

SIMIAN MALARIA IN MALAYSIA WITH SPECIAL REFERENCE TO *PLASMODIUM KNOWLESI*

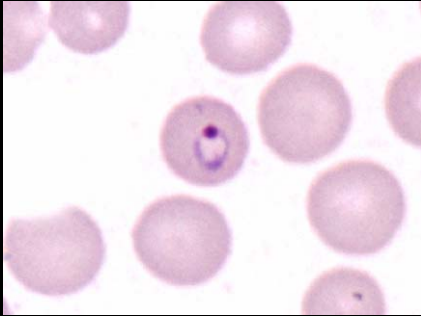
***Indra Vythilingam, Parasitology Unit,
Institute for Medical Research,
Kuala Lumpur. Malaysia***



INTRODUCTION

- 1st natural infection of *P. knowlesi* in humans was reported from Pahang - 1965
- 2nd case was reported from Johore in 1971.

Plasmodium knowlesi



Macaca fascicularis, *M. nemestrina*, *Presbytis melalophos*

Vectors – Peninsular Malaysia

Anopheles hackeri

- natural vector of *P. knowlesi*
- highly zoophagic

Anopheles cracens (as *An. balabacensis*)

- vector of *P. inui* and *P. cynomolgi*

Anopheles latens (as *An. leucosphyrus*)

- vector of *P. inui*

REF: Wharton *et al* 1961, 1962; Cheong *et al* 1965

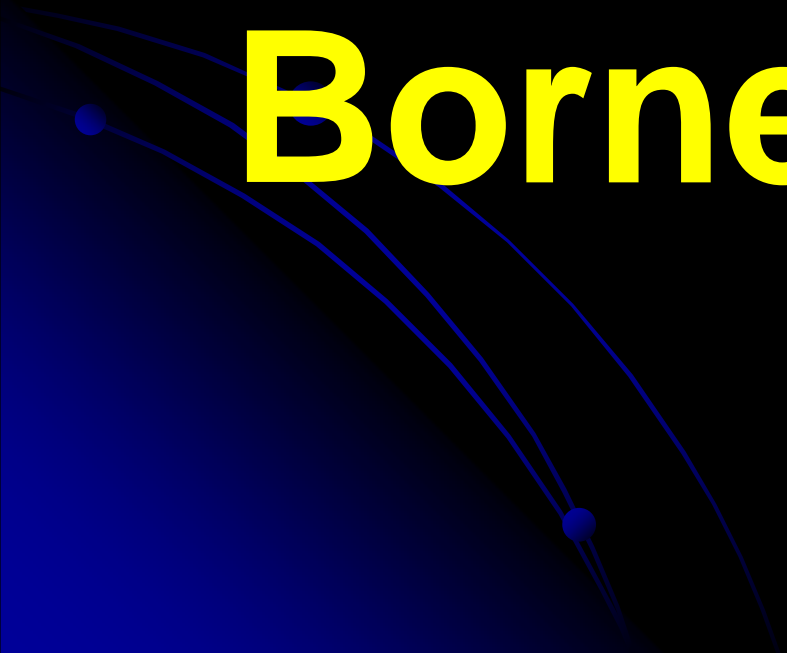
INTRDOUCTION

.In 2004 Singh *et al* reported a large focus of *P. knowlesi* in Sarawak Malaysian Borneo

(Lancet 2004)

