MALARIOMETRIC SURVEY IN KEOUDOM DISTRICT, LAOS: SENSITIVITY OF *PLASMODIUM FALCIPARUM* TO ANTI-MALARIALS AND AUTOMEDICATION WITH CHLOROQUINE

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Abstract. A malariometric survey was conducted in the Keoudom District, in the northern part of Vientiane Province, Laos, where an artificial dam-lake on the Nam River is located. The parasite rate of the whole cohort representing 1,105 subjects was 2.44% with the predominance of *Plasmodium vivax* (70%), while *P. falciparum* represented 30% with the average parasite density index 3. The low spleen rate (2.3%) characterized the study area as a hypoendemic zone. IFAT antibodies were examined in 419 subjects. The seropositivity of 195 persons < 15 years was 13.7% while in > 15 year old subjects seropositivity was 61.6% with a low GMRT in both groups (140 : 148). Automedication with aminoquinoline was assayed by urinary analysis in 125 outpatients. Of these, 36 (28.8%) were positive, 89 (71.2%) negative. The frequency of positive blood films for *P. falciparum* was higher in subjects with aminoquinoline in the urine (36.1%) than in those without (10.1%). Chloroquine sensitivity assay of 15 strains of *P. falciparum* displayed resistance in 39.3%.

INTRODUCTION

Laos is situated in the monsoon zone, its climate is mostly warm and humid. The coolest month is January, with an average temperature in the south of about 25°C, in the north 15°C. The dry winter season occurs between November and April, the wet season between May and October.

Malarial transmission is perennial with its peak between July and August, the warmest months of the year.

Plasmodium falciparum has been regarded as the dominant species in Laos, with the proportion reaching 75-95% (Pholsena, 1988). However, these data do not appear to be uniform throughout the country over time. Acheson (1970) in screening subjects participating in the construction of a dam on the Nam Ngum River in the Keoudom District, and members of their families, reported that the dominant species of the area was *P. vivax* (64%).

Most of the data on prevalence of malaria in Laos have originated in diagnostic laboratories (passive case detection, PCD). Statistics of the Institute of Malaria and Parasitic Diseases (IMPD) in Vientiane indicated a slide-positivity rate (SPR) of 13.5% in Luang Prabang, 18% in Vientiane, 21.8% in Savanakhet, 5.6% in Champassak Provinces (Pholsena, 1988).

Our investigation conducted in the Keuodom District in the area of the Nam Ngum dam-lake followed these aims: (1) determination of endemicity of malaria in the area - blood examination for parasitemia, sera for antimalarial antibody; (2) sensitivity testing *in vivo* of *P. falciparum* to chloroquine and quinine; (3) to establish the extent of automedication with chloroquine which is freely available on the market.

MATERIALS AND METHODS

Description of the study area

The Keoudom District occupies the northern part of Vientiane Province, at about 80 km from the capital, Vientiane. A major part of the District is occupied by lowlands with dispersed forest stands. The District holds the largest body of water in Laos - an artificial dam-lake on the Nam

Ngum River, covering about 420 km^2, its water table being about 200 m above sea level. The dam was constructed between 1968 and 1971. At the time of its construction, numerous Japanese, Thai and Lao workers acquired malaria (Ebisawa *et al,* 1970). The dam-lake is surrounded by mountains, of which the highest is Phadang, altitude 1,621 m and its banks are lined by small villages.

Baseline data for a malariometric survey were obtained from: (a) parasitological examination of blood films of all subjects present at the time in the villages (active case detection, ACD), (b) classification of spleen size according to Hackett's method (WHO, 1963) and (c) determination of antibody against *P. falciparum* antigen by a single cross-sectional survey. Our study was performed in August 1989 at the peak of the wet season, in four selected villages:

1. Khet Song - 975 inhabitants, 112 houses, 43% of inhabitants were examined.
2. Donmai -241 inhabitants, 50 houses, 83% of inhabitants were examined.
3. Nanin - situated in a forest-covered plane directly adjoining the Nam Ngum River, 430 inhabitants, 81 houses, 43% inhabitants were examined.
4. Camps of the power plant workers (PPW) and their families living under different hygienic conditions, 39% of the camp population were examined.

