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National Center for Global Health and Medicine

Experience with Malaria-LAMP in Laos and Haiti:
Importance of submicroscopic/asymptomatic malaria detection toward malaria elimination

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91 countries and territories are endemic

216 million episodes (90% in s-S Africa, 3% in S-E Asia)

445,000 death (91% in s-S Africa, 6% in S-E Asia),

estimated in **2016**

TABLE 6.4.

Estimated number of malaria deaths by WHO region, 2010–2016 *Source: WHO estimates*

	Number of deaths						
	2010	2011	2012	2013	2014	2015	2016
African	538 000	484 000	445 000	430 000	423 000	409 000	407 000
Eastern Mediterranean	7 200	7 100	7 700	7 800	7 800	7 600	8 200
European	0	0	0	0	0	0	0
Americas	830	790	630	620	420	450	650
South-East Asia	41 700	34 000	29 000	22 000	25 000	26 000	27 000
Western Pacific	3 800	3 300	4 000	4 300	2 900	2 600	3 300
World	**591 000**	**529 000**	**487 000**	**465 000**	**459 000**	**446 000**	**445 000**

Challenges to elimination

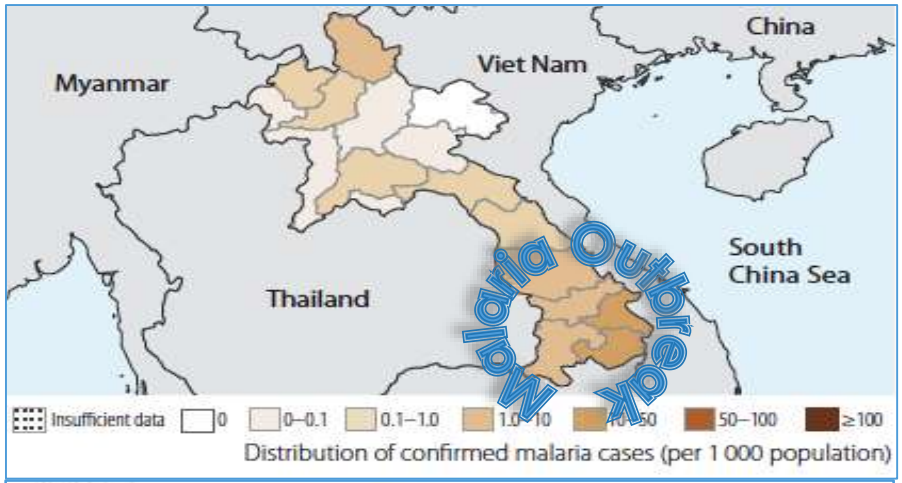
- Asymptomatic reservoir
- Drug resistance
- Vivax hypnozoites

- Exophilic (outdoor) biting vectors

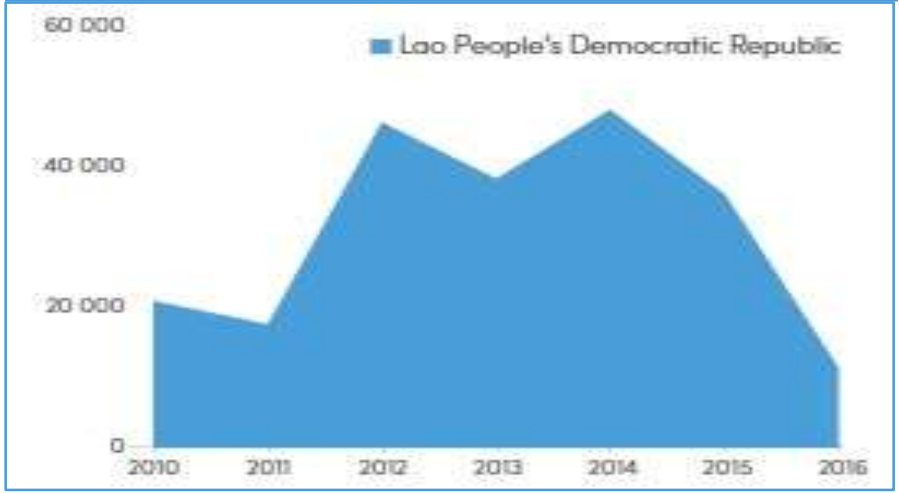
- Movement and migration
- Logistics in remote areas

Science and Technology Research Partnership for Sustainable Development (SATREPS)

- Number of cases: **11,753** (2016)
- Number of death: **1**(2016)
- Reported outbreaks in south provinces caused by **migration** of people
- Emerence and spread of **artemisinin resistance**



