | 26 | 10.3% | 65 | 18.1% |
| <i>philippinensis</i> | 31 | 28.4% | 68 | 27.1% | 99 | 27.5% |
| <i>sawadwongporni</i> | | | 3 | 1.2% | 3 | 0.8% |
| <i>cell. sp.</i> | 1 | 0.9% | 2 | 0.8% | 3 | 0.8% |
| <i>vagus</i> | 2 | 1.8% | 1 | 0.4% | 3 | 0.8% |
| <i>dirus</i> | | | 1 | 0.4% | 1 | 0.3% |
| <i>kochi</i> | 4 | 3.7% | 3 | 1.2% | 7 | 1.9% |
| (Anopheles) | | | | | | |
| <i>hyrcanus gr.</i> | 17 | 15.6% | 44 | 17.5% | 61 | 16.9% |
| <i>umbrosus gr.</i> | 1 | 0.9% | | | 1 | 0.3% |
| <i>barbirostirs gr.</i> | 1 | 0.9% | | | 1 | 0.3% |
| <i>ano. sp.</i> | | | 1 | 0.4% | 1 | 0.3% |
| Total | 109 | 100% | 251 | 100% | 360 | 100% |

We did not conduct mosquito collection in *remote* because of the severe condition of roads.

4. DISCUSSION

In 1999, the distribution of 40,000 insecticide-treated nets (ITNs) was started in Vientiane, Bolikhamxay and Khammouanne Provinces. By the end of 2000, the average number of persons per net was 3.3 in the Xebangfay and 3.9 in Boualapha districts [6]. The results of the present study revealed an improvement in these numbers in 2006: the figure was 1.8-2.1 in the Xebangfay (*near city*) and 1.2-2.7 in the Boualapha district (*rural*). In contrast, the number of persons per net ranged from 3.1 to 5.9 in *remote* reflecting the small number of nets distributed.

It appears that a low density of persons per net is associated with a reduced number of malaria cases. This trend was particularly prominent in Phan Tien village, Vietnam, where the density was less than 2.0 [3]. In Lao P.D.R, a dramatic decrease in the annual malaria incidence and slide positivity rate was observed from 1999 to 2006. This trend was also confirmed by ACD in *near city* and *rural*, where the density of persons per net was around 2.0. In contrast, twelve malaria-positive cases were documented in *remote*, where the density of persons per net was 3.1 to 5.9. Our results were consistent with this report.

Several community-randomized trials have shown a significant reduction of the pediatric morbidity and mortality from malaria following ITN distribution in sub-Saharan Africa [4,9]. It has been confirmed by assessment of the parous and sporozoite density of mosquitoes that ITNs reduce the survival of anthropophilic malaria vectors [13]. In our study, a low density of persons per net of ITNs was

associated with an effectively reduced malaria morbidity in *near city* and *rural*. Thus, it is necessary to distribute more ITNs especially in *remote*, but also in other areas.

After the distribution of the ITNs, malaria health education activities for the villagers, including group discussions, video programmes and posters were also started in *near city* and *rural*. These activities were undertaken once in each village [5]. However, the present study results reveal that more than 60% of the respondents of *near city* and *rural* still had incorrect knowledge about malaria transmission, quite similar to that reported in a previous study in the same areas [17]. In Indonesia and Vietnam, however, the favorable results of health education were reported [11,14]. Hung reported that a continuous health education programme focusing on malaria as well as other aspects of health and hygiene is important, especially for ethnic minority communities in Vietnam [3]. Our results suggest that only one session of health education failed to improve the knowledge of villagers about malaria. Interestingly, more than 90% of the villagers in *near city* and *rural* were using mosquito nets throughout the year even without a knowledge of malaria, probably to avoid nuisance from insects. In *remote*, knowledge about the measures for prevention of malaria and behavior with respect to the use of mosquito nets was significantly lower than that in the other areas, probably because of the high rate of illiteracy. Thus, health education regarding malaria should be strengthened, especially in *remote*, but also in other areas.

An. dirus and *An. minimus* are suspected to be the vectors transmitting malaria in Thailand, Vietnam and Cambo-

dia [12,16]. *An. minimus* has been reported to be the important vector in the dry season, while *An. dirus* appears to play the predominant role in the rainy season in Khammouanne Province of Lao P.D.R [15]. *An. nivipes* is also suspected to be a malaria vector in the same areas [17]. In our entomological survey, *An. nivipes* accounted for most of the collected mosquitoes in *near city* and *An. minimus* for most of those collected in *rural*. Only one *An. dirus* was collected by human-baited adult collection in *rural*. We cannot provide an estimate of the prevalence of *An. dirus* in 2006, because our survey was conducted only in the dry season. Our results indicate that malaria vectors were still present in these areas. Thus, the continuous use of mosquito nets should be recommended. *An. dirus* was also confirmed as an important vector in the southern parts of Lao P.D.R [18,19]. In 2006, distribution of ITNs was started in these provinces by the Global Fund Program [10], and it is expected that the expansion of the ITN distribution programme in the southern provinces may lead to success in malaria control in future years.

In conclusion, our findings indicate that a low density of persons per net is associated with reduced malaria morbidity in *near city* and *rural* areas. However, it is imperative that ITN distribution and health education on malaria be strengthened, especially in *remote*, but also in other areas.

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