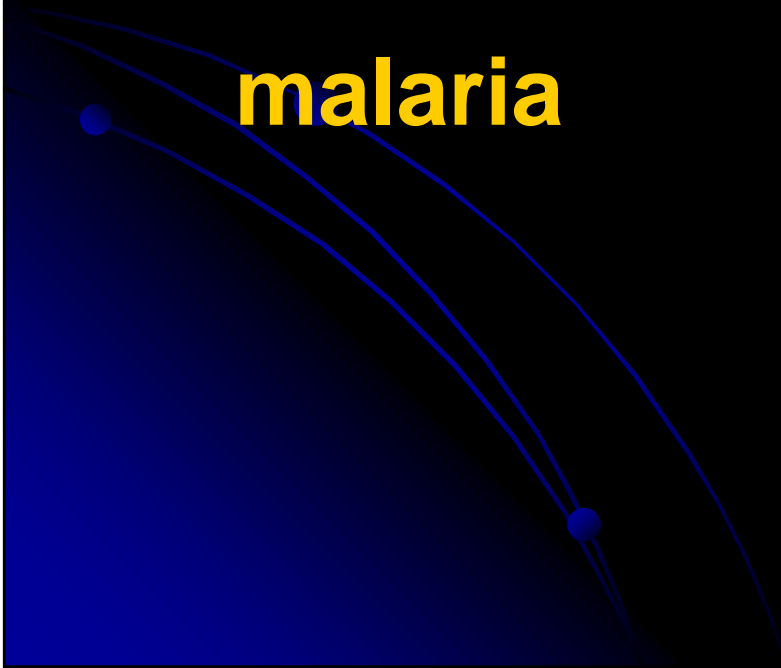


**Is *P. knowlesi*
occurring only
in Malaysian
Borneo ?**




GENERAL OBJECTIVES

- **To study simian malaria in humans and non human primates**
- **To elucidate the vectors of simian malaria**



MATERIALS & METHODS

- **Samples received since July 2005**
 - **Extraction of DNA from whole blood or blood film**
 - **Nested PCR following protocol of Singh et al 2004**
- 

Species specific primers used in the Nested-PCR Assay

rPLU1&5

Human malaria

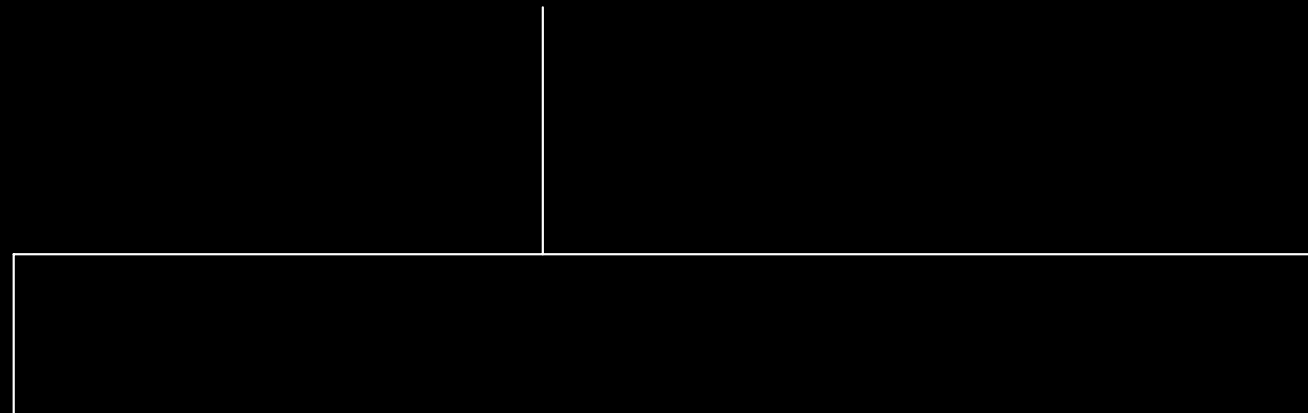
P. falciparum

P. vivax

P. malariae

Simian malaria

P. knowlesi



CASES BY MICROSCOPY AND PCR JULY 2005-DEC. 2007

	Cases detected by microscopy					Cases detected by PCR
PCR results	Pf	Pv	Pm	Pf+Pm	Pm+Pv	
Pf	4	1	1			6
Pv		6	3	1	1	11
Pm			6			6
Pk	2	1	58			61
Pf+Pm			1			1
Pf+Pk			1			1
Pv+Pk		1	1			2
Pk+Pm			2			2
Total	6	9	73	1	1	90

2008
Jan- Aug.

	MALARIA CASES BY MICROSCOPY							
PCR	Pf	Pv	Pm	Pf+Pv	Pm+Pv	Pf+Pm	Negative	Cases by PCR
Pf	2		2			1		5
Pv		4	12		1		5	22
Pm			2					2
Pk	3		40	2		1		46
Pf+Pv				1				1
Pf+Pk	1		2	1		1		5
Pv+Pk			12	1	1		1	15
Pm+Pk			1					1
Pv+Pf+Pk							1	1
Pf+Pm+Pk						1		1
Total	6	4	71	5	2	4	7	99