Parasitological examination

A malariological team examined thick and thin blood films of residents of each locality present at that time but a preference was give to the younger age groups.

Spleen examination

Splenic enlargement was assessed by palpation with the subject in an upright position, in every individual whose blood had been tested.

***In vivo* test for response of *P. falciparum* to chloroquine and quinine.**

The test was made with outpatients of IMPD between June and July 1989. Chloroquine (25 mg chloroquine base/kg body wt) was administered in a three day course.

Quinine at a daily dose of 1.5 g was administered for 7 days. The results of the 10 days follow-up of the parasitemia was taken for the final evaluation of the *in vivo* test (Myint Lwin *et al,* 1985; Giboda and Denis, 1988).

Automedication with chloroquine

Prior to microscopic examination of the blood, all outpatients of the IMPD were screened for 4-aminoquinoline in the urine using the Dill-Glasko test. If the blood film was positive for *P. falciparum* or/and *P. falciparum* gametocytes, the record was made complete by adding the name of the locality in which infection had been contracted.

RESULTS

In 1988 the population of the Keoudom District numbered 15,970, of which 1,105 subjects (7%) were examined for the presence of plasmodia in the blood.

Summarized results are presented in Table 1. The parasite rate of the whole cohort was 2.44%. A low prevalence rate was recorded in age group 0-2, regarded as the best indicator of a recent transmission of malaria. There was a predominance of *P. vivax* (70%), while *P. falciparum* represented 30%. The average parasite density index of *P. falciparum* was 3 representing 201-400 asexual parasites in 1 µl of blood. A comparison of data on *P. falciparum* parasitemia in patients examined at the IMPD, Vientiane is presented in Table 2. Parasitemia up to 10,000/µl of blood occurred in 71.6% of the 67 positive subjects. High parasitemia ($>100,000$/µl) was clustered in the age group <10 years.

Using the indirect immunofluorescence test (IFAT), 419 sera were examined for IgG antibody against the antigen of *P. falciparum.* Of these, 195 sera were from patients below the age of 15 years and 224 were from patients above this age (Table 3). In the group <15 years, 86% of sera were negative, while in the group >15 years, no antibody was detected in as few as 30% of the subjects examined. Serum negativity was highest in both age groups from the PPW camps. In the remaining three localities, most sera were obtained from subjects older than 15 years. The highest serum positivity was in the locality of Donmai, but the

Table 1

Malariometric survey in Keoudom District, Vientiane Province, Laos. Total 1,105 examined subjects = 7.03% of the district population.

Age groups	Blood film examined	Blood film positive	Parasite rate	Plasmodium species			Class of spleen enlargement after Hackett					
				Pf	Fg	Pv+	0	1	2	3	4	5
0-11 months	31	0	0	0	0	0	-					
12-23 months	44	1	2.27	0	0	1	42	1	1			
2-4 years	167	3	1.79	0	0	3	163	4				
5-9 years	264	7	2.65	3	1	3	256	5	2	1		
10-14 years	187	4	2.13	1	1	2	186				1	
>15 years	412	12	2.91	2	0	10	-					
Total	1,105	27	2.44	6++	2	19	647	10	3	1	1	
				0.54%	0.18%	1.71%			Spleen rate = 2.3%			
									Average enlarged spleen = 1.5			

\+ Pf = *P. falciparum* asexual forms, Fg = gametocytes of *P. falciparum*, Pv = *P. vivax*
++ Average parasite density index = 3. Ratio of *P. falciparum* (70 : 30)

Table 2

Parasite count per mm^3 of blood by age group in 67 cases diagnosed at the Institute of Malaria and Parasitic Diseases, Vientiane, May - June 1989.

