

Human genetic contribution to clinical malaria in a Karen population in Thailand

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MATERIALS & METHODS

A. Phenotypic preparation

Study site : 7 hamlets in Tanaosri subdistrict , Suan Phung, Ratchaburi

Family data : family structure of 2,447 participants from 244 families were constructed by using a questionnaire.

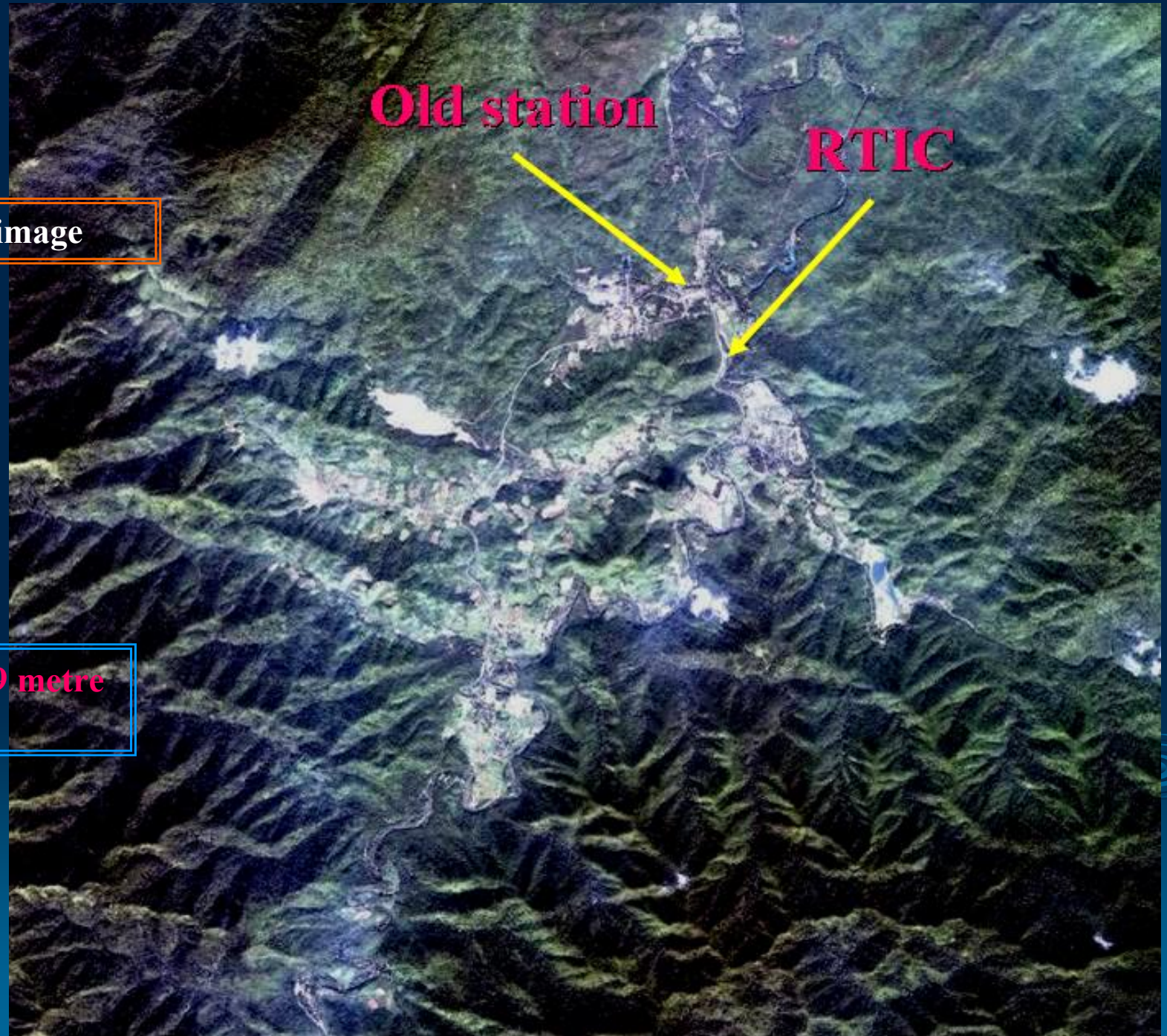
Data collection : Clinical malaria episodes associated with a slide positive for blood-stage asexual and sexual PF, PV, PM, PO and Mixed parasites at any density were collected.