Institut Pasteur du Laos (IPL)
Lao-Japan Joint Parasitology Laboratory



SATREPS
Science and Technology Research Partnership for Sustainable Development Program

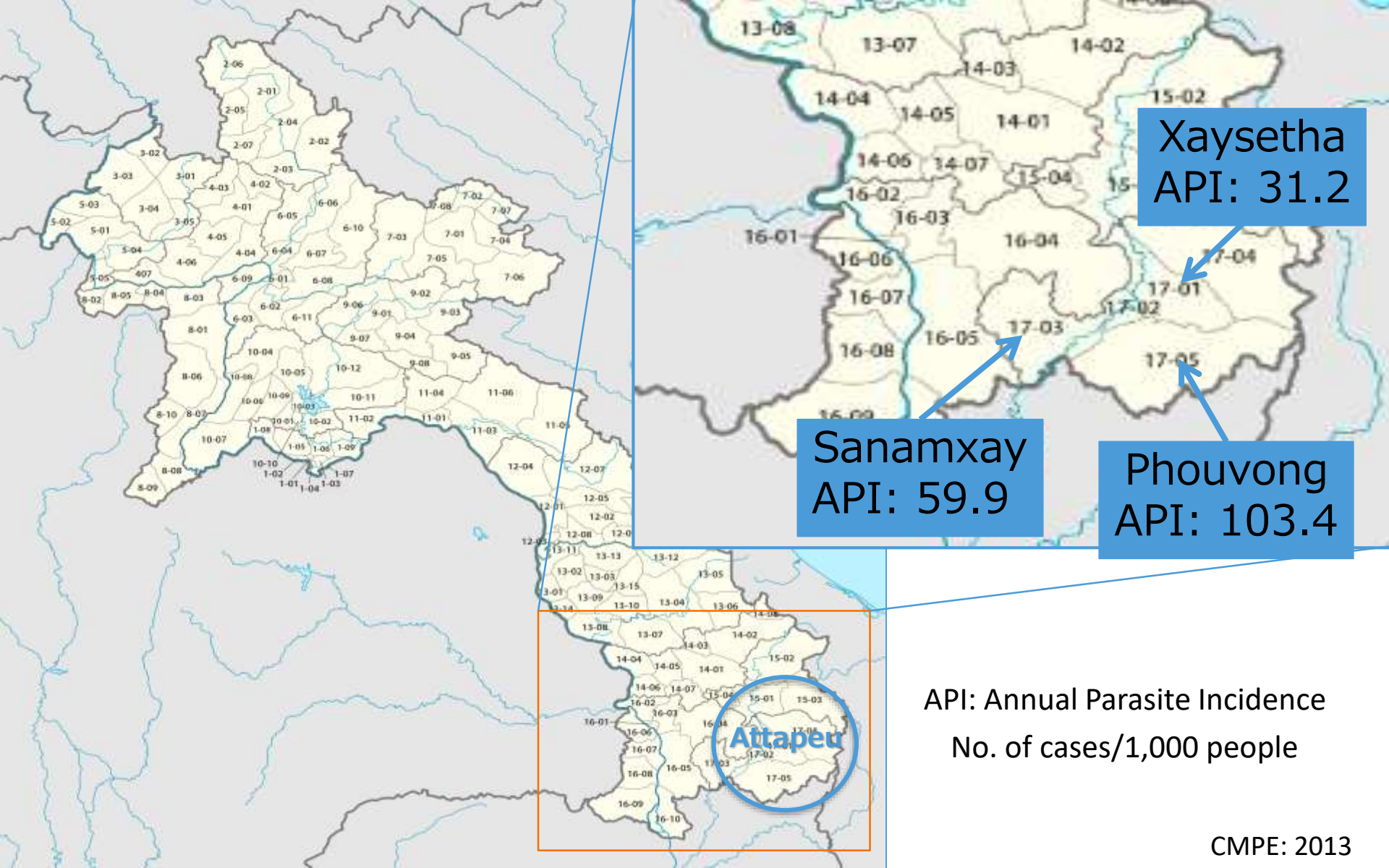
Ministry of Health

INSTITUT PASTEUR DU LAOS

JICA

AMED

NCGM
National Center for Global Health and Medicine



API: Annual Parasite Incidence

No. of cases/1,000 people

CMPE: 2013

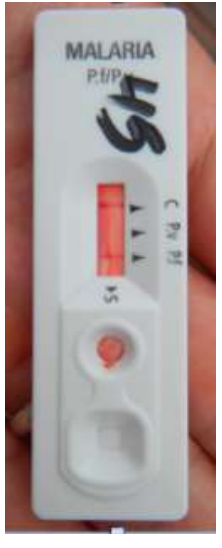
Study population

- 381 healthy inhabitants
- female 193、male 188
- Average age=29（1～90yo)
- Occupation：72% of them are farmers
- Average BT ：36.7°C