Age group (years)	No. of infections	⩽1,000	1,001-9,999	10,000-19,999	20,000-30,000	30,001-50,000	50,001-100,000	>100,000
1-5	2							832,000 232,000
6-10	5		1					713,000 136,000 1,778,000 500,000
11-20	13	5	6	2				
21-30	31	4	12	10	1	4		
31-40	13	1	8	4				
41-50	2		1					280,615
>50	1				1			
Total	67	10	28	16	2	4		7
		+14.9%	56.7%	80.6%	83.6%	89.5%		100%

\+ cumulative%

Table 3

IFAT antibodies against *P. falciparum* antigen in the sera of 419 inhabitants of 4 villages in Keoudom District, Vientiane Province, August 1989.

Locality	Age	Serology negative	Reciprocal titers number of sera with final dilution (%). 32	64	128	256	512	1,024	2,048	4,096	8,192	Total positive	GMRT+
PPW Camps	<15 years n=189	181 (91.9)	6 (6.7)		2 (2.2)							8 (9.0)	44
	>15 years n=117	59 (50.4)	23 (19.6)	2 (1.7)	22 (18.8)	5 (4.3)	5 (4.3)	1 (0.8)	0	0	0	58 (49.6)	88
Keth Song	<15 years n=0												0
	>15 years n=45	19 (42.4)	7 (15.5)	2 (4.4)	9 (20.0)	4 (8.9)	1 (2.2)	1 (2.2)	1 (2.2)	1 (2.2)		26 (57.8)	133
Donmai	<15 years n=2	0		1		1						2 (100)	125
	>15 years n=27	3 (11.1)	4 (14.8)	2 (7.4)	10 (37.0)	2 (7.4)	1 (3.7)	4 (14.8)	0	1 (3.7)		24 (88.9)	173
Nanin	<15 years n=4	1 (25.0)							1 (25.0)	2 (50.0)		3 (75.0)	3,235
	>15 years n=35	5 (14.3)	1 (2.8)	0	9 (25.7)	4 (11.4)	7 (20.0)	4 (11.4)	4 (11.4)	0	1 (2.8)	30 (85.7)	400
Total	<15 years n=195	182 (86.3)	6 (6.3)	1 (1.0)	2 (2.0)	1 (1.0)	0	0	1 (1.0)	2 (2.0)	0	13 (13.7)	140
	>15 years n=224	86 (38.9)	35 (15.6)	6 (2.7)	50 (22.3)	15 (6.7)	14 (6.2)	10 (4.5)	5 (2.2)	2 (0.9)	1 (0.45)	138 (61.1)	148

+ from positive cases only

Table 4

Parasitemia and IFAT antibodies in four localities tested, August 1989.

Locality	Parasitemia: Blood films examined		Parasite rate		Plasmodium species		IFAT antibodies: No. tested		Positive for antibodies		GMRT	
	<15 yr	>15 yr	<15 yr	>15 yr	Pf	Pv	<15 yr	>15 yr	<15 yr	>15 yr	<15 yr	>15 yr
PPW Camps	144	175	2 (1.4)	6 (3.4)	2	6	189	117	8 (9.0)	58 (49.6)	44	88
Keth Song	273	152	6 (2.2)	2 (1.3)	2	6	0	45	0	26 (57.8)	0	133
Donmai	106	94	2 (1.9)	2 (2.1)	1	3	2	27	2 (100)	24 (88.9)	125	173
Nanin	106	28	5 (3.1)	2 (7.1)	3	4	4	35	3 (75.0)	30 (85.7)	3,235	400

Table 5

Presence of aminoquinolines in urine samples of 125 examined at the Institute of Malaria and Parasitic Diseases, Vientiane, June - July 1989.

	Dill - Glazko positive n = 36/28.8%	Dill - Glazko negative n = 89/71.2%
Positive for Pf	10/27.7%	9/10.1%
Positive for Fg only	3	0
Average parasitedensity index	6.4	6.3

Table 6

Data on sensitivity of *P. falciparum* to chloroquine reported by different authors.

Tested strains	RI	RII	RIII	Authors
n = 48	20.8%	14.6%	4.6%	Al Tawil (1977)
n = 202	13.8%	26.3%	2.8%	Pholsena (1988)
n = 15	13.3%	13.3%	13.3%	Giboda *et al* (1988)

antibody level (GMRT) was highest in the locality of Nanin, corresponding to the highest prevalence of malaria (Table 4). GMRT for outpatients of the IMPD was 368, GMRT of hospitalized malaria patients was 1,893.