Passive case detection ; June 1998-May 2005

Active case detection ; June 2003-August 2004, every 45 days

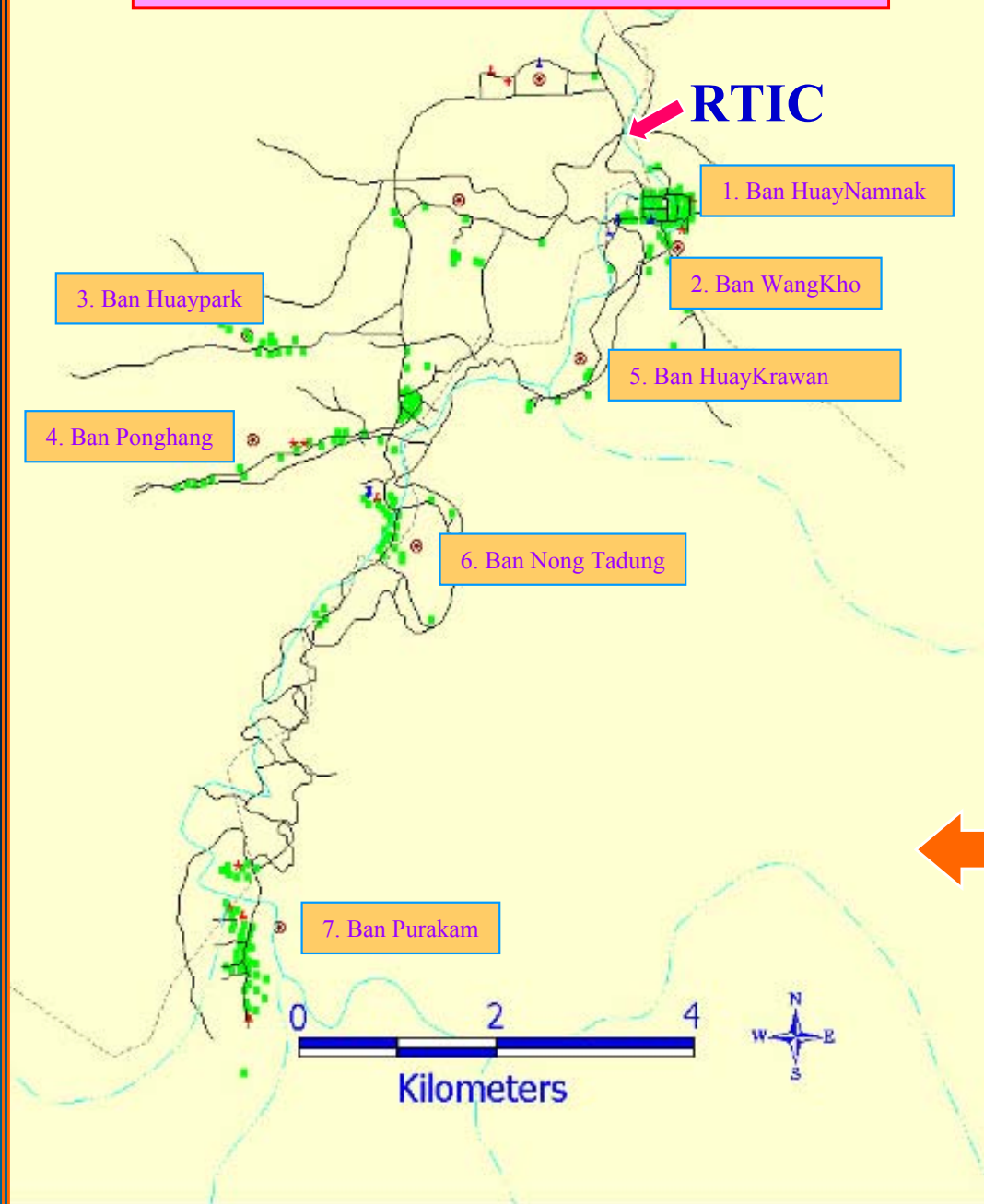
+
Crossectional study ; 2002, for pedigree and genotypic assay