CASES OF *P. KNOWLESI* IN P. MALAYSIA



NON HUMAN PRIMATES





Species specific primers used in the Nested-PCR Assay

rPLU1&5

Simian malaria

P. knowlesi

P. coatneyi

P. inui

P. fieldi

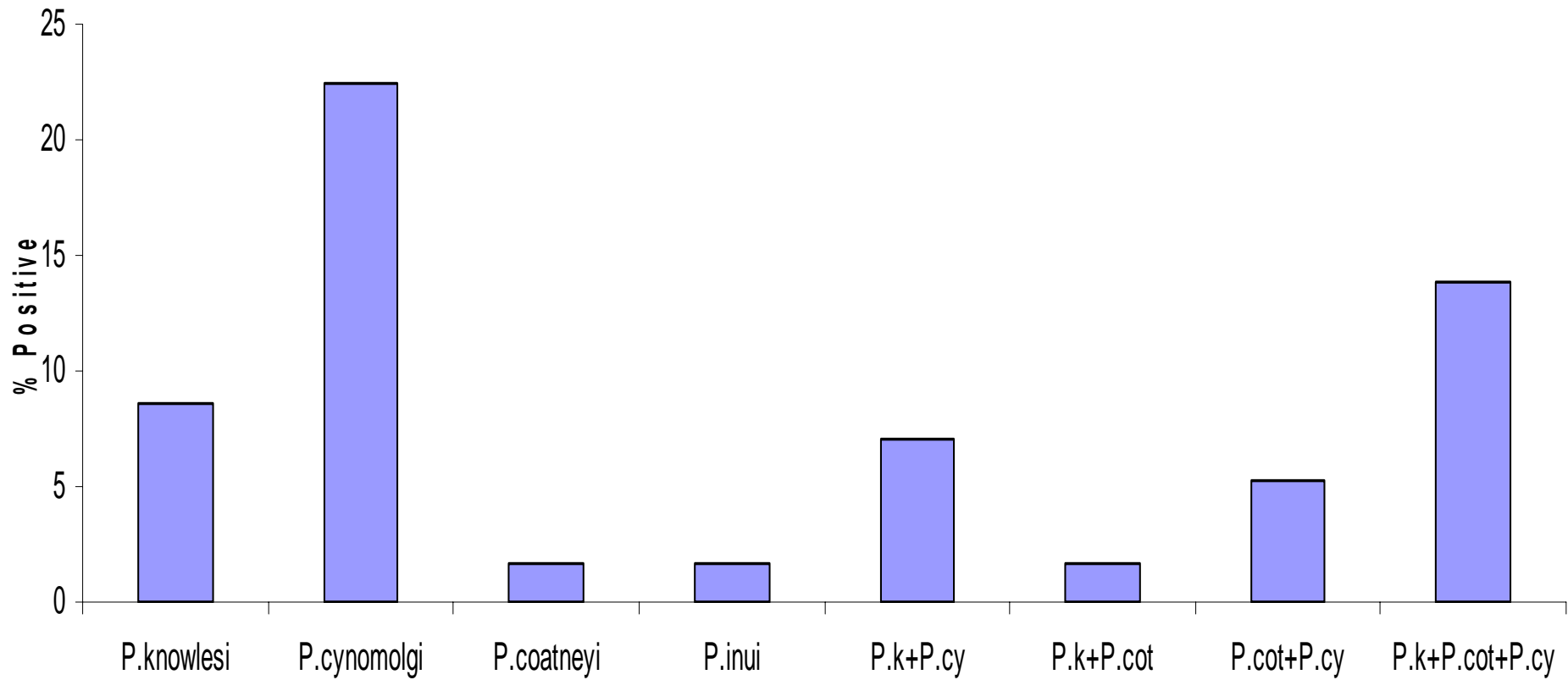
P. cynomolgi

P. fragile

RESULTS OF MONKEY SAMPLES

Locality	Samples collected	BFMP Positive (%)	PCR Positive Genus (%)
Selangor	60	0	0 (0)
Kuala Lipis	104	102 (98.1)	95 (91.3)
Temerloh	11	11 (100)	11 (100)
Kuala Lumpur	29	2 (6.9)	2 (6.9)
Total	214	115 (56.7)	108 (53.2)