Results of automedication with 4-aminoquinoline obtained from outpatients of the IMPD are shown in Table 5. Using the Dill-Glasko test, we examined 125 patients. Of these, 36 (28.8%) were positive, 89 (71.2%) negative. The frequency of positive blood films for *P. falciparum* was higher in subjects with 4-aminoquinoline in the urine than in those without it (36.1% : 10.1%). The parasite density index was identical in both groups (6.4 : 6.3). In addition, urine examinations of 40 other subjects from the Keoudom District were all negative.

Sensitivity of *P. falciparum* to chloroquine was tested in 33 subjects, but the tests could be completed in only 15 of these. Six showed resistance, 2 each at RI, RII and RIII levels.

DISCUSSION

The prevalence of malaria was surprisingly low in our study groups. In July 1969, infection with malaria was found in 21.5% of subjects working on the dam-lake and in 24.7% of subjects inhabiting the village of Thinkeo in the neighbourhood of the dam-lake; the rate of infection was reduced by 1.6% and 7.2% respectively after one year of antimalarial control measures. *P. vivax* was predominant in both localities, 60% *P. vivax;* 40% *P. falciparum* (Acheson, 1970). However, in countries of Southeast Asia in which *P. falciparum* is regarded as the predominant species (Kondrashin, 1986) the ratio may be reversed in favor of *P. vivax.* During an ACD survey in Cambodia, provinces Takeo and Kompong Som, infection with *P. vivax* was predominant (80%) in 360 subjects from areas with a low prevalence of malaria (1-2%) (unpublished data). A similar distribution was reported from the Lower Mekong Basin in Vietnam among 95,979 persons screened. (Anonymous, 1971).

A longitudinal serological survey of populations in endemic areas is needed to obtain information on how much a given pupulation is exposed to malaria infection. Our single cross-section survey gives only a window at one point in time. The results achieved showed that antibody levels in the inhabitants were directly proportional to the malaria endemicity in area. The low seropositivity

in the age group < 15 years (Table 3) as well as low parasitemia in age group 0-2 years (Table 1) suggested that both the intensity of transmission and the exposure must have been low. Serological surveillance is valid in this area where the parasite rate dropped below a value of 1-2% and *P. vivax* prevails (Wernsdorfer, 1979). To assess the trend of the malaria situation in Keoudom District further serological study is needed, particularly in the lower age groups.

Automedication with antimalarials is common in endemic areas where the population is familiar with malaria symptoms (Facer *et al,* 1990). Automedication may have a negative impact on the spread of resistant strains of *P. falciparum.* In respect to this statement it is interesting that *P. falciparum* gametocytes were present only in the blood of Dill-Glasko positive persons.

Ebisawa *et al* (1970) were the first to point out a reduced sensitivity chloroquine in strains of *P. falciparum* from Laos. The first reliable report was made by Al Tawil (1977) who determined *in vivo* chloroquine sensitivity of 48 strains of *P. falciparum* from the surroundings of Vientiane using a 7 days follow-up observation period. Data on chloroquine sensitivity of 202 strains of *P. falciparum* were collected in IMPD between 1980 and 1986 (Pholsena, 1988). A comparison of earlier tests with recent ones indicated the relative number of resistant strains did not increase over more than 13 years except for a slight shift to category R III (Table 6). Because Vientiane Province was the site of origin of most of the tested strains in the three studies, the results gave a picture of dynamics/stability of resistance. The apparently constant level of resistance as well as degree of resistance in the zone of Vientiane Province is somewhat surprising but RIII degree of resistance is relatively rarely seen while RI and RII responses seem to remain more prevalent even in the oldest know foci (Wernsdorfer, 1981). The *in vitro* testing of sensitivity is necessary to determine the proportion of resistant/sensitive clones within *P. falciparum* strains circulated in the areas.

So far quinine-resistant strains have not been reported from Laos, but we observed a reduced sensitivity to this drug evidencing itself in a prolonged disappearance of asexual parasites from the blood.

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