IKONOS Satellite image

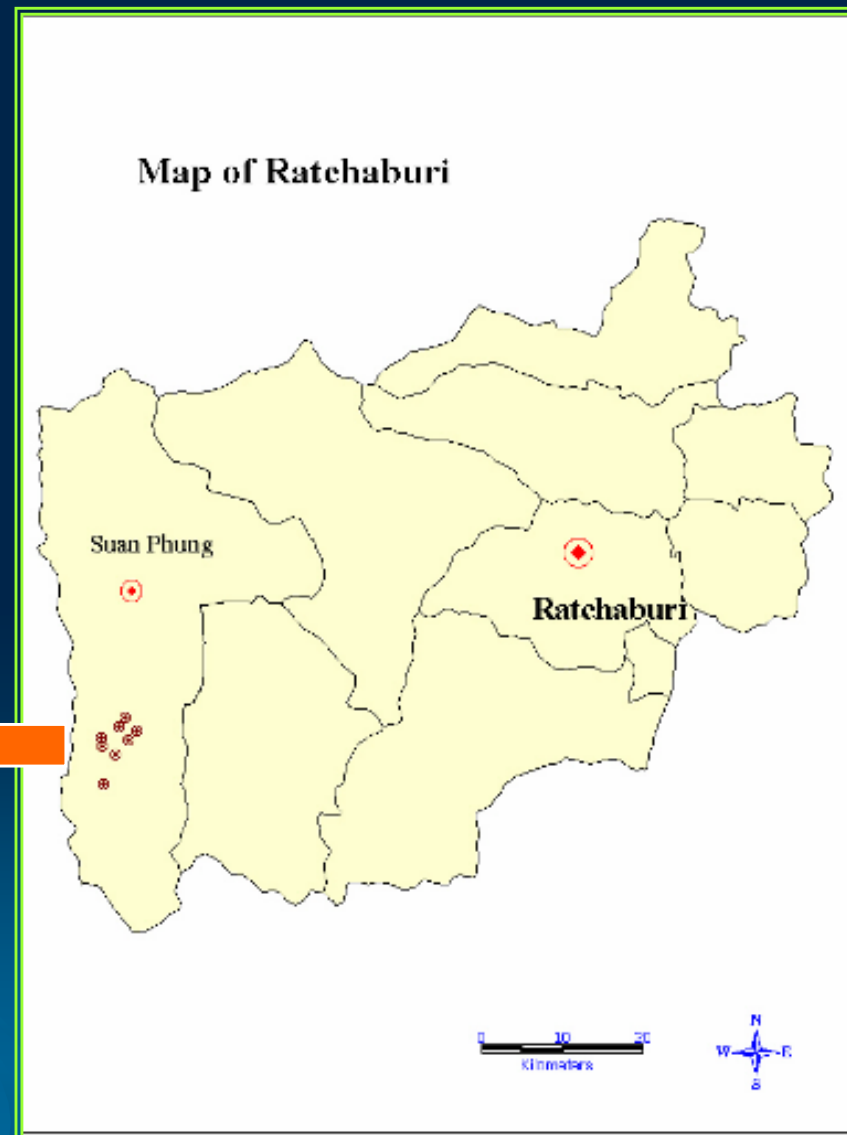


**Height of area 245.9 metre
from sea level**

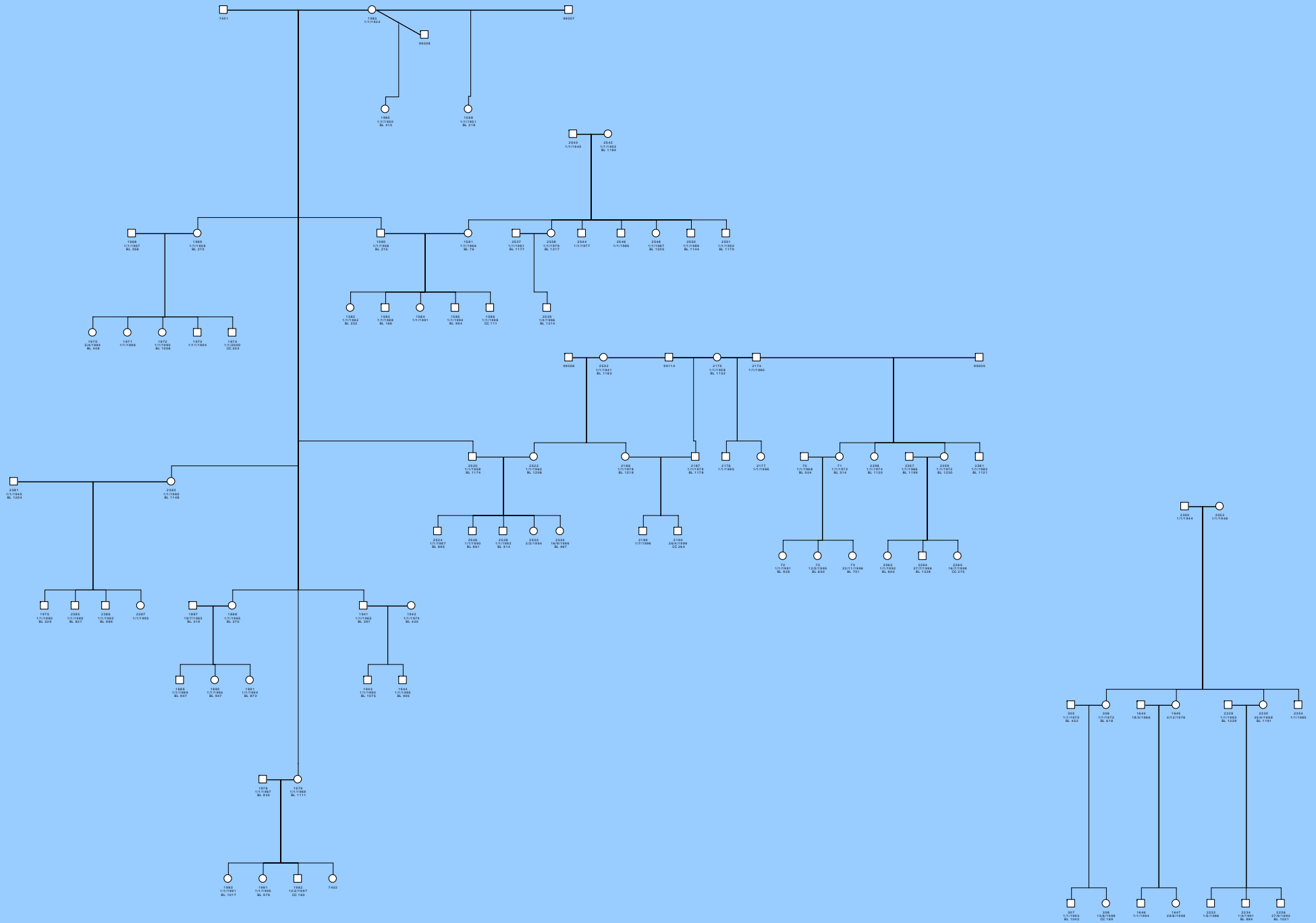
Map of Tanawasri Sub-district



Location







MATERIALS & METHODS (Cont.1)

Clinical malaria phenotype and data transformation

Clinical malaria attacks ;

- number of all malaria attacks
- *P. falciparum*
- *P. vivax*

Trophozoite and gametocyte density ;

- *P. falciparum*
- *P. vivax*

MATERIALS & METHODS (Cont.2)

Phenotypes

Transformation

- | | | |
|---|---|---|
| 1. Number of clinical attack | } | Negative binomial regression |
| 2. Number of <i>P. falciparum</i> infection | | |
| 3. Number of <i>P. vivax</i> infection | | |
| 4. <i>P. falciparum</i> trophozoite density | } | Log10 linear regression |
| 5. <i>P. vivax</i> trophozoite density | | |
| 6. <i>P. falciparum</i> gametocyte density | } | Log10 and Log10 +1
linear regression |
| 7. <i>P. vivax</i> gametocyte density | | |

Covariates

Age-group, sex, hamlet and year of study (dummy variable)

(Stata v.8.0)

MATERIALS & METHODS (Cont.3)

B. Genotypic preparation

Blood samples ; 10 ml of venous blood was checked for CBC, Blood chemistry, Hemoglobin typing, G6PD and DNA extraction.