Diagnosis

Field

- **Rapid diagnostic test (RDT)**
  Malaria Ag *Pf*/*Pv*, Standard Diagnostics, Inc. Korea
  HRP-II Antigen (*Pf*), pLDH Antigen (*Pv*)



IPL

- **Microscopy**
- **PCR: Nested Real-Time PCR** (*cytochrome b* gene)
  SaoAdvanced™ Universal CYBR Green Supermix, BioRad
  Primary real-time PCR: universal primer set
  Secondary real-time PCR: species specific primer sets (*Pf*/*Pv*)

District	village	Age	Sex	Body tem.	Microscopy	
					P.f	*P.v*
Saysetha	Hard Sun	60	M	36.6		
Saysetha	Hard Sun	48	M	36.9		
Phouvong	Vong Say	27	M	36.5	+	
Phouvong	Vong Say	26	M	36.0		+
Phouvong	Vong Say	35	M	36.4		
Phouvong	Vong Say	29	F	36.9		
Phouvong	Vong Say	23	F	36.7		
Phouvong	Vong Say	45	F	36.3		
Phouvong	Vong Say	30	M	36.4		
Phouvong	Vong Say	25	F	36.5		
Phouvong	Vong Say	24	M	36.6		
Phouvong	Vong Say	47	M	37.1		
Phouvong	Vong Say	41	M	36.7		
Phouvong	Vong Say	14	M	36.9		
Sanamxay	Som Poi	22	M	36.9		
Sanamxay	Som Poi	23	M	36.6		+
Sanamxay	Som Poi	34	M	36.7		
Sanamxay	Som Poi	22	M	36.3		
Sanamxay	Som Poi	22	M	36.7		
Sanamxay	Som Poi	14	M	37.0		
Sanamxay	Som Poi	15	M	36.7		
Sanamxay	Som Poi	21	F	36.9		
Sanamxay	Som Poi	22	M	36.9		
Sanamxay	Som Poi	30	M	36.8		
Sanamxay	Som Poi	18	M	36.8		
Sanamxay	Som Poi	24	M	37.1		
Sanamxay	Som Poi	30	M	36.5		
Sanamxay	Som Poi	29	M	36.6		
Sanamxay	Som Poi	42	F	36.8		
Sanamxay	Som Poi	16	M	36.2		
Sanamxay	Som Poi	17	M	37.0		
Sanamxay	Som Poi	41	M	36.3		
Sanamxay	Som Poi	13	M	36.7		
Sanamxay	Som Poi	23	M	36.6		
Sanamxay	Som Poi	46	M	36.8		
Sanamxay	Som Poi	24	M	36.4		
Sanamxay	Som Poi	36	M	36,7		
Sanamxay	Som Poi	57	M	36.7		
Sanamxay	Som Poi	33	M	36.9		
Sanamxay	Som Poi	20	M	36.8		
Sanamxay	Som Poi	15	M	36.9		
Sanamxay	Som Poi	35	M	36.9		

Results of Microscopy, RDT and Real-Time PCR

381 Samples

Microscopy: 3 +

RDT:

RT-PCR:

RDT: Rapid Diagnostic Test

P.f* : *P. falciparum

P.v* : *P. vivax

District	village	Age	Sex	Body tem.	Microscopy		RDT	
					P.f	*P.v*	*P.f*	*P.v*
Saysetha	Hard Sun	60	M	36.6				
Saysetha	Hard Sun	48	M	36.9				
Phouvong	Vong Say	27	M	36.5	+		+	
Phouvong	Vong Say	26	M	36.0		+		+
Phouvong	Vong Say	35	M	36.4				
Phouvong	Vong Say	29	F	36.9				
Phouvong	Vong Say	23	F	36.7				
Phouvong	Vong Say	45	F	36.3				
Phouvong	Vong Say	30	M	36.4				
Phouvong	Vong Say	25	F	36.5				
Phouvong	Vong Say	24	M	36.6				
Phouvong	Vong Say	47	M	37.1				
Phouvong	Vong Say	41	M	36.7				
Phouvong	Vong Say	14	M	36.9				
Sanamxay	Som Poi	22	M	36.9				
Sanamxay	Som