Malaria Species in Monkeys by PCR



MOSQUITO COLLECTION STUDY SITE





05/09/2007





14/08/2007





15/08/2007



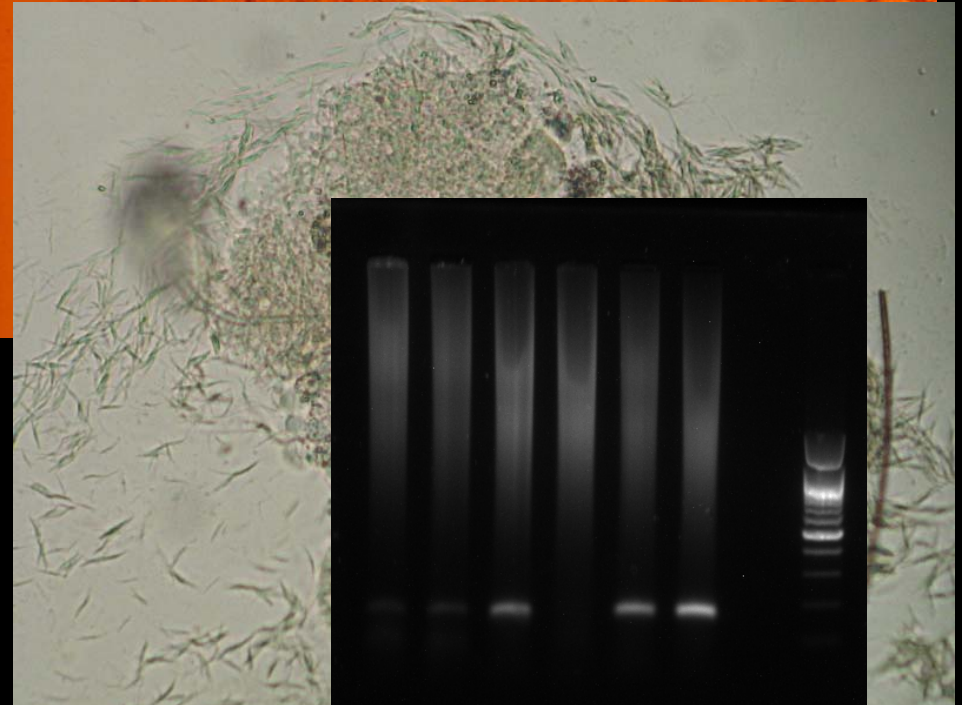
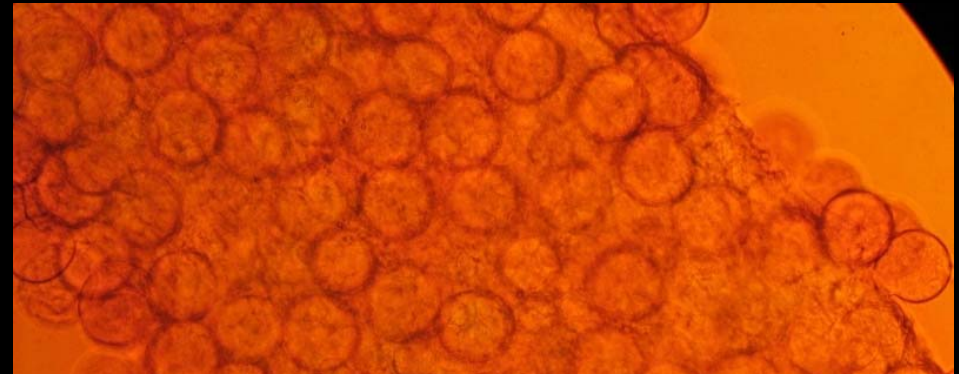
15/08/2007

RESULTS

Predominant *Anopheles* species in study site Aug 2007-Aug 2008

Anopheles species	Mela		Sg Ular	Total (%)
	BLC	MBT	BLC	
<i>An. aconitus</i>	0	0	6	6 (0.46)
<i>An. barbirostris</i> gr	10	11	4	25 (1.93)
<i>An. cracens</i>	186	77	584	847 (65.55)
<i>An. hyrcanus</i> gr	34	15	1	50(3.86)
<i>An. kochi</i>	2	51	1	54 (4.17)
<i>An. maculatus</i>	99	1	163	263 (20.35)
<i>An. phillippinenssis</i>	6	0	6	12 (0.92)
<i>An. pujutensis</i>	0	1	0	1 (0.07)
<i>An. separatus</i>	2	1	5	8 (0.61)
<i>An. tesselatus</i>	7	10	5	22 (1.70)
<i>An. umbrosus</i>	1	1	0	2 (0.15)
<i>An. vagus</i>	1	1	0	2 (0.15)
Total	348	169	775	1292