DNA extraction and Quantitation ; 3 ml of EDTA blood and buccal swab (children < 5yrs) was extracted by Phenol-chloroform method and then quantified by Picogreen® Fluorimetry

Genotyping ; 400 microsatellite markers were genotyped by CNG, France.

MATERIALS & METHODS (Cont.4)

Phenotypic preparation

Clinical attack

Parasite density

Genotypic preparation

DNA extraction

400 microsatellite markers
genotyping

Heritability estimation (h^2)

Pedigree-based variance component model

Estimation of heritability

Variance component models

$$H_0: V_p = V_e$$

$$H_1: V_p = V_e + V_g$$

statistic χ^2 , $df = 1 = 2 \ln(L_1/L_0)$

V_p = variation of phenotype

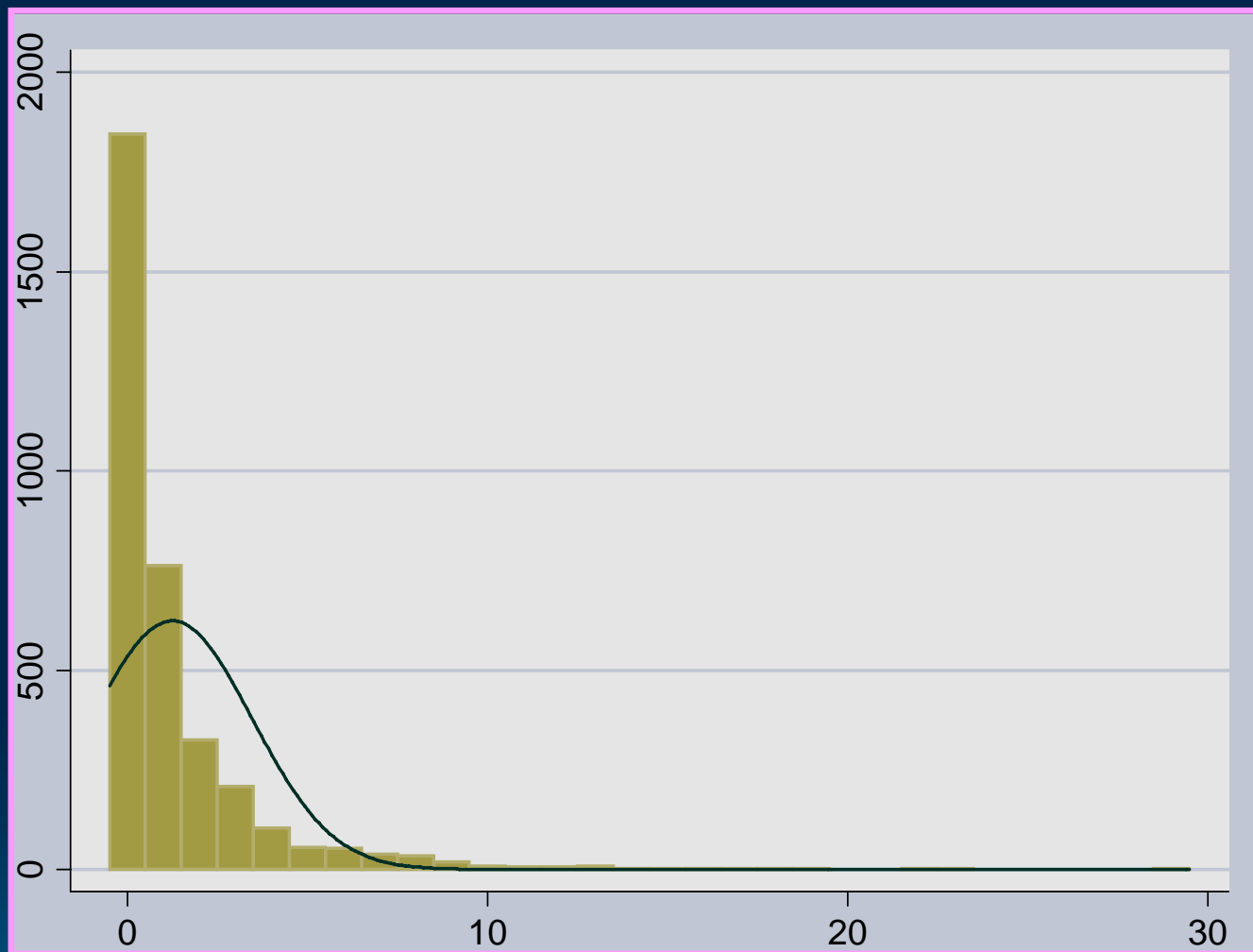
V_e = variation due to environment

V_g = variation due to genetics

$$\text{Heritability} = V_g/V_p$$

Statistic package: SOLAR version 2.1.4

Number of clinical malaria attacks



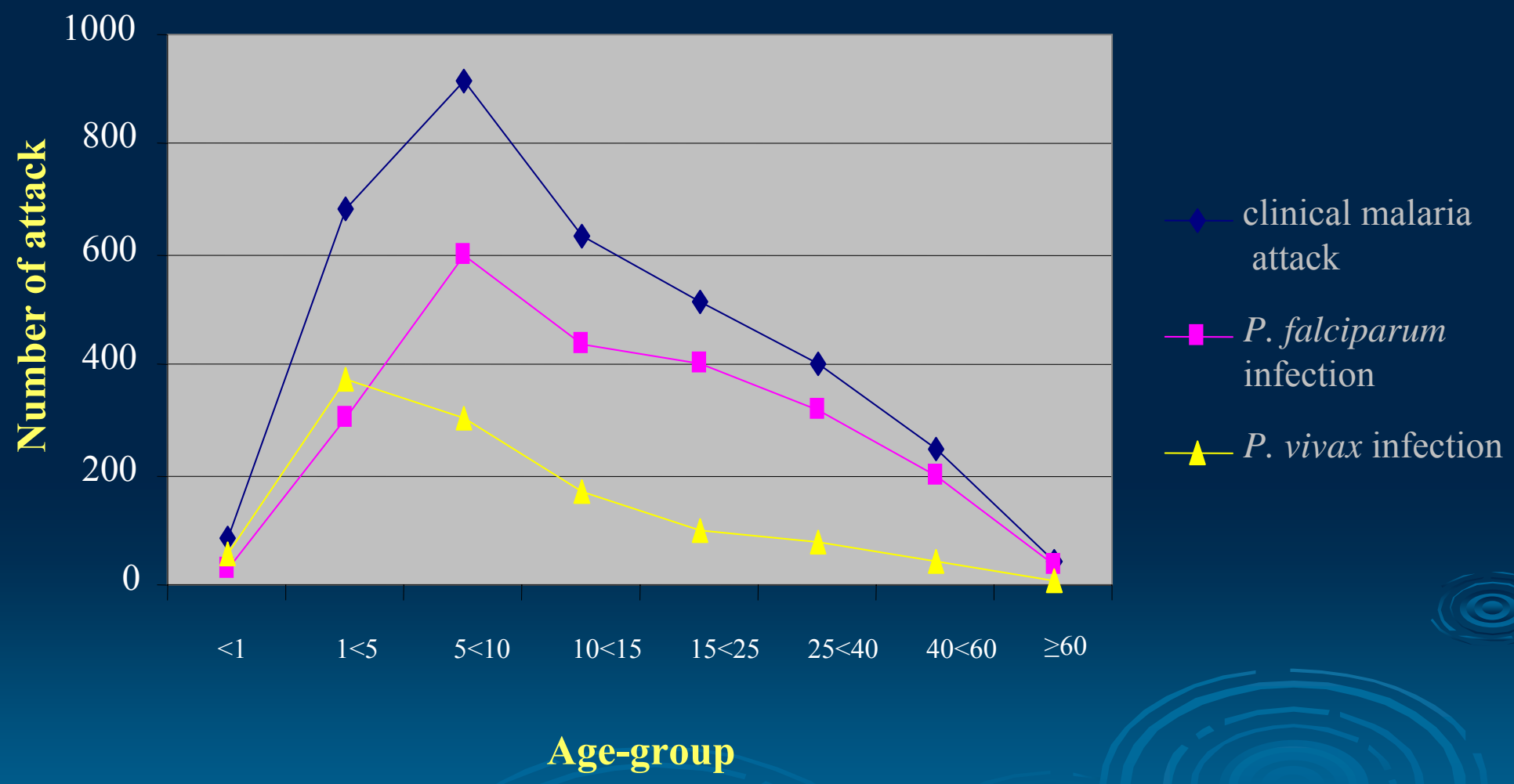
❖ From 3,484 individuals, 1,638 (47%) experienced at least 1 clinical attack

❖ Average number of attacks per person was 1.24

❖ The incident rate 2.4 cases per 1000 person-year in *P. falciparum* and 1.1 cases per 1000 person-year in *P. vivax*

Passive case detection (1998-2005)

Number of clinical malaria attack among age-groups



RESULTS

Correlation between number of clinical attacks, *P. falciparum* and *P. vivax* attacks between <10 and ≥ 10 age-groups

- ❖ The RR of no. of clinical attacks was **1.2** (95%CI 1.12, 1.28) and
The RR of no. of *P. vivax* infections was **2.34** (95%CI 2.06, 2.66) in
