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MYCOBACTERIUM TUBERCULOSIS AND *gyrA* VARIATION IN ZAMBIA

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Abstract: *M. tuberculosis* strains were isolated from clinically and bacteriologically confirmed patients to evaluate the susceptibility of clinical *M. tuberculosis* isolates to fluoroquinolone and to obtain molecular epidemiological information in Zambia,. The pathogens were subjected to susceptibility testing with isoniazid, rifampicin, ethambutol and streptomycin. The minimum inhibitory concentrations to ciprofloxacin, sparfloxacin and levofloxacin were also evaluated. The *gyrA,* fluoroquinolone resistance-determining region (QRDR), was sequenced and analysed. As a result, three of the 16 strains examined were resistant to isoniazid, rifampicin and/or streptomycin. All of the strains were susceptible to ciprofloxacin, levofloxacin and sparfloxacin. However, a unique *gyrA* gene variation of *M. tuberculosis* was identified in the isolates. One strain had a mutation (T73A) in QRDR. Additionally, 81.25% (13/16) of the strains tested had Thr at codon 88. Several variations of *gyrA* gene have been reported in relation to drug resistance. The *gyrA* variation data will be useful as epidemiological information. It may be important to monitor fluoroquinolone susceptibility even in developing countries for use against resistant *M. tuberculosis* infection, even though no fluoroquinolone resistance was observed in this study.

INTRODUCTION

Multi-drug resistant *Mycobacterium tuberculosis* (MDRTB) is one of the major obstacles to effective control of tuberculosis [1,2] in both developed and developing countries. The emergence of MDRTB is believed to be due, in part, to incomplete anti-tuberculosis treatment. In Sub-Saharan African countries, the incidence of tuberculosis has rapidly increased mainly due to the human immunodeficiency virus epidemic [3]. Thus, the incidence of resistant *M. tuberculosis* infections can be expected to increase in these countries.

It is thought that some drugs, i.e., rifamycin derivatives and fluoroquinolones (FQs), are potentially effective treatments for MDRTB. Even in Zambia, it is relatively easy to obtain ciprofloxacin (CIP) and other FQs in general/private clinics. Therefore, as part of a drug resistance investigation in Zambia, we evaluated the drug susceptibility of *M. tuberculosis* to FQs.

MATERIALS AND METHODS

Clinical isolates and drug susceptibility tests

A total of 16 *M. tuberculosis* strains were isolated from 16 patients with pulmonary tuberculosis at the University Teaching Hospital Chest Clinic in Lusaka, Zambia. All strains were recovered on Lowenstein-Jensen (L-J) medium from sputum specimens. The drug susceptibility of each strain to isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and streptomycin (STR) was determined by the resistant ratio method using L-J media. The critical concentrations for each drug were as follows: INH 0.05, 0.1 and 0.2 μg/ml; RIF 12.5, 25 and 50 μg/ml; EMB 0.8, 1.6 and 3.2 μg /ml; STR 10, 20 and 40 μg/ml.

Minimum inhibitory concentrations of fluoroquinolones

FQs were kind gifts from Bayer (ciprofloxacin: CIP),

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Dainippon Pharmaceutical (sparfloxacin: SPX) and Daiichi Pharmaceutical (levofloxacin: LVX). These drugs were tested for minimum inhibitory concentration of each strain in Middlebrook 7H9 broth with albumin-dextrose-catalase (ADC). The isolates were sub-cultured in Middlebrook 7H9 with ADC broth and prepared in the same medium to the turbidity of McFarl and 1. The suspension was further diluted 10^2 to 10^3 times resulting in a concentration of approximately 10^5 CFU/ml. One ml of each suspension was inoculated into 1 ml of Middlebrook 7H9 broth with either no antibiotic or with serial two-fold dilutions of each antibiotic, with the final concentrations ranging from 0.1 to 25 μg /ml. The tubes were incubated at 37℃ for 2 to 4 weeks until sufficient bacterial growth was evident in the control tubes.

PCR and sequencing of *gyrA* region

The tubercle bacilli were harvested from the colonies on L-J medium. DNA extraction was performed using 100 μg/ml of proteinase K and 2% SDS, followed by ordinary solvent assay with phenol, phenol/chloroform and chloroform. After re-suspension of each DNA sample, the reaction mixture was added to 1 μg of DNA template to a final concentration of 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.0 mM $MgCl_2$, 200 μM of each of the dNTPs, 25 pmole of each of primers and 2.5 units of *Taq* DNA polymerase in a total volume of 50 μl and subjected to 30 cycles of PCR. The primers for *gyrA* region were as follows: gyrAF1; 5' G GTGCTCTATGCAATGTTCG 3' and gyrAR1; 5' GGATAT TGGTTGCCATGCC 3'. DNA specimens were incubated at 94℃ for one minute to melt the DNA, cooled to 62℃ for one minute to allow for annealing, and incubated for one minute at 72℃ for DNA amplification. The PCR product was recovered from gels and subjected to direct sequencing. The amino acid sequences of GyrA deduced from nucleotide sequence were numbered in the system used for E.coli. The complete nucleotide sequence has been deposited in GenBank under accession number AF400983.

RESULTS

Susceptibility to anti-tuberculosis drugs

One strain (no. 99-1049) was resistant to INH, RIF, and STR by the resistant ratio method. Two strains (no. 99-979 and 99-1201) were solely resistant to INH. No tested strain showed resistance to CIP, SPX or LVX (Table 1). SPX showed the lowest MIC, followed by LVX and CIP.

Sequence analysis of *gyrA* region

A 387 bps of PCR product from *gyrA* involving the quinolone resistance-determining region (QRDR) was amplified and sequenced. The sequences were analysed as shown in Figure 1. Strain 99-1183, which showed significant susceptibility to FQs, was found to have a mutation resulting in a Thr to Ala substitution at codon 73. Furthermore, a Ser > Thr substitution at codon 88 was found in 13 out of the 16 strains examined, all of which were susceptible to FQs.

Table 1. Drug susceptibilities of clinical *M. tuberculosis* isolates

Strain no.	Resistant ratio method				MIC (μg/ml)		
	INH	RIF	EMB	STR	CIP	SPX	LVX
H37Rv	1 (S)	1 (S)	1 (S)	1 (S)	0.39	0.1	0.2
99-794	2 (S)	1 (S)	1 (S)	2 (S)	0.2	<0.1	<0.1
99-800	1 (S)	1 (S)	1 (S)	1 (S)	<0.1	<0.1	0.2
99-804	1 (S)	1 (S)	1 (S)	1 (S)	ND	<0.1	<0.1
99-978	2 (S)	1 (S)	1 (S)	1 (S)	0.2	0.1	0.2
99-979	8 (R)	1 (S)	1 (S)	1 (S)	0.2	<0.1	<0.1
99-980	2 (S)	2 (S)	1 (S)	1 (S)	0.39	<0.1	0.2
99-983	1 (S)	1 (S)	1 (S)	1 (S)	ND	ND	ND
99-995	2 (S)	2 (S)	1 (S)	2 (S)	0.2	ND	0.2
99-999	2 (S)	1 (S)	1 (S)	1 (S)	0.2	<0.1	0.2
99-1049	4 (R)	4 (R)	1 (S)	8 (R)	0.39	0.2	0.2
99-1053	2 (S)	2 (S)	1 (S)	1 (S)	ND	<0.1	<0.1
99-1058	2 (S)	2 (S)	1 (S)	2 (S)	ND	ND	ND
99-1128	2 (S)	1 (S)	1 (S)	1 (S)	0.2	<0.1	0.2
99-1136	2 (S)	1 (S)	1 (S)	1 (S)	ND	<0.1	0.2
99-1183	1 (S)	1 (S)	1 (S)	1 (S)	0.2	<0.1	0.2
99-1201	4 (R)	1 (S)	1 (S)	1 (S)	0.39	<0.1	0.2

S: Sensitive R: Resistant ND: No data

```
H37Rv  5'- GCCCGGTCGG TTGCCGAGAC CATGGGCAAC TACCACCCGC ACGGCGACGC GTCGATCTAC
99-983     ---------- --------A- ---------- ---------- ---------- ----------
99-995     ---------- --------A- ---------- ---------- ---------- ----------
99-1049    ---------- --------A- ---------- ---------- ---------- ----------
99-1053    ---------- --------A- ---------- ---------- ---------- ----------
99-1058    ---------- --------A- ---------- ---------- ---------- ----------
99-1183    ---------- --------G- ---------- ---------- ---------- ----------
99-1201    ---------- --------A- ---------- ---------- ---------- ----------
Amino acid AlaArgSerV alAlaGluAl aMetGlyAsn TyrHisProH isGlyAspAl aSerIleTyr
                          *Th r

H37Rv      GACAGCCTGG TGCGCATGGC CCAGCCCTGG TCGCTGCGCT ACCCGCTGGT GGACGGCCAG -3'
99-983     ----G----- ---------- ---------- ---------- ---------- ----------
99-995     ----G----- ---------- ---------- ---------- ---------- ----------
99-1049    ----G----- ---------- ---------- ---------- ---------- ----------
99-1053    ----C----- ---------- ---------- ---------- ---------- ----------
99-1058    ----C----- ---------- ---------- ---------- ---------- ----------
99-1183    ----C----- ---------- ---------- ---------- ---------- ----------
99-1201    ----C----- ---------- ---------- ---------- ---------- ----------
Amino acid AspThrLeuV alArgMetAl aGlnProTrp SerLeuArgT yrProLeuVa lAspGlyGln
```

Figure 1. Nucleotide and amino acid sequences of QRDR in *gyrA* gene by direct sequencing. Sequences of other strains that are not shown here were completely consistent with 99-1201 in the sequenced region.