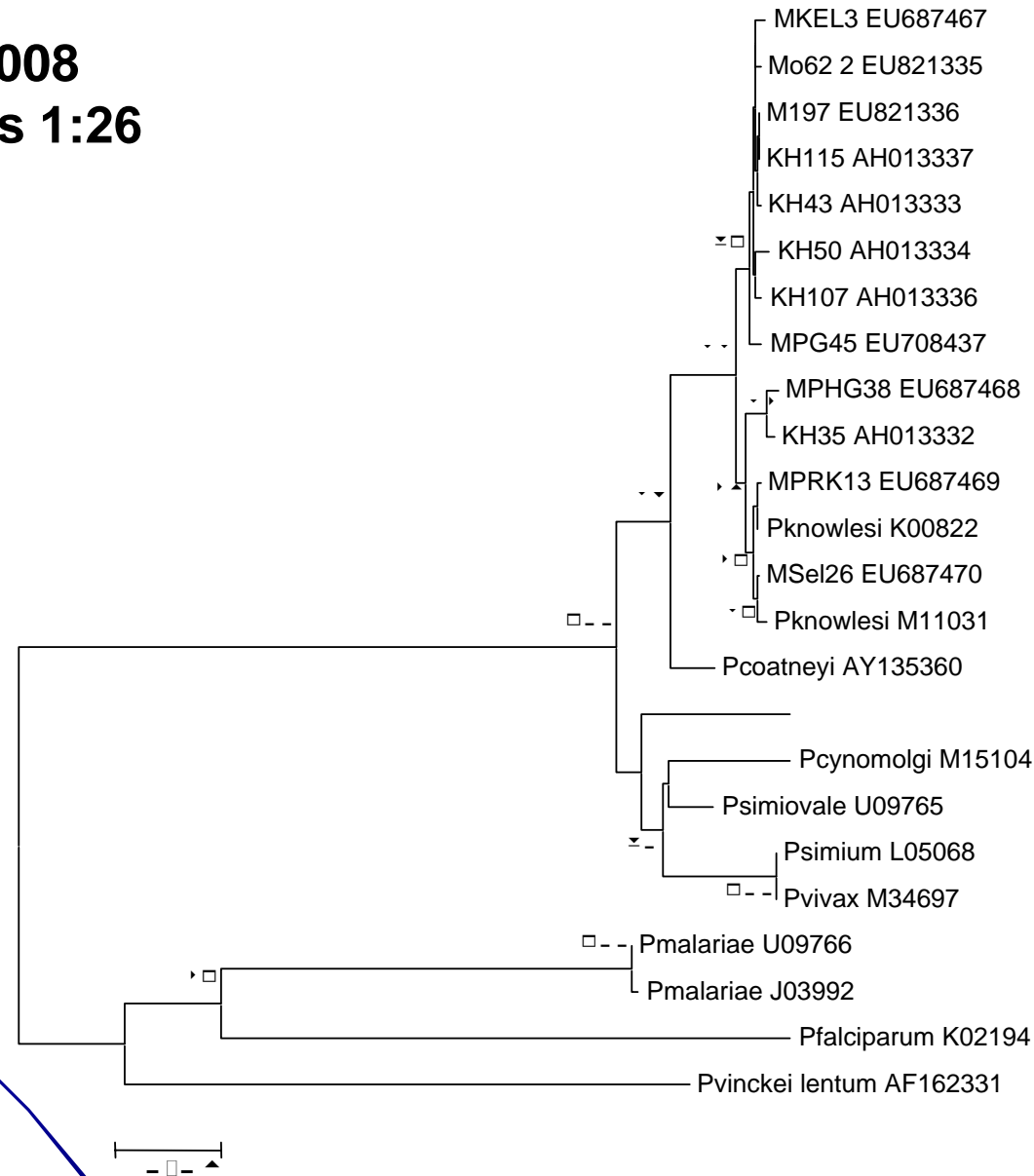
RESULTS





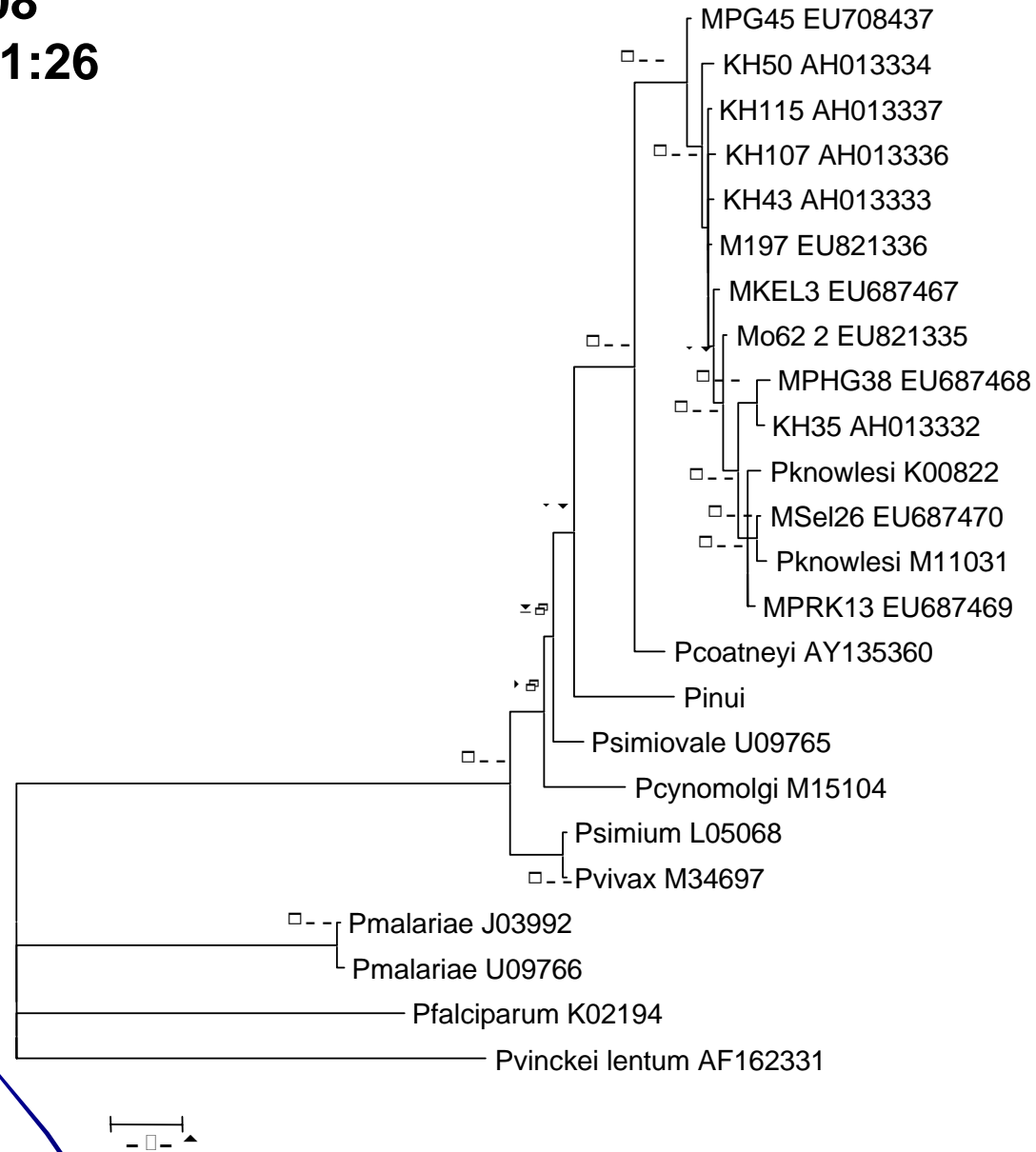
To verify species of *Plasmodium* found, cloning and sequencing of the circumsporozoite protein (*csp*) genes were conducted...

Vythilingam *et al* 2008
Parasites & Vectors 1:26



Phylogenetic tree based on the non-repeat region of the circumsporozoite (*csp*) genes of malaria parasites produced by the neighbor-joining method. Figures on the branches are bootstrap percentages based on 1000 replicates and only those 70 and above shown.