children <10 yrs old times **greater than** the age ≥ 10 yrs old.
- ❖ The RR of no. of *P. falciparum* infection (RR 0.94 95%CI 0.86, 1.03, NS).

Estimation of heritability according to no. of clinical attacks, *P. falciparum* and *P. vivax* infections, after adjusted for confounding factors by using Negative binomial regression models

Trait	Model*											
	1		2		3		4		5		6	
	h^2 (SE)	<i>p</i> -value	h^2 (SE)	<i>p</i> -value	h^2 (SE)	<i>p</i> -value	h^2 (SE)	<i>p</i> -value	h^2 (SE)	<i>p</i> -value	h^2 (SE)	<i>p</i> -value
No. of clinical attack	0.23 (0.04)	5.47x10 ⁻¹⁴	0.23 (0.04)	2.15x10 ⁻¹⁴	0.10 (0.03)	4.65x10 ⁻⁵	0.10 (0.03)	9.33x10 ⁻⁵	0.10 (0.04)	4.53x10 ⁻⁴	0.11 (0.04)	2.98x10 ⁻⁴
No. of <i>Pf</i> attack	0.22 (0.04)	2.11x10 ⁻¹³	0.23 (0.04)	2.47x10 ⁻¹⁴	-	-	-	-	0.21 (0.04)	7.74x10 ⁻¹³	0.22 (0.04)	1.12x10 ⁻¹³
No. of <i>Pv</i> _{28d} attack	0.08 (0.03)	0.002	0.08 (0.03)	0.004	0.08 (0.03)	0.004	0.07 (0.03)	0.008	-	-	-	-
No. of <i>Pv</i> _{90d} attack	0.07 (0.03)	0.005	0.07 (0.03)	0.007	0.07 (0.03)	0.011	0.06 (0.03)	0.015	-	-	-	-

- Covariates in each model are as follows. Number of clinical attack traits;
- model 1: hamlet, age-group, year and sex;
- model 2: hamlet, age-group and year;
- model 3: no. of *P. falciparum* infection, hamlet, age-group, year and sex;
- model 4: no. of *P. falciparum* infection, hamlet, age-group and year;
- model 5: no. of *P. vivax* infection, hamlet, age-group, year and sex;
- model 6: no. of *P. vivax* infection, hamlet, age-group and year.