DISCUSSION

Many mutations in residues 81 to 87 (88 to 94 in other systems) have been identified in quinolone resistant *M. tuberculosis* isolates [4-11], suggesting that this region of the genome is associated with susceptibility to FQs. The mutation at codon 73 in strain 99-1183, which may be involved in the susceptibility to FQs, has not been registered in GenBank as far as the authors could determine.

The patients had no a history of frequent and/or continuous use of any FQs for the treatment of either tuberculosis or more common bacterial infections. The strain was susceptible to all four first line anti-tuberculosis drugs. It is known that certain natural variations exist in *M. tuberculosis* with some probability [12]. Strain 99-1183 seemed to be one such strain, but further investigation is required to confirm the role of this mutation. The new mutation identified in this report will provide additional information for analysis of the effects of quinolone on *M. tuberculosis*, and it may also serve as an epidemiological marker. The amino acid at codon 88 was mainly Thr in these strains. It is thought that *M. tuberculosis* is a relatively new species and evolutionarily has a few silent variations. Codon 88 is one of the genetic markers and Thr is assumed to be common in ancestral strains. Here, the higher ratio of S88T strains will be informative for future epidemiological research [13,14].

There is intense interest in the FQs as anti-tuberculosis drugs because of the emergence of MDRTB. The use of FQs for the treatment of MDRTB infection has been shown to be effective in some reports [15,16]. The FQs must be used carefully because resistance may develop soon after clinical application as an anti-tuberculosis drug. The *gyrA* variation found in this study in pathogenic strains may provide useful epidemiological information for future studies implemented to investigate the use of fluoroquinolone to treat resistant *M. tuberculosis* infection.

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A NEW SPECIES OF *SIMULIUM (SIMULIUM)* FROM NORTHERN THAILAND (DIPTERA: SIMULIIDAE)

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Accepted 19, May, 2005

Abstract: *Simulium* (*Simulium*) *phukaense* sp. nov. is described on the basis of the observation of females and males (both sexes of adults reared from pupae) and pupae collected in Nan Province, northern Thailand. This new species is assigned to the *griseifrons* species-group of the subgenus *Simulium* (*Simulium*) and is easily distinguished from other known species of this species-group by the simple shoe-shaped cocoon, as well as the arrangement of the six gill filaments.
Key words: *Simulium*, black fly, Simuliidae, Thailand, new species

During recent surveys on black flies in northern Thailand, we collected a new species which is assigned to the *griseifrons* species-group of the subgenus *Simulium* (*Simulium*) Latreille s. str., redefined by Takaoka and Davies [1]. This new species is very similar to *S*. (*S*.) *maenoi* Takaoka and Choochote, described from Thailand [2] but differs from the latter species and all the other species of the same species-group by its shoe-shaped cocoon.

The terms for morphological features used here follow those of Takaoka [3]. Holotype and paratype specimens of the new species are deposited at the Department of Infectious Disease Control, Faculty of Medicine, Oita University, Oita, Japan.

Simulium (*Simulium*) *phukaense* sp. nov.

DESCRIPTION. **Female.** Body length 2.8−3.0 mm. ***Head.*** Narrower than thorax. Frons brownish black, shiny, thickly white pruinose, with several dark stout hairs along lateral margins; frontal ratio 1.3:1.0:0.9−1.0; frons-head ratio 1.0: 3.4−3.8. Fronto-ocular area (Fig. 1A) well developed, rounded apically. Clypeus brownish black, thickly white pruinose, moderately covered with dark stout hairs except upper 1/2 narrowly bare medially. Labrum about 0.7 times as long as clypeus. Antenna (Fig. 1B) composed of 2+9 segments, medium to dark brown except scape, pedicel, and basal 1/3 (or up to 1/2) of 1st flagellar segment yellow;1st flagellar segment slightly wider than long, and about 1.8 times as long as 2nd flagellar segment. Maxillary palp with 5 segments, light to dark brown, proportional lengths of 3rd, 4th, and 5th segments 1.0:1.1:2.3; 3rd segment (Fig. 1C) of normal size, with sensory vesicle oblong, 0.34 times as long as 3rd segment. Maxillary lacinia with 11−13 inner and 15 outer teeth. Mandible with about 34 inner and 15 or 16 outer teeth. Cibarium (Fig. 1D) with short blunt median projection directed dorsally at its dorsal margin, with many tubercles on its surface. ***Thorax.*** Scutum black, slightly shiny at certain angles of light, densely covered with yellow recumbent short hairs interspersed with dark brown short hairs on anterior surface, and also with dark long upright hairs on prescutellar area; when illuminated in front and viewed dorsally, scutum thickly whitish-grey pruinose, with 5 longitudinal unpruinose vittae, of which 1 median vitta is very narrow, 2 submedian and 2 lateral vittae rather wide, all vittae united with broad transverse band on prescutellar area; when illuminated from behind, scutum shows a reversed color pattern. Scutellum medium brown, covered with dark brown long upright hairs as well as yellow short hairs. Postnotum dark brown, shiny, whitish-grey pruinose, and bare. Pleural membrane bare. Katepisternum longer than deep, and bare. ***Legs.*** Foreleg: coxa whitish yellow; trochanter yellow with apical 1/3 or 1/2 dark yellow; femur yellow with apical tip light brown; tibia white except apical 1/4 brownish black; tarsus brownish black, with moderate dorsal hair crest; basitarsus greatly dilated, 6.1 times as long as its greatest width. Midleg: coxa brownish black; trochanter yellow except apical 1/2 dark yellow or light brown; femur yellow except apical tip light brown; tibia white to yellowish white except a little more than apical 1/4 blackish brown; tarsus dark brown to brownish black except basal

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1/5 (or up to 1/2) of basitarsus yellow. Hind leg: coxa blackish brown; trochanter yellow; femur yellow except apical cap light to medium brown; tibia white to yellowish white with a little less than apical 1/4 brownish black; tarsus blackish brown except a little less than basal 2/3 of basitarsus and basal 1/2 of 2nd segment yellowish white; basitarsus (Fig. 1E) nearly parallel-sided, about 6.6 times as long as wide, and 0.71 and 0.61 times as wide as greatest widths of hind tibia and femur, respectively; calcipala (Fig. 1E) moderately developed, nearly as long as wide; pedisulcus (Fig. 1E) well developed at basal 1/3 of 2nd tarsal segment. All tarsal claws simple. ***Wing.*** Length 2.6 mm. Costa with dark spinules and hairs; subcosta haired except apical 2/5 to 1/2 bare; basal section of radial vein bare, or with 2−4 hairs near apex; R_1 with dark spinules and hairs; R_2 with hairs; hair tuft on stem vein dark brown. ***Abdomen.*** Basal scale dark brown, with fringe of pale yellow hairs as well as dark brown hairs. Dorsal surface of abdomen dark brown to brownish black, with short dark hairs; tergite 2 shiny, silvery iridescent at certain angles of light, and tergites 6−8 shiny. Ventral surface of 7th segment with a pair of small submedian sternal plates. ***Genitalia.*** Sternite 8 (Fig. 1F) moderately sclerotized, with 16−20 long and medium-long stout hairs as well as a few short fine hairs on each lateral surface. Ovipositor valves (Fig. 1F) nearly triangular, membranous, covered with 11−15 short or medium-long hairs as well as numerous microsetae; narrow bare area along inner and posterior margins very thin and transparent (though very lightly darkened anteriorly along inner margin). Genital fork (Fig. 1G) of inverted-Y form, with narrow, well sclerotized stem; arms of moderate width, each with distinct projection directed anteriorly. Paraproct in ventral view (Fig. 1H) nearly as long as wide, with distinct concavity on anteroventral surface; paraproct in lateral view (Fig. 1I) about half as long as wide, moderately protruding ventrally beyond cercus forming round unpigmented apex, with numerous stout hairs on lateral and ventral surfaces. Cercus in lateral view (Fig. 1I) rounded posteriorly, about half as long as wide, with numerous stout hairs. Spermatheca (Fig. 1J) globular or somewhat ovoid, well sclerotized (except portion of junction to duct unsclerotized), polygonal surface patterns faintly visible, and with internal setae; accessory ducts subequal in thickness to major duct.