Vythilingam *et al* 2008
Parasites & Vectors 1:26

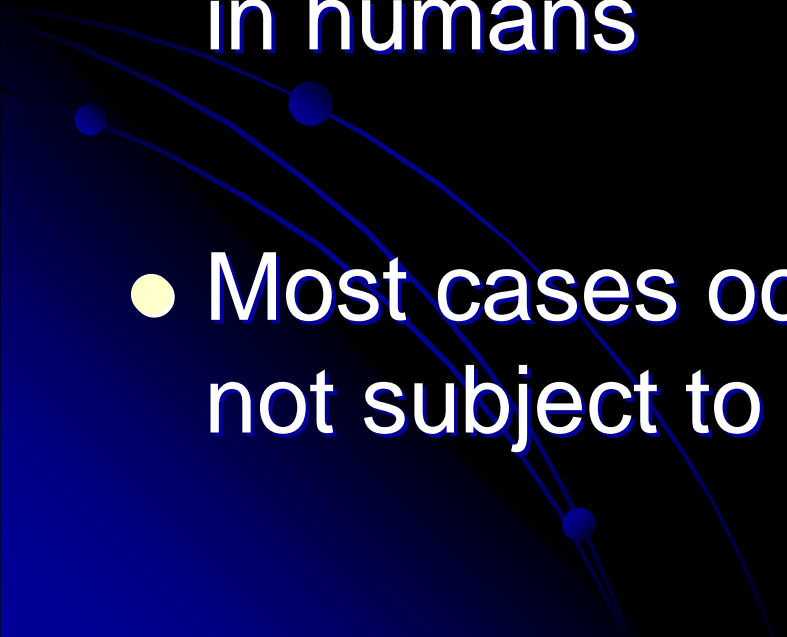


Phylogenetic tree based on the non-repeat region of the circumsporozoite (csp) genes of malaria parasites produced by the Bayesian method. Figures on the branches are the posterior probabilities from the Bayesian analysis.

DISCUSSION

- *Knowlesi* malaria occurs in this region (Jongwutiwes et al 2004, Zhu et al 2006, Cox –Singh et al 2008, Ng et al 2008, Luchavez et al 2008, Vythilingam et al 2008)
- With better molecular techniques one can differentiate between *P. malariae* and *P. knowlesi*

DISCUSSION

- Chin *et al* 1968: Experimental transmission studies
 - Due to declining anti-plasmodial immunity in humans
 - Most cases occurring in malaria free areas not subject to control activities
- 

DISCUSSION

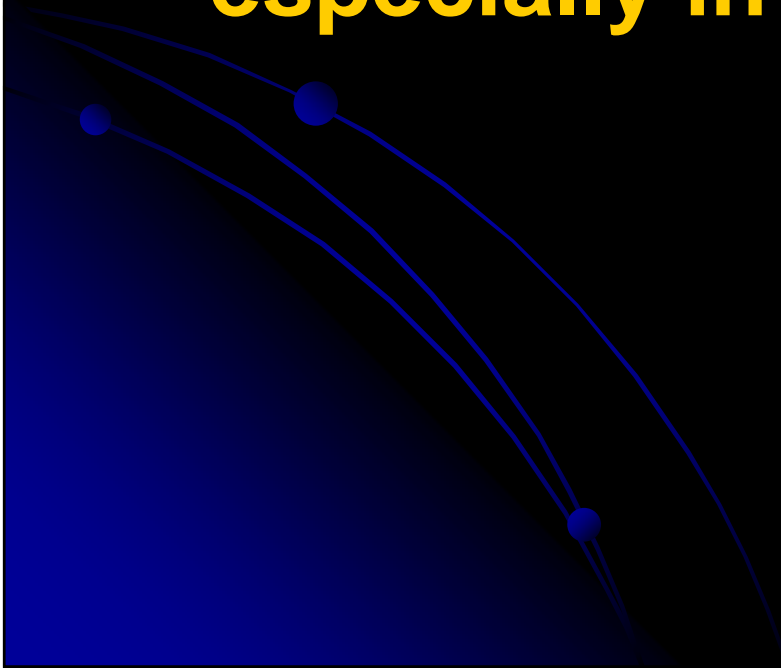
- This study has shown a link between *An. cracens*, humans and monkeys
- Early workers in Malaysia felt that simian malaria transmission to humans will be remote
- Development and deforestation-macaques close to human habitation

DISCUSSION


- In the rain forests of Southeast Asia natural hosts of *P. knowlesi* – macaques abound so does the *An. leucosphyrus* group of mosquitoes
- Current control strategies are not going to work. Zoonotic transmission has to be widely publicized

CONCLUSION

- **Fifth human malaria parasite has been established and thus new strategies for malaria control should be considered especially in the elimination of malaria**



ACKNOWLEDGEMENTS

- Grant from the NIH, MOH Malaysia
 - Staff from Vector Borne Disease Control Program Kuala Lipis, Pahang
 - Wildlife Department, Malaysia
- 





THANK YOU