Estimation of heritability according to mean and maximum trophozoite and gametocyte density of *P. falciparum* and *P. vivax* after adjusted for confounding factors by using Log₍₁₀₎ linear regression models

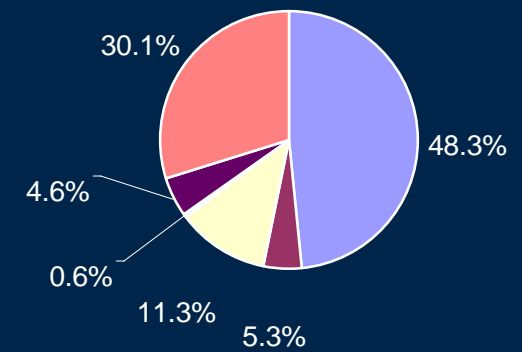
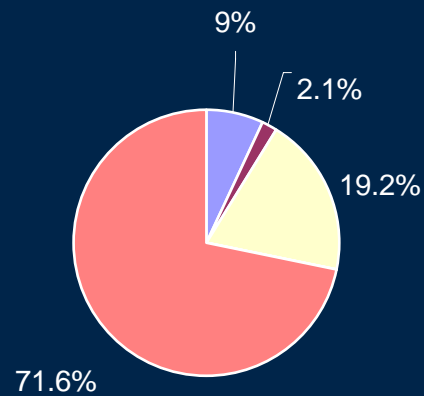
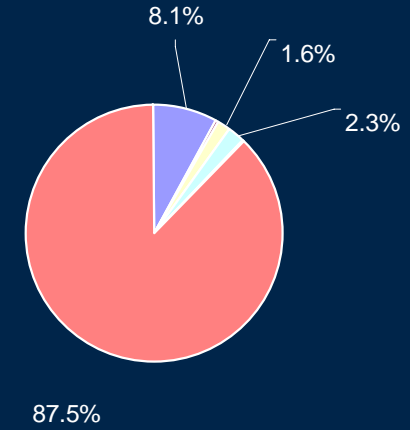
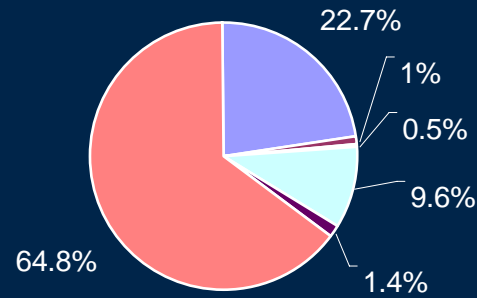
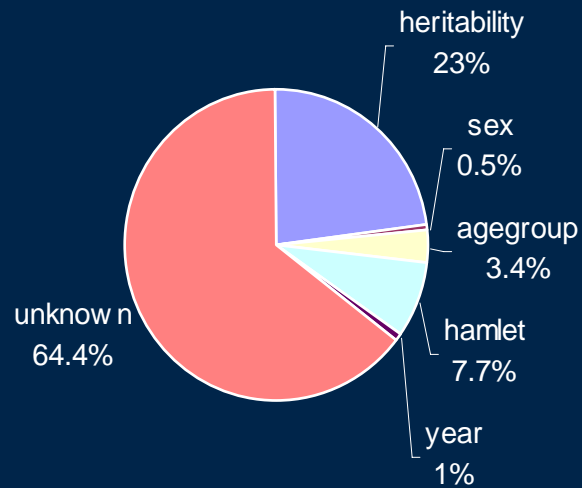
Trait	<i>P.falciparum</i>				<i>P.vivax</i> _{28d}				<i>P.vivax</i> _{90d}			
	Model*				Model*				Model*			
	1		2		1		2		1		2	
	<i>h</i> ² (SE)	<i>p</i> -value	<i>h</i> ² (SE)	<i>p</i> -value	<i>h</i> ² (SE)	<i>p</i> -value	<i>h</i> ² (SE)	<i>p</i> -value	<i>h</i> ² (SE)	<i>p</i> -value	<i>h</i> ² (SE)	<i>p</i> -value
Trophozoite density (Log₁₀)												
Max trophozoite density	0.07 (0.06)	NS	0.07 (0.06)	NS	0.46 (0.14)	2.71x10 ⁻⁴	0.52 (0.14)	3.92x10 ⁻⁵	0.48 (0.14)	1.5x10 ⁻⁴	0.50 (0.14)	7.5x10 ⁻⁵
Mean trophozoite density	0.07 (0.06)	NS	0.07 (0.06)	NS	0.48 (0.14)	1.61x10 ⁻⁴	0.53 (0.14)	2.73x10 ⁻⁵	0.48 (0.14)	1.6x10 ⁻⁴	0.50 (0.14)	7.5x10 ⁻⁵
Gametocyte density (Log₁₀)												
Max gametocyte density	0.47	NS	0.09 (0.06)	NS	NE	-	NE	-	NE	-	NE	-
Mean gametocyte density	0.60 (0.36)	0.044	0.60 (0.36)	0.045	NE	-	NE	-	NE	-	NE	-
Gametocyte density (Log₁₀ +1)												
Max gametocyte density	0.09 (0.06)	0.044	0.09 (0.06)	0.036	0.16 (0.13)	NS	0.17 (0.13)	NS	0.12 (0.12)	NS	0.12 (0.12)	NS
Mean gametocyte density	0.09 (0.06)	0.039	0.10 (0.06)	0.031	0.17 (0.13)	NS	0.18 (0.13)	NS	NE	NS	0.14 (0.13)	NS