Male. Body length 3.0−3.2 mm. ***Head.*** Nearly as wide as thorax. Upper eye with large facets in 20 or 21 vertical columns and in 21 or 22 horizontal rows. Face and clypeus black, thickly white pruinose, sparsely covered with dark brown long hairs. Antenna (Fig. 2A) composed of 2+9 segments, medium to dark brown except base of 1st flagellar segment yellowish white; 1st flagellar segment elongate, twice as long as wide, and 2.5 times as long as 2nd flagellar segment. Maxillary palp with 5 segments, medium to dark brown, proportional lengths of 3rd, 4th, and 5th segments 1.0:1.1:2.2; sensory vesicle (Fig. 2B) small, oblong, 0.2 times as long as 3rd segment. ***Thorax.*** Scutum black, densely covered with golden-yellow recumbent short hairs interspersed with brown similar hairs anteriorly, and with dark brown long upright hairs on prescutellar area; scutum at certain angles of light with whitish-grey pruinose spots, i. e., anterolateral pair of rectangular spots on shoulders which connect widely to posterior large spot on prescutellar area through spots along lateral margins; all these spots usually indistinct due to thick covering of golden-yellow short hairs. Scutellum brownish black or black, with golden-yellow short hairs as well as dark brown long upright hairs. Postnotum black, white pruinose, and bare. Pleural membrane and katepisternum as in female. ***Legs.*** Foreleg: coxa yellow; trochanter and femur medium to dark brown; tibia dark brown to brownish black with large median area on outer surface medium brown; tarsus brownish black, with moderate dorsal hair crest; basitarsus somewhat dilated, 7.0 times as long as its greatest width. Midleg: coxa brownish black; trochanter dark brown except base narrowly yellowish; femur medium brown; tibia medium to dark brown (though somewhat pale at extreme base); tarsus dark brown to blackish brown. Hind leg: coxa brownish black; trochanter dark yellow to yellowish brown; femur medium brown except base yellow, and apical cap dark brown to brownish black; tibia and tarsus dark brown to brownish black except minute base of tibia white; basitarsus (Fig. 2C) enlarged, spindle-shaped, 4.0 times as long as its greatest width, and very slightly narrower than greatest width of hind tibia, which is as wide as greatest width of hind femur; calcipala well developed, nearly as long as its width at base; pedisulcus well developed. ***Wing.*** Length 2.6 mm; other characters as in female except subcosta with 0−6 hairs on basal 1/2, and basal portion of radial vein bare or with 1 or 2 hairs near apical tip. ***Abdomen.*** Basal scale black, with fringe of dark brown long hairs. Dorsal surface of abdominal segments black, with dark short hairs; tergites 2, 6 and 7 each with a pair of dorsolateral whitish spots, those on tergite 2 connected broadly to each other medially. ***Genitalia.*** Coxite in ventral view (Fig. 2D) subquadrate, with stout hairs on posterior 1/2 of ventral surface. Style (Fig. 2D) elongate, much longer than coxite, gently curving inwards, moderately covered with stout hairs on ventral and lateral surfaces, and with single subterminal spine; style in ventrolateral view (Fig. 2E) very slightly narrowed from base to apical 1/3, then slightly widened near apex; style in medial view (Fig. 2F) with distinct basal protuberance directed dorsomedially, with several small cone-shaped spines at and near apex. Ventral plate in ventral view (Fig. 2D) with its

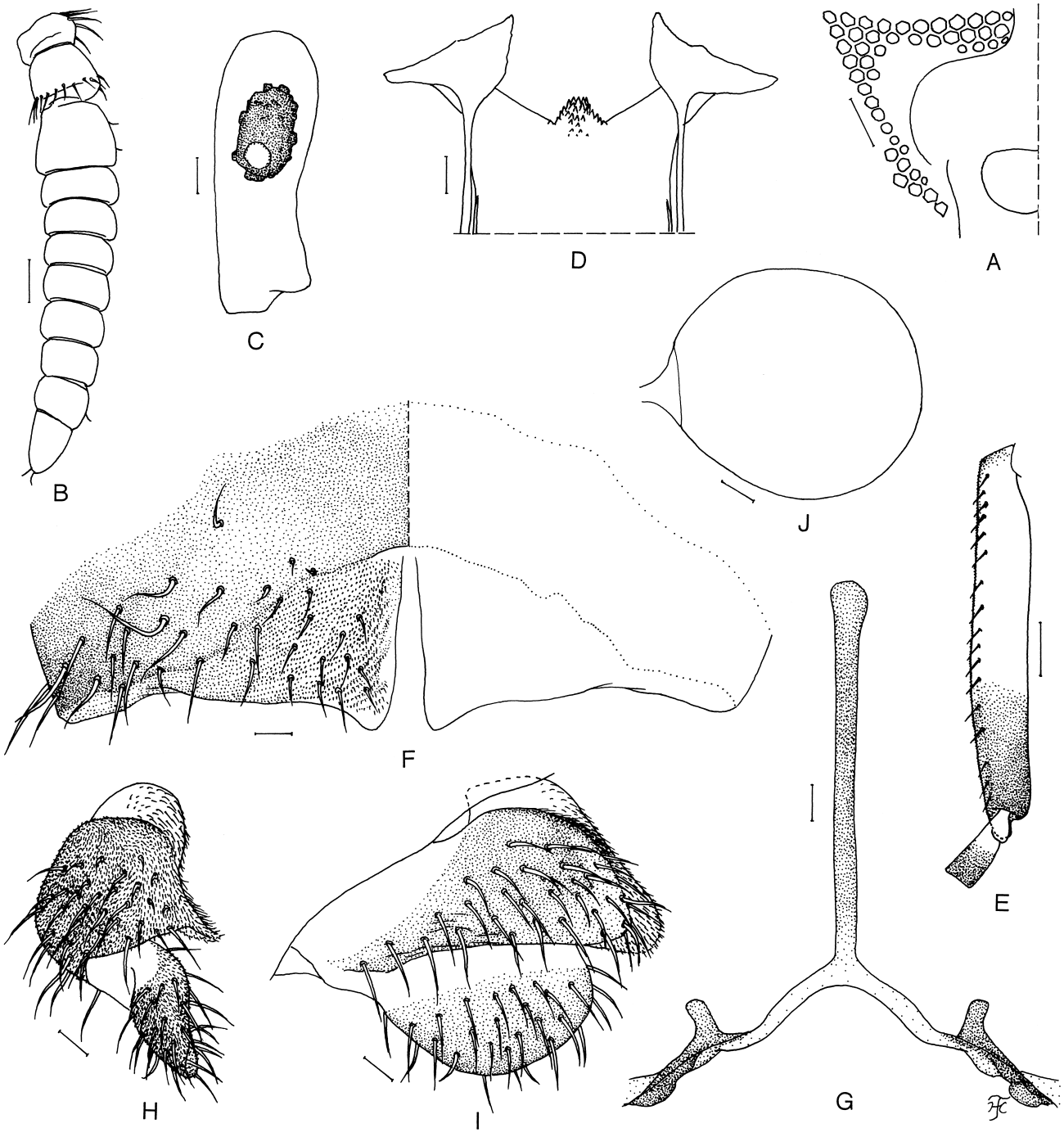

Fig. 1. Female of *Simulium* (*Simulium*) *phukaense* sp. nov. A, fronto-ocular area (right side); B, antenna (left, outer view); C, 3rd segment of maxillary palp with sensory vesicle (right side, front view); D, cibarium; E, basitarsus and second tarsal segment of hind leg showing calcipala and pedisulcus (left side, outer view); F, 8th sternite and ovipositor valves *in situ* (ventral view); G, genital fork (ventral view); H and I, paraprocts and cerci (right side; H, ventral view; I, outer view); J, spermatheca. Scale bars. 0.1 mm for E; 0.05 mm for A and B; 0.02 mm for C, D and F–J.

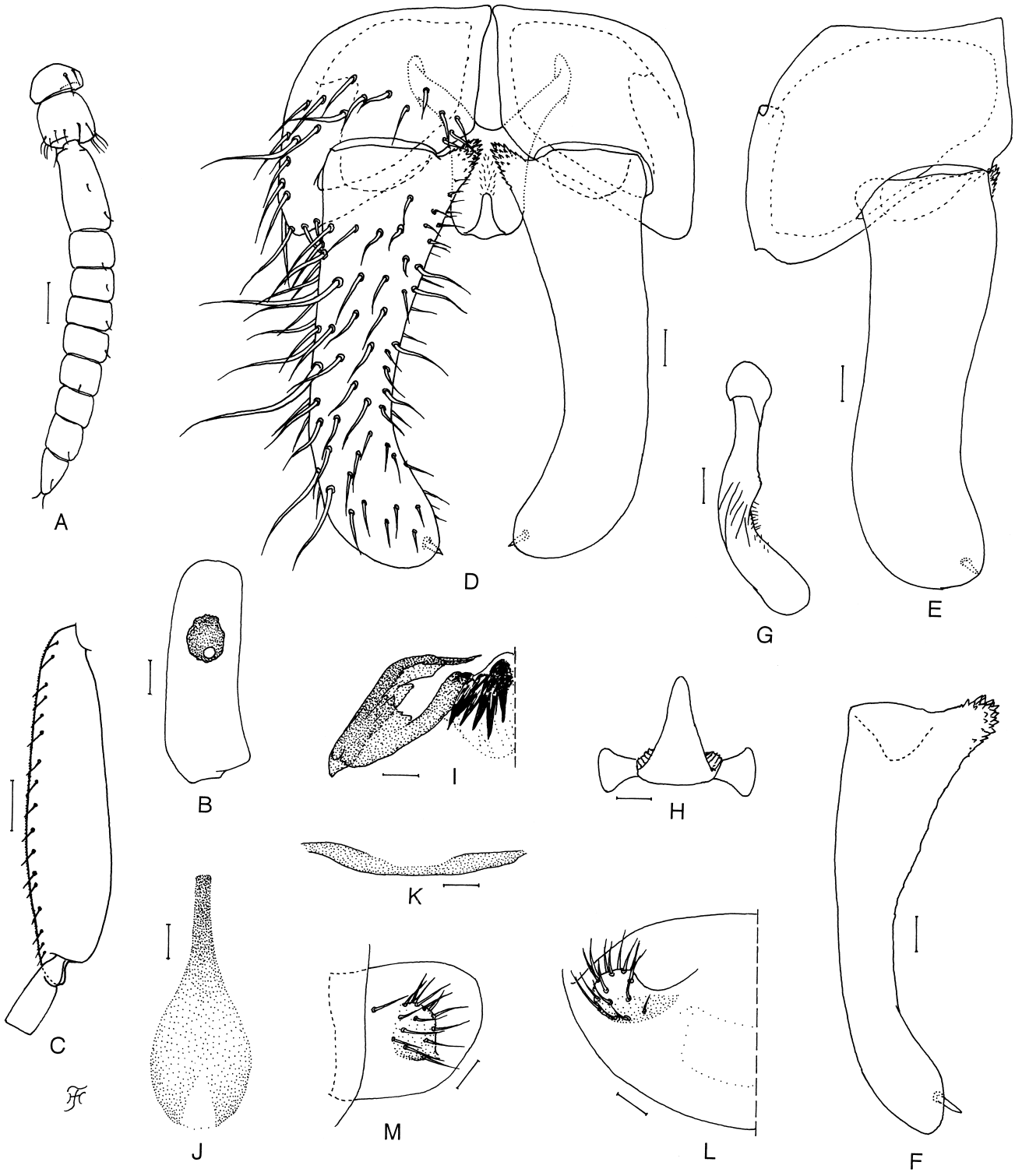

Fig. 2. Male of *Simulium* (*Simulium*) *phukaense* sp. nov. A, antenna (left side, inner view); B, 3rd segment of maxillary palp with sensory vesicle (right side, front view); C, basitarsus and second tarsal segment of hind leg showing calcipala and pedisulcus (left side, outer view); D, coxites, styles and ventral plate *in situ* (ventral view); E, coxite and style (right side, ventrolateral view); F, style showing basal protuberance (right side, medial view); G and H, ventral plates (G, lateral view; H, end view); I, paramere (right side, end view); J, median sclerite (posteroventral view); K, dorsal plate (ventral view); L and M, 10th abdominal segments and cerci (L, end view; M, outer view). Scale bars. 0.1 mm for C; 0.05 mm for A; 0.02 mm for B and D−M.

body rectangular (though the posterolateral corners are rounded), much longer than wide, and basal arms strongly sclerotized and divergent from each other; ventral plate in lateral and end views (Fig. 2G, H) with long narrow median process directed ventrally, nearly bare except anteroventral surface with several microsetae. Paramere (Fig. 2I) large basally, with several hooks. Median sclerite in ventroposterior view (Fig. 2J) narrow on basal 1/3, then widened toward apical 1/3, then somewhat narrowed apically with round apex, moderately sclerotized and brown except medial narrow portion near apex not sclerotized and transparent. Aedeagal membrane densely setose; dorsal plate (Fig. 2K) moderately sclerotized. Abdominal segment 10 (Fig. 2 L, M) without any distinct hairs on each posterolateral corner. Cercus (Fig. 2L, M) small, short, rounded, with 8−16 hairs.

Pupa. Body length 3.0−3.5 mm. ***Head.*** Integument medium brown, moderately covered with rather large tubercles on frons each with minute secondary projections on its surface (Fig. 3A); antennal sheath with 9 ridges corresponding to 9 flagellar segments, each ridge covered densely or moderately with round tubercles, while interridge spaces bare; face with a pair of long fan-like trichomes each with 12−15 slender branches (Fig. 3B), frons with 2 pairs of long fan-like trichomes (upper one with 16−22 slender branches (Fig. 3C), lower one with 23−30 slender branches). ***Thorax.*** Integument medium brown, moderately covered with tubercles of various sizes, larger ones with secondary projections (similar to those on frons) on anterior 1/2, as well as smaller cone-shaped tubercles on posterior 1/2; thorax on each side with 5 long fan-like trichomes each with 13−22 branches anterodorsally and anterodorsolaterally (similar in shape to, but somewhat longer than, those on frons−Fig. 3C), 1 medium-long stout trichome with 2−4 branches (Fig. 3D) posterolaterally, and 3 trichomes (2 medium-long, with 2−4 branches, 1 simple or bifid, long and stout) (Fig. 3E, F, G) ventrolaterally. Gill (Fig. 3H) with 6 slender thread-like filaments in 3 pairs (middle and lower pairs almost sessile, dorsal pair very short-stalked); outer filament of dorsal pair longest (1.8−2.0 mm) and thickest of all, followed by inner filament of dorsal pair, then by outer filament of middle pair, and 3 other filaments subequal to one another, and shortest (1.2−1.4 mm) and thinnest; all filaments dark brown, tapered toward apex, with distinct annular ridges and furrows, and densely covered with minute tubercles. ***Abdomen.*** Dorsally, segment 1 well sclerotized, medium brown, sparsely covered with small dark brown cone-shaped tubercles, and with 1 simple or bifid medium-long slender seta on each side; segment 2 transparent, with 1 simple or bifid medium-long slender seta and 5 short stout spines on each side; segments 3 and 4 transparent, each with 4 hooks and 1 short stout spine on each side; segments 5 and 6 transparent and bare; segment 7 with comb-like groups of minute spines on weakly sclerotized light brown narrow transverse band near anterior margin on each side; segment 8 with a transverse row of distinct spine-combs as well as comb-like groups of minute spines on weakly sclerotized light brown narrow transverse band near anterior margin on each side; segment 9 with or without comb-like groups of minute spines on weakly sclerotized light brown narrow transverse band near anterior margin on each side; posterior 1/2 of segment 9 very weakly to moderately sclerotized, yellowish to medium brown, and sparsely covered with small round tubercles, and lacking terminal hooks. Ventrally, all segments nearly transparent except segment 9 yellowish; segments 3−8 each with comb-like groups of minute spines; segment 4 with 1 simple stout hook (similar in shape and size to those on segments 5−7), 1 simple slender hooklet submedially and a few simple slender setae on each side; segment 5 with a pair of bifid and simple hooks submedially and a few simple slender setae on each side; segments 6 and 7 each with a pair of simple hooks and a few simple (or bifid) slender setae on each side. ***Cocoon*** (Fig. 3I, J). Shoe-shaped, tightly woven, not extending ventrolaterally; individual threads invisible; 3.5−4.0 mm long by 1.7−2.0 mm wide.

Mature larva. Unknown.

TYPE SPECIMENS. Holotype female with its associated pupal exuvia and cocoon, collected in Doi Phu Ka National Park, Nan Province, northern Thailand, 2.XII. 2004, by Wej Choochote. Paratypes: 3 females and 3 males, all with their associated pupal exuviae and cocoons, same data as holotype; 5 females and 11 males, all with their associated pupal exuviae and cocoons, same data as holotype except date, 23. XII. 2004.

ECOLOGICAL NOTES. The pupae of this new species were found attached to the surface of rocks in a fast flowing cascading stream (width ca. 1.5 m, depth ca. 15 cm, exposed to sun, water temperature 18 ℃, altitude 1,250 m above sea level) in a sparsely forested area. This species was found together with *Simulium* (*Daviesellum*) *courtneyi* Takaoka and Adler, *S*. (*Gomphostilbia*) *inthanonense* Takaoka and Suzuki and *S*. (*S*.) *fenestratum* Edwards.

ETYMOLOGY. The specific name *phukaense* refers to the name of the national park, Phu Ka, where this new species was collected.

REMARKS. *Simulium* (*Simulium*) *phukaense* sp. nov. is assigned to the *griseifrons* species-group of the subgenus *Simulium* (*Simulium*) by having the simple female claws,

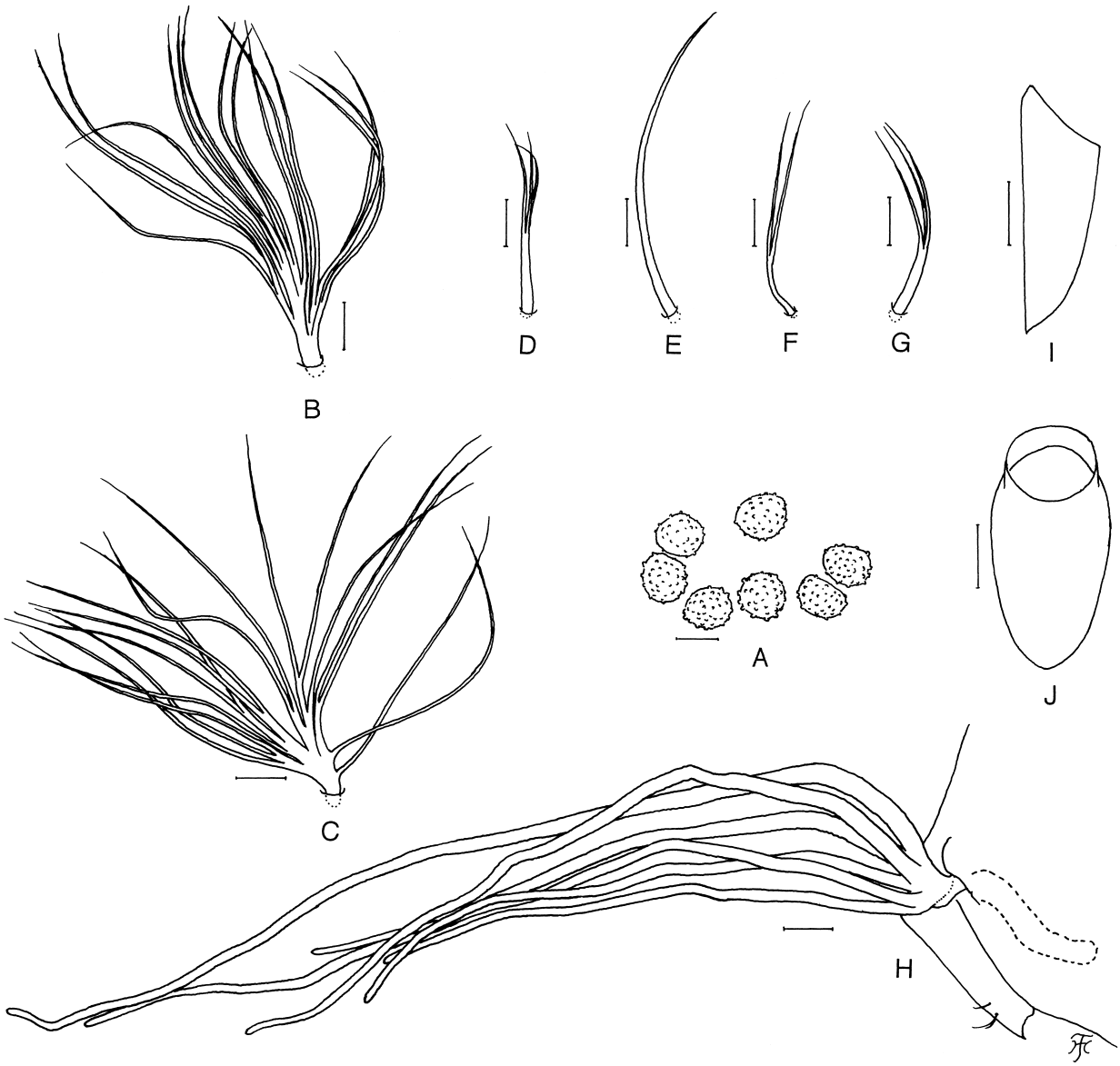

Fig. 3. Pupa of *Simulium* (*Simulium*) *phukaense* sp. nov. A, large tubercles on frons; B, facial trichome; C, frontal trichome; D–G, thoracic trichomes (D, posterolateral; E–G, ventrolateral); H, gill filaments (left side; outer view); I and J, cocoons (I, lateral view; J, dorsal view). Scale bars. 1.0 mm for I and J; 0.1 mm for H; 0.02 mm for B–G; 0.01 mm for A.

the male ventral plate without teeth (Fig. 2D), and the pupal gill with six filaments (Fig. 3H).

This new species appears to be closely related to *S*. (*S*.) *maenoi* described from Thailand [2] in that adults of both species have similarly shaped genitalia and a similar color pattern of the legs. However, *S*. (*S*.) *phukaense* sp. nov. is distinguished from the latter species as follows (characteristics of *S*. (*S*.) *maenoi* in parentheses): in the female by the median projection on the cibarium short (long), and the second abdominal segment entirely dark brown (pale yellow on anterior 3/4), in the male by the hind basitarsus spindle-shaped (wedge-shaped), a pair of dorsolateral whitish spots present only on tergites 2, 6 and 7 (present also on tergite 5) and the body of the ventral plate in ventral view nearly parallel-sided (gradually narrowed toward posterior tip), in the pupa by the facial fan-like trichome with 12–15 slender branches (4 or 5 branches), the inner filament of the middle pair apparently thinner than the outer one of the same pair (both filaments subequal in thickness to each other), the terminal hooks absent (present), and the cocoon

shoe-shaped (wall-pocket-shaped with a small anterolateral window on each side).

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A PILOT FIELD SURVEY ON THE *IN VITRO* DRUG SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* IN LAO PDR

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In Southeast Asia, malaria has presented a major public health problem, and the spread of drug-resistant falciparum malaria is making the problem more serious in this region. Thus, evidence-based detection of drug-resistant parasites is important for the accurate evaluation of susceptibility to antimalarial drugs. Lao PDR (Lao People's Democratic Republic) is a developing country in which about 70% of the population lives in malaria endemic areas. Because of the lack of information on the *in vitro* drug susceptibility of parasites in this country, chloroquine (CQ) is still the drug of choice for uncomplicated falciparum malaria [1]. This report is a pilot field survey on the *in vitro* CQ- and mefloquine (MQ)-susceptibility of falciparum malaria using AnaeroPack® gas system in Saravan province, Lao PDR.

Saravan province is located in the southern part of Lao PDR. The survey in this province was conducted from August 8 to 16, 2003. Blood samples were successfully obtained from nine Laotian patients suffering from falciparum malaria. The samples were collected by the staff of the Center of Malariology, Parasitology and Entomology, after explaining the purpose of the study to the patients. The survey was conducted in accordance with the ethical guidelines for epidemiological studies established by the Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour and Welfare of Japan. The *in vitro* drug susceptibility test was administered using the AnaeroPack® malaria culture system with a thermostat portable incubator as described previously [2, 3]. The AnaeroPack® CO_2 (Mitsubishi Gas Co., Tokyo, Japan) is a foil-packed paper sachet that on exposure to air immediately absorbs atmospheric O_2 and simultaneously generates CO_2 until a condition of 15% O_2 and 5% CO_2 is attained. The microaerophilic atmosphere produced within a sealed jar (AnaeroPack® Kakugata jar, SUGIYAMA-GEN Co., Ltd., Tokyo, Japan) can be maintained for at least 24 hours. The temperature inside the portable thermostat incubator (SUGIYAMA-GEN Co., Ltd.) was adjusted to 37℃. During *P. falciparum* cultivation, the sachet inside the jar was replaced every day when the culture medium was changed. The WHO semi-micro test method was used for evaluation of *in vitro* drug susceptibility [4]. Briefly, blood samples (0.1 ml) were resuspended in RPMI 1640 (GIBCO BRL), pH 7.4, supplemented with 25 mM HEPES, and sodium bicarbonate. To monitor parasite growth, six wells per plate served as controls without antimalarials. When the schizonts were fully grown in the control wells, the culture plate was removed from the incubator. Thin-smear specimens stained with Giemsa solution were made from each well. We defined parasites as schizonts when they had both dark brown pigment and more than three nuclei [5]. The effect of antimalarials on parasite growth was evaluated by the WHO standard evaluation method.

The results of this study are shown in Table 1. When complete schizont inhibition is observed at a CQ amount of

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Table 1: The results for *in vitro* drug susceptibility

No.	Parasitemia (%)	Chloroquine	Mefloquine
A	0.015	Susceptible	Susceptible
B	0.36	Susceptible	Susceptible
C	1.97	Susceptible	Susceptible
D	0.91	**Resistant**	Susceptible
E	0.01	**Resistant**	Susceptible
F	0.13	**Resistant**	Susceptible
G	0.002	**Resistant**	Susceptible
H	0.004	Susceptible	Susceptible
I	0.007	Susceptible	Susceptible

80 nM or less, the parasite is considered susceptible. If schizont formulation is observed at an MQ amount of 640 nM or more, the parasite can be considered resistant. In the present study, four (44%) of the nine isolates were resistant to CQ, while all the isolates were susceptible to MQ. There was no correlation between the parasitemia and CQ-resistance.

The results of this study suggest that CQ-resistant parasites have increased even though CQ is commonly used as the first-line drug for treatment of uncomplicated falciparum malaria in Lao PDR. In neighboring countries such as Thailand and Cambodia, high-grade multi-drug resistant parasites are reported to be spreading and, indeed, *in vivo* CQ-resistant falciparum malaria has already been reported in Lao PDR [6]. Dedicated efforts have to be made to determine the *in vitro* drug susceptibility of *P. falciparum* in Lao PDR as a way to prevent the spread of multi-drug resistant parasites in the near future. This is the first test report on *in vitro* drug resistance in Lao PDR.

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Review

MALARIA ENDEMIC PATTERNS ON LOMBOK AND SUMBAWA ISLANDS, INDONESIA

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ABSTRACT: Nusa Tengara Barat (NTB) province consists of two main islands, Lombok and Sumbawa, to the east of Bali Island, Indonesia. Most of the area is known to be moderately malaria endemic, but the exact malaria epidemiology has not been elucidated. At least 30 deaths per year are thought to be caused by falciparum malaria in Lombok alone, judging from the hospital data. According to the Gebrak Malaria Team in West Lombok, the annual incidence in the district of West Lombok from 1996 to 1999 was consistently over 40‰.

In the present report, we describe the small malaria endemic foci in the West Lombok and Sumbawa districts. Falciparum malaria is predominant over vivax malaria and other types of malaria. There are 11 species of *Anopheles* vector, but three of these species, *An. subpictus*, *An. maculates* and *An. barbirostris*, are of primary importance in malaria transmission and *An. sundaicus* and *An. aconitus* are of secondary importance. Our data from Sekotong, West Lombok, and Sumbawa supported the importance of *An. subpictus* in coastal areas but suggested the existence of different transmission peaks according to environmental conditions. The usual transmission peak comes in the dry season but is affected by climatic and geographical conditions. Although there were many malaria endemic foci along the coast, the width and grade of the foci varied widely. The presence of malaria endemic foci inland, although likely, has not been definitively reported to date.

INTRODUCTION

Indonesia is known as a country where tourists are at a high risk for malaria infection. But the incidence of malaria varies widely among different islands and even among different areas of the same island. It is important to obtain exact information on the epidemiological conditions of malaria on each island. In this report, we describe the epidemiology of malaria in parts of Lombok and Sumbawa islands on the basis of our experience and local data (Fig.1).

1) Malaria situation in Indonesia;

Malaria is still a major public health problem in Indonesia. In 1995, the National Health Household Survey estimated that around 32,000 deaths were caused by malaria. [1]. Indonesia is a large archipelago consisting of 12,508 islands of various sizes and shapes located along the equator and had a total population of 209 million in 1999. About 70% of the population live in Java and Bali, where malaria has been mostly eradicated, although even today small outbreaks are reported every year. In the outer islands, however, a much higher incidence of malaria is seen in general. But the incidence of malaria varies from hypo-to hyper-endemic depending on the environmental and socio-economic conditions of an area. The natural and social environment of the Indonesian islands varies widely, resulting in different malaria conditions. Furthermore, even on a single island, malaria endemic situations vary in degree and

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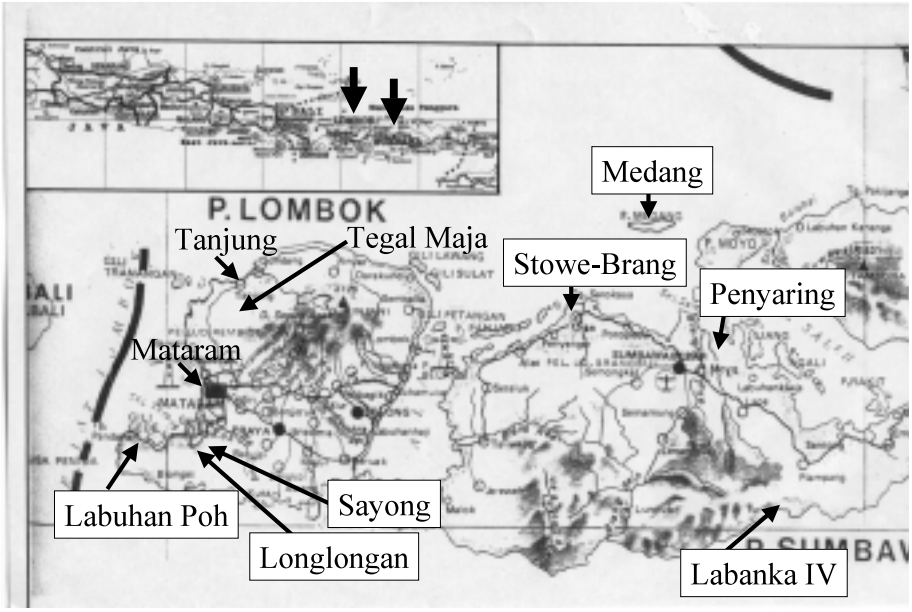

Fig. 1 Location of places described in the text on Lombok and Sumbawa islands, Indonesia

size according to geographical conditions. No exact information on malaria epidemiology in each area has been published, especially in English. In this review, we introduce the conditions of malaria in Lombok and Sumbawa islands based on our epidemiological studies conducted under Japan Society for the Promotion of Science (JSPS) sponsorship, and on local data and reports offered by concerned health organizations.

2) The collaborative survey of Indonesian and Japanese researchers; In 1991 we had an opportunity to conduct an epidemiological malaria survey on Lombok island as one of the collaborative works in a large scale cooperative study between Kobe University School of Medicine, Japan and the Tropical Disease Center (TDC), Airlangga University, Surabaya, Indonesia under the sponsorship of JSPS. The malaria epidemiological study was carried out continuously in Lombok and later in Sumbawa for ten years until the JSPS project reached completion in 2000.

3) Malaria situation in West Lombok; The West Lombok district is located in the western part of Lombok island, NTB province. The province is composed of two major islands, Lombok and Sumbawa, each of them containing three districts. The capital of the province is the municipality Mataram, which is located in the center of West Lombok district. West Lombok consists of nine subdistricts, in all of which malaria is endemic. According to the report by Gerback Malaria Team in West Lombok [2], the annual incidence in the district was over 40 per 1,000 population every year from 1996 to 1999. Falcipaum malaria is more common than vivax and other types of malaria. The transmission peak is usually observed between July and September. There are 11 species of *Anopheles* vector but, of these, three, *An. subpictus*, *An. maculates* and *An. barbirostris*, are of primary importance in malaria transmission and *An. sundaicus* and *An. aconitus* are of secondary importance.
Figure 2 shows the monthly slide-positive cases observed in 2000 and 2001 in Tanjung and Tegal Maja villages by the health center in Tanjung, which is located in the northernmost part of West Lombok. The village of Tanjung lies on the northern coast while the village of Tegal Maja is located south of it in the inland. At the former, the clear peak of malaria cases was seen in October, which was two months later than the usual transmission peak between July and

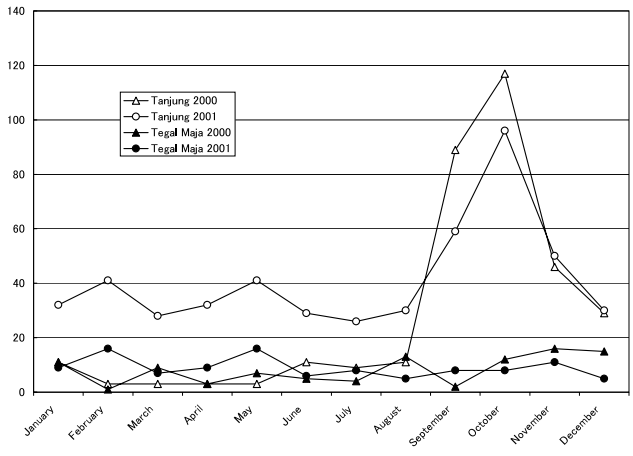

Fig. 2 Number of slide positive cases in two villages of Tanjung health center

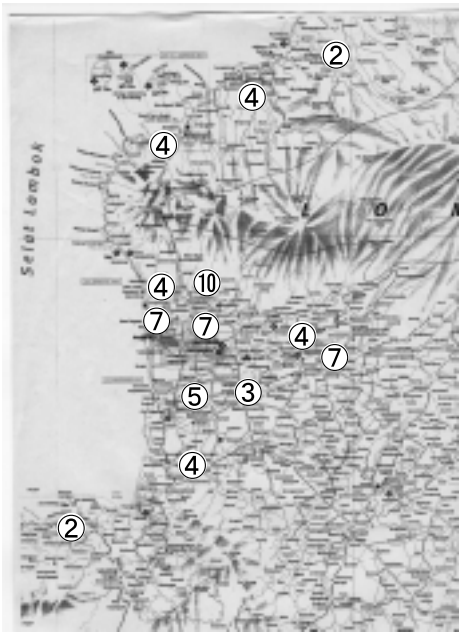

Fig. 3 Number of cerebral malaria cases at Mataram hospital shown by the place of residence

September mentioned in the local report [2]. This type of transmission is thought to be caused by *An. sundaicus* rather than *An. subpictus* according to a previous study by one of the present authors [3]. We obtained the data on malaria patients hospitalized in the Mataram hospital in 2001 and the first half of 2002. In total, 809 malaria patients were hospitalized. These were composed of 580 falciparum malaria patients including 71 with cerebral malaria, 54 vivax malaria patients and 175 clinical malaria patients. A total of 39 died of falciparum malaria, and 29 of these were cerebral malaria patients. We marked the number of cerebral malaria patients on a map according to the place of residence recorded in their patient-reports (Fig. 3). The cases were distributed equally in coastal and inland areas. This indicates that more careful attention should be given to the inland areas to identify malaria endemic foci.

STUDIES IN WEST LOMBOK

1) Survey areas in West Lombok

Malaria epidemiological study target areas were selected on the basis of discussions between TDC and the Nusa Tenggara Barat (NTB) provincial health office. The subdistricts (kecamatan) Batulayar and Sekotong were selected for the preliminary survey. Subjects for blood and spleen examination were randomly selected from different subvillages (dusun) in the two subdistricts. A total of 36 subvillages, or 10 from Batulayar and 26 from Sekotong, were subjected to the survey. Although the number of persons examined in each subvillage was too small for evaluation, we selected three subvillages in Sekotong for the longitudinal survey, namely, dusun Labuhan Poh from desa (village) Sekotong Barat, and dusun Longlongan and Sayong from desa Sekotong Tengah. (Fig. 1, Fig. 4 and Table 1).

2) Geographical differences among subvillages (Table 1)

At subvillage Labuhan Poh, the land gradually rises away from the coast toward the inland. The largest river in this village passes through the subvillage and creates a wide lagoon during the dry season (Photo. 1). Many branches of

Fig. 4 Geographical distribution of the three subvillages selected for malaria survey at Sekotong

Table 1 Geographical features and population of three subvillages of Sekotong Barat and Tengah in 1992

Subvillage	hill	wet rice field	beach	dry field	population
Labuhan Poh	16.5%	0	21.3%	62.2%	855
Sayong	30.6%	67.7%	1.7%	0	1,247
Longlongan	71.5%	4.3%	16.1%	8.1%	805

Photo 1. A huge lagoon formed after closing the river's exit to the sea at Labuhan Poh, Sekotong, West Lombok

the river extend into mangrove areas where large mangrove trees have been cut for fuel, leaving many water pools exposed to sunshine and resulting in breeding places for brackish species of *Anopheles* mosquitoes such as *An. sundaicus* and *An. subpictus*. Subvillage Sayong lies on flat ground. Most of the area is occupied by wet rice fields, and in the coastal part of the flat land once covered by mangrove forest, fish ponds were made after the removal of mangrove trees. Subvillage Longlongan has a complex topography; the narrow flat land along the coast, originally mangrove forest, was developed for fish ponds, and the following sharp sloping land leads to a rather flat hilly area where rice fields were developed between stands of grass or bush in the rainy season.

3) Survey methods and subjects

The longitudinal survey was started in August 1992 and carried out five times until June 1993 [4]. At the initial step, in order to determine the seasonal changes in malaria transmission, we intended to collect blood samples from the same subjects randomly selected from all age groups through all the surveys in a year. After the third survey, however, we had to replace the subjects with a new group composed of almost the same proportion of age groups, because of difficulties encountered in obtaining informed consent and cooperation from the former subjects. In the survey, the subjects were usually gathered in one place such as a school or a village health office (pustu) on an appointed date, and a 1−2 ml venous blood sample was obtained from each person along with in a syringe, a drop of blood for thin smear and another drop for thick smear on separate slide-glasses. The blood in a syringe was transferred into a small tube for serum collection. All the samples were carried to TDC for parasitological and serological examination. Medical examination was administered to each person after blood collection, and if necessary, medicines were given. On the same day the entomological survey was conducted, consisting of the examination of breeding sites and larva collection in the daytime, and adult mosquito collection at night.

4) Malaria prevalence in the survey areas

Table 2 and Fig. 5 present the results of the blood examinations. We cannot accurately compare the results of the first three surveys with those of the last two surveys because of the replacement of subjects. In total, the malaria positive rate gradually declined after the first survey in August 1992. However, the malaria transmission trend in each subvillage differed from that in others (Fig. 5). A relatively stable slide positive rate was found in dusun Labuhan Poh in August, October and December 1992, while in other subvillages it varied by month, especially in dusun Longlongan.

Table 2 Results of blood examinations in a longitudinal malaria survey conducted in three subvillages of Sekotong, Lombok from August 1992 to June 1993

	August 1992					October 1992					December 1992				
Subvillage	PR	Number of positive cases				PR	Number of positive cases				PR	Number of positive cases			
	%	Pf	Pv	Pm	Mix	%	Pf	Pv	Pm	Mix	%	Pf	Pv	Pm	Mix
Labuhan Poh	11.5 (14/122)	10	4	0	0	9.8 (12/122)	9	2	0	1	12.3 (15/122)	11	3	0	1
Sayong	11.9 (18/151)	6	11	0	1	6.0 (9/150)	4	5	0	0	6.0 (9/150)	4	5	0	0
Longlongan	21.1 (20/95)	12	8	0	0	6.4 (6/95)	5	1	0	0	2.1 (2/95)	1	1	0	0
Total	14.1 (52/368)	28	23	0	1	7.4 (27/367)	18	8	0	1	7.1 (26/367)	16	9	0	1

	April 1993					June 1993				
Subvillage	PR	Number of positive cases				PR	Number of positice cases			
	%	Pf	Pv	Pm	Mix	%	Pf	Pv	Pm	Mix
Labuhan Poh	3.7 (4/107)	3	1	0	0	1.1 (1/89)	0	0	1	0
Sayong	0.72 (1/139)	1	0	0	0	0 (0/129)	0	0	0	0
Longlongan	1.0 (1/98)	1	0	0	0	9.0 (8/89)	5	2	0	1
Total	1.7 (6/344)	5	1	0	0	2.9 (9/307)	5	2	1	1

PR, positive rate; Pf. *Plasmodium falciparum*; Pv, *Plasmodium vivax*; Pm, *Plasmodium malariae*; Mix, mix infection; ( ), actual number

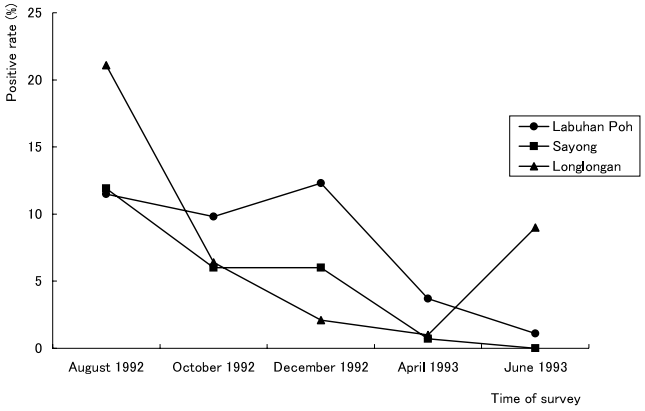

Fig. 5 Slide positive rates in the three subvillages of Sekotong, Lombok from August 1992 to June 1993

This difference may be attributable to the different environmental and geographical conditions of each subvillage (Table1). Especially in dusun Longlongan, the malaria in the hilly area may have a different transmission mode.

5) Entomological observation in the survey area

The results of the entomological examination also showed a wide variety (Table 3 and 4). As expected, a relatively stable number of adult *Anopheles subpictus* mosquitoes were captured at all three subvillages, especially at dusun Labuhan Poh (Table 3), but *An. sundaicus*, *An. barbirostris* and *An. aconitus*, which have been recognized as malaria vectors in Indonesia, were captured sporadically once or twice in five surveys conducted one year except *An. sundaicus* at dusun Labuhan Poh [4, 5]. The fluctuation in the number of captured mosquitoes suspected to be malaria vectors did not correspond to the parasitological data (Table 2, 3 and Fig. 5). The most stable larva collection of the brackish *Anopheles* species was obtained at lagoon and mangrove areas in dusun Labuhan Poh but at fish-ponds in dusun Sayong and Longlongan (Table 4). These results, taken with the parasitological data, indicate that *An. subpictus* (and additionally *An. sundaicus*) play a major role in malaria transmission in these subvillages. The previous intensive study on mosquito fauna in Lombok island by Lee et al.

Table 3 Adult collection of *Anopheles* species known as malaria vector in three subvillages of Sekotong (1992-1993)

SUBVILLAGE	COLLECTION METHOD	Aug 92	Oct 92	Dec 92	Apr 93	Jun 93
		(species / no. mosq. collected per night*)				
LABUHAN POH	Outdoor Human Bait (OHB)	sub/14 sun/4	sub/9 sun/8	sub/6 sun/2	bar/14 sub/4	sub/6 sun/3
	Indoor Human Bait (IHB)	sub/2	sub/11 sun/22	sub/6 sun/5	0 0	sub/4
	Indoor Resting (IR)	0	sun/5	0	0	0
	Bednet Trap (BT)	0	sub/7 sun/16	0	0	0
	Cattle Bait (CB)	sub/11 sun/1 bar/1	sub/12	sub/1	sub/2 acon/1	sub/5 sun/1
SAYONG	OHB	sub/7	sub/14 sun/3	sub/10 acon/5	sub/8 acon/1	sub/1
	IHB	0	sub/4	sub/9 acon/2	acon/2	0
	IR	0	0	0	0	0
	BT	bar/1	0	0	0	0
	CB	sub/5	sub/16	sub/6	acon/4 sub/9	sub/9
LONGLONGAN	OHB	sun/3	sub/15 sun/3	sub/4	sub/19	0
	IHB	0	sub/3 sun/1	sub/17 sun/1	sub/3	sub/1
	IR	0	0	0	0	sub/1
	BT	sun/1	0	0	0	—
	CB	sub/7 bar/1	sub/17	sub/8	sub/10 acon/7	sub/9 acon/1

*40 min per hour from 6 pm−12 pm.

sub, *Anopheles subpictus*; sun, *An. sundaicus*; acon, *An. aconitus*; bar, *An. barbirostris*

Table 4 Type of breeding place and density of *Anopheles* larvae (per dip) in three subvillages of Sekotong

Type Br. Pl.	*Anoph*. Species	Aug. 92	Oct. 92	Dec. 92	Apr. 93	Jun. 93
Labuhan Poh						
I. Lagoon	1. *An. subpictus*	n. d.	1.87	1.30	1.40	0.95
	2. *An. sundaicus*	n. d.	0.70	0.40	0	0.30
II. River	1. *An. flavirostris*	1.00	0	0	0	0
	2. *An. minimus*	0.50	0	0	0.70	0
	3. *An. vagus*	0	0	0	0.11	0.10
	4. *An. subpictus*	1.56	0	1.50	0	0
	5. *An. sundaicus*	0.12	0	0	0	0
III. Mangrove	1. *An. subpictus*	1.00	0	1.60	0.67	(−)
	2. *An. sundaicus*	0	0	0.40	0	(−)
IV. Rice field	1. *An. vagus*	(−)	(−)	1.40	(−)	0.10
V. Fishpond	1. *An. subpictus*	(−)	(−)	(−)	0.10	0
Sayong						
I. Fishpond	1. *An. subpictus*	0.80	0.80	1.00	0.05	0.90
	2. *An. sundaicus*	0	0	0	0.05	0
	3. *An. annularis*	0	0	0	0.05	0
II. Rice field	1. *An. aconitus*	0	0	0.40	0.60	0.25
	2. *An. barbirostris*	0.70	0	0	0	0
	3. *An. vagus*	0	1.50	2.20	0	0.28
III. Fresh water	1. *An. barbirostris*	0.60	0	0	0	0
	2. *An. annularis*	0	0	3.40	0.60	0
Longlongan						
I. Well	1. *An. barbirostris*	0.40	0	0	0	0.35
	2. *An. annularis*	0	0	0	0.10	0
	3. *An. vagus*	0	0	1.20	3.00	0.70
II. River	1. *An. aconitus*	0.02	0	0	0	0
	2. *An. barbirostris*	0.77	0	0	0.40	0
	3. *An. vagus*	0.85	0.67	1.80	0	0.23
III. Ricefield	1. *An. barbirostris*	0	0.05	0	0.25	0.01
	2. *An. annularis*	0	0	0	0.01	0.01
	3. *An. vagus*	1.00	0.80	4.20	2.07	0.55
IV. Fishpond	1. *An. subpictus*	0.08	1.38	1.20	1.80	1.03

(−), no water at the time examined; Br. Pl., Breeding Place; n. d., not done

identified three *Anopheles* species, *An. annularis*, *An. barbirostris* and *An. subpictus*, as potential vectors [6]. Recently, Miyagi et al also found *An. subpictus* and *An. sundaicus* in coastal areas and *An. barbirostris*, *An. leucosphyrus* group and *An. minimus* in fresh water and cited them as potential vectors [7]. In 2001, Sukowati, S. et al., Health Ecology Research Center, NIHR&D found *Plasmodium falciparum* (*P. f.*) sporozoite-positive *An. subpictus* in this area (report to the Indonesian health ministry). In the subvillage Longlongan, in addition to the coastal area, malaria was found in the hilly area where more than half of the population of this subvillage live, but we could not determine the vector mosquitoes there. Because of windy conditions and the collection confined to one night during the survey, the entomological staff were able to capture only a few adult mosquitoes. From the larvae examination we inferred two probable transmission vectors. One is *An. barbirostris*, the larvae of which were found in rice-fields, stagnant water along small rivers and wells, and the other is *An. subpictus*, which was consistently found in fish ponds along the coast and is thought to be able to move back and forth between the coast and the hills with the wind.

6) Endemic situation of malaria

We selected subjects equally from all the age groups to determine the degree of endemicity. Our results showed no difference in malaria prevalence among age groups [4] (data, not shown), indicating a hypo-or meso-endemic pattern in the area. The additional serological examination of antibodies to *P. f.* crude antigens using ELISA also demonstrated a meso-endemic pattern at dusun Labuhan Poh (Fig. 6), that is, the positive rate was low (about 20%) at the age of 0 but rose to nearly 100% at the age of 6 or over. In this area, three *Plasmodium* species were detected, that is, about

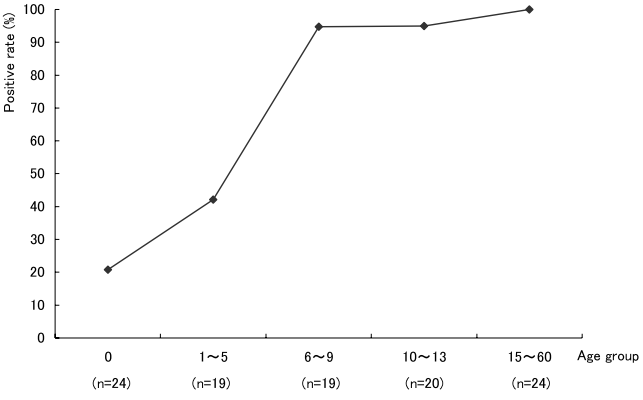

Fig. 6 Sero-positive rate to P. f. crude antigens among persons examined in subvillage Labuhan Poh in October 1992

60% *P. falciparum*, 40% *P. vivax* and only one *P. malariae* (Table 2). The *P. malaria* case was confirmed by PCR using the ribosomal DNA sequence [8]. Our results did not confirm the peak of transmission between July and September as described in the local report [2].

STUDIES IN SUMBAWA

1) Survey areas in Sumbawa

In Sumbawa, four subvillages in different subdistricts were examined for prevalence of malaria from 1996 to 1999 (Fig. 1). One subvillage, dusun Medang, is a small island accessible in one hour from Sumbawa Besar by small motorboat. In this subvillage, the preliminary spleen examination was

Table 5 Parasite positive rate and spleen rate in the longitudinal survey conducted in three subvillages of Sumbawa

	December 1996					July 1997				
Subvillage	Positive Rate (%)	Number of positive cases Pf	Pv	Mix	Spleen Rate (%)	Positive Rate (%)	Number of positive cases Pf	Pv	Mix	Spleen Rate (%)
Penyaring	7.1 (8/112)	7	1	0	0	—	—	—	—	—
Labangka IV	14.3 (16/112)	10	6	0	8.0	7.1 (8/112)	4	4	0	14.3
Stowe Brang	33.9 (38/112)	8	25	5	25.9	15.3 (17/111)	13	3	1	34.2
Total	18.5 (62/336)	25	32	5	11.3	11.2 (25/223)	17	7	1	16.0*
	March 1998					October 1998				
Subvillage	Positive Rate (%)	Number of positive cases Pf	Pv	Mix	Spleen Rate (%)	Positive Rate (%)	Number of positive cases Pf	Pv	Mix	Spleen Rate (%)
Penyaring	1.8 (2/112)	2	0	0	0	0.9 (1/112)	1	0	0	0
Labangka IV	0.9 (1/112)	1	0	0	0	13.0 (9/69(74))**	4	3	2	23.0
Stowe Brang	8.0 (9/112)	7	2	0	12.5	1.8 (2/112)	1	1	0	4.5
Total	3.6 (12/336)	10	2	0	4.2	14.1 (12/293)	6	4	2	7.4
	December 1998					February 1999				
Subvillage	Positive Rate (%)	Number of positive cases Pf	Pv	Mix	Spleen Rate (%)	Positive Rate (%)	Number of positive cases Pf	Pv	Mix	Spleen Rate (%)
Penyaring	0.9 (1/112)	1	0	0	0	3.6 (4/111)	3	1	0	0
Labangka IV	5.3 (5/95)	1	4	0	1.1	5.4 (6/111)	2	3	1	0
Stowe Brang	0 (0/112)	0	0	0	0	ND (−/108)	ND	ND	ND	0.9
Total	1.9 (6/319)	2	4	0	0.3	4.5 (10/222)	5	4	1	0.3

P. f., *Plasmodium falciparum*; P. v., *Pl. vivax*; Mix, mix infection

−,data lost

* The number of subjects in Penyaring was assumed to be 112.

** 74 persons were subjected to spleen examination, but only69of these underwent blood examinations.

ND, not done

conducted on 161 1st and 2nd grade school-children and showed a 42.9% spleen rate (meso-endemic), but afterwards neither blood nor mosquito examinations was conducted because of the risk of the available boat capsizing. Therefore, three subvillages were selected for the longitudinal survey. The methods were the same as those used in Lombok.

2) Malaria prevalence in the Sumbawa survey areas

The slide positive rates and spleen rates at three subvillages are shown in Table 5. All three subvillages are located along the coast. Subvillage Penyaring and Stowe Berang face the ocean to the north and subvillage Labangka IV to the south. The former two subvillages are geographically similar. They have mangrove beaches and flat lands. The mangrove beaches were developed for fish ponds in both subvillages. Despite the environmental similarities, subvillage Penyaring showed a very low slide-positive rate and 0% spleen rate, while dusun Stowe Berang showed rather high positive rates for both examinations. Subvillage Labangka IV showed a medium endemic pattern with seasonal epidemics.

3) Entomological observation and epidemiological analysis

The entomological examination clearly demonstrated a high density of adults and larvae of *An. subpictus* at subvillage Stowe Berang but a very low density at subvillage Penyaring (data, not shown). This was due to the difference in breeding sites between the two subvillages, namely, many abandoned fish-ponds with algae and weeds were found at the former (Photo. 2) while most of the fish ponds were well maintained at the latter. The sharp decline in positive rates for spleen and blood examinations at dusun Stowe Berang from October 1998 was due to two malaria control projects conducted from January 1998 for a year, that is, the distribution of insecticide impregnated mosquito-nets and the cleaning of abandoned fish-ponds (Fig. 7). Our reports in 1996 and 1997 note that these control projects were conducted by the Sumbawa district health office, and suggest that the control methods worked effectively. In subvillage Labangka IV, an outbreak of malaria was observed just before our survey in October 1998. This subvillage has a very narrow sandy beach with a steep cliff rising behind. A rather flat hilly area spreads away from the cliff. The entomological survey found that the captured *Anopheles* mosquitoes were exclusively *An. subpictus* and that there were several lagoons on a small beach where *An. subpictus* larvae bred. According to staff in the Sumbawa district health office, the outbreak may be related to the custom of villagers to gather around the cliff (cape) at night to catch a species of bird during this season.

Photo 2. An abandoned fish pond at Stowe Brang, Utan, Sumbawa

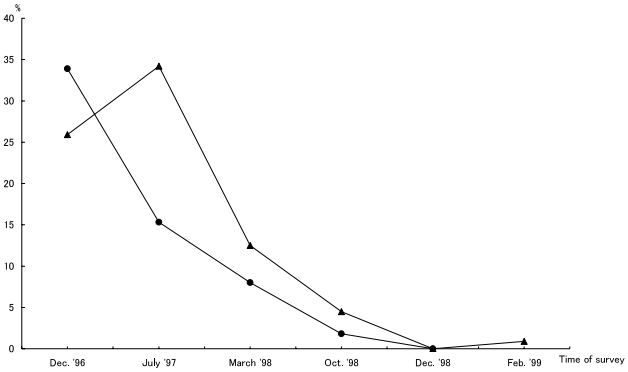

Fig. 7 Changes in slide−positive rate (●) and spleen rate (▲) in Stowe Brang, Sumbawa during the three−year period after malaria control activities in January 1998

SUMMARY

Although our data are still insufficient to determine the full range of epidemiological features, we can draw the following conclusions about malaria in Lombok and Sumbawa.

1) Malaria endemic areas are located mainly along the seacoast and less frequently inland.

2) The degree of endemicity is hypo-endemic to meso-endemic.

3) The main transmission vectors are *Anopheles subpictus* and *An. sundaicus*, which breed in brackish water.

4) Although similar species of vector play a role in transmission in coastal endemic foci, the mode and the season of transmission vary with the ecological characteristics of the vector and social and environmental conditions.

5) Small endemic foci are found in hilly areas inland, but the responsible vector species have not been determined.

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