- Covariates in each model are as follows. Trophozoite and gametocyte density traits:
- model 1, hamlet, age-group, year and sex; model 2, hamlet, age-group and year

Number of *P. falciparum* infection

Number of *P. vivax* infection

Heritability to clinical malaria



P. falciparum gametocyte density

P. vivax trophozoite density

LIMITATIONS

1. Because of our cohort study has been conducted during a long period, so we could not control all of the environmental factors that were related to changes over time and we also could not adjust all of confounding factors from our phenotypes.
2. The h^2 to clinical malaria only estimated the genetic contributions that act additively but some known resistance genes (Williams *et al.*, 2005) and variability in parasite virulence genes interacts with host genetic polymorphisms (Kwiatkowski, 2005), will not contribute to the h^2 estimation here.

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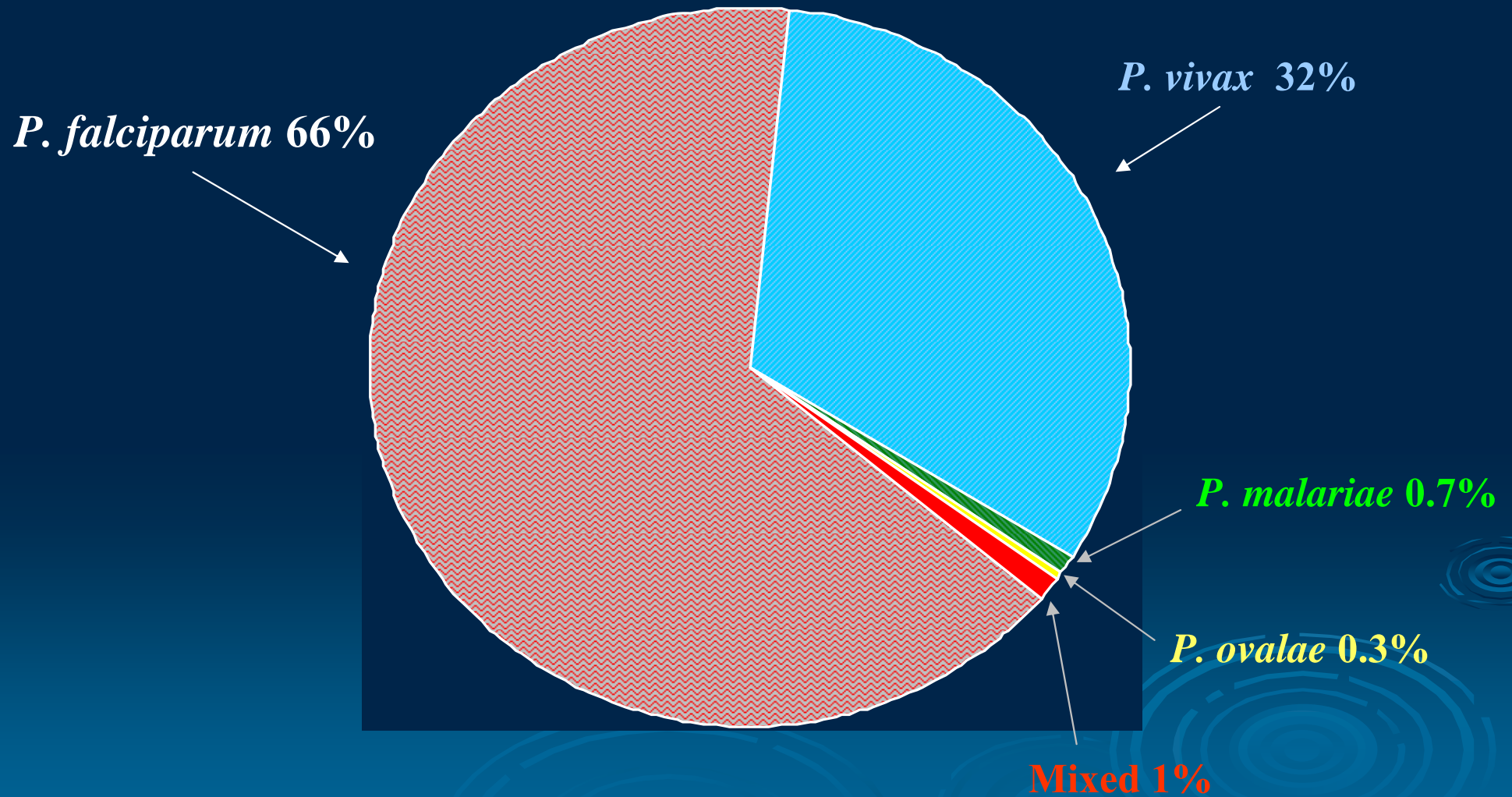
(RGJ) Thailand Research Fund

Biotech



Thank you

Proportion of malaria cases by *Plasmodium* spp.



Number of clinical malaria episodes by years during 1998-2005

Year	Person ^a	Episode ^b	Clinattack ^c (%)	Pf ^d (%)	Pv ^e (%)	Pm ^f	Po ^g	Mixed ^h
1998	760	1487	451 (30.3)	298 (66.0)	140 (31.0)	2	6	5
1999	1258	3482	921 (26.5)	580 (63.0)	319 (34.6)	6	2	14
2000	1795	4170	950 (22.8)	621 (65.4)	298 (31.4)	7	0	24
2001	1903	4167	785 (18.8)	521 (66.4)	255 (32.5)	4	1	4
2002	1538	2917	500 (17.1)	334 (66.8)	164 (32.8)	2	0	0
2003	1339	2246	325 (14.5)	226 (69.5)	94 (28.9)	4	1	0
2004	1370	2421	364 (15.0)	243 (66.8)	113 (31.0)	6	1	1
2005	146	151	7 (4.6)	2 (28.6)	5 (71.4)	0	0	0
All	10109	21041	4303	2825	1388	31	11	48

^a Number of person, ^bmalaria episode, ^cclinical malaria attack, ^d*P. falciparum*, ^e*P. vivax*, ^f*P. malariae*, ^g*P. ovalae* and ^hmixed infection respectively.

Number of clinical malaria episodes among 7 hamlets

Hamlet	Episode ^a	Clinattack ^b (%)	Pf ^c	Pv ^d	Pm ^e	Po ^f	Mixed ^g
1 (A)	6736	1059 (15.7)	642	393	9	1	14
2 (B)	1123	353 (31.4)	238	108	3	1	3
3 (C)	2740	607 (22.1)	431	161	6	2	7
4 (D)	1780	370 (20.8)	246	115	3	2	4
5 (E)	2686	680 (25.3)	438	229	2	2	9
6 (F)	2782	556 (20.0)	363	184	2	2	5
7 (G)	3194	678 (21.2)	467	198	6	1	6
All	21041	4303	2825	1388	31	11	48

^a Number of malaria episode, ^bclinical malaria attack, ^c*P. falciparum*, ^d*P. vivax*, ^e*P. malariae*, ^f*P. ovalae* and ^gmixed infection respectively.

Number of clinical malaria attacks due to sex

Sex	Episode ^a	Clinattack ^b (%)	Pf ^c	Pv ^d	Pm ^e	Po ^f	Mixed ^g
1 (Male)	9330	2181 (50.7)	1451	685	18	6	21
2 (Female)	7497	1341 (31.2)	865	451	6	3	16
9 (unknown)	4214	781 (18.1)	509	252	7	2	11
All	21041	4303	2825	1388	31	11	48

^a Number of malaria episode, ^bclinical malaria attack, ^c*P. falciparum*, ^d*P. vivax*, ^e*P. malariae*, ^f*P. ovalae* and ^gmixed infection respectively.

Estimation of the genetic contribution to the number of clinical malaria attacks and blood-stage parasite density among Karen in Ratchaburi, Thailand

Trait	No. of records ^a	Percentage of Genetic h^2 (SE)					
		Model*					
		1A	2A	3A	4A	5A	6A
Number of clinical attack							
All species	1623	23(4)	23(4)	10(3)	10(3)	10(4)	11(4)
<i>P. falciparum</i>	1623	22(4)	23(4)	-	-	21(4)	22(4)
<i>P. vivax</i>	1623	8(3)	8(3)	8(3)	7(3)	-	-
<i>P. vivax</i> (90 days)	1623	7(3)	7(3)	7(3)	6(3)	-	-

^a Number of records recruited from 2,027 individuals of total pedigrees

* Covariates in each model are as follows. Number of clinical attack traits: model 1A, hamlet, age-group, year and sex; model 2A, hamlet, age-group, year; model 3A, number of *P. falciparum* infection, hamlet, age-group, year and sex; model 4A, number of *P. falciparum* infection, hamlet, age-group, year; model 5A, number of *P. vivax* infection, hamlet, age-group, year and sex; model 6A, number of *P. vivax* infection, hamlet, age-group, year.

Estimation of the genetic contribution to the number of clinical malaria attacks and blood-stage parasite density among Karen in Ratchaburi, Thailand (cont.)

Trait	No. of records ^a	Percentage of Genetic h^2 (SE)			
		Model*			
		1B	2B	3B	4B
Trophozoite density					
<i>P. falciparum</i>	868	NE	NE	NE	NE
<i>P. vivax</i>	475	46(14)	52(14)	48(14)	53(14)
<i>P. vivax</i> (90 days)	475	48(14)	50(14)	48(14)	50(14)
Gametocyte density					
Negative results excluded					
<i>P. falciparum</i>	92	NE	NE	60(36)	60(36)
<i>P. vivax</i>	206	NE	NE	NE	NE
<i>P. vivax</i> (90 days)	206	NE	NE	NE	NE
Negative results included					
<i>P. falciparum</i>	868	9(6)	9(6)	9(6)	10(6)
<i>P. vivax</i>	475	NE	NE	NE	NE
<i>P. vivax</i> (90 days)	475	NE	NE	NE	NE

^a Number of records recruited from 2,027 individuals of total pedigrees

* Covariates in each model are as follows. Trophozoite and gametocyte density traits: model 1B, maximum parasite density by hamlet, age-group, year and sex; model 2B, maximum parasite density by hamlet, age-group and year; model 3B, mean parasite density by hamlet, age-group, year and sex; model 4B, mean parasite density by hamlet, age-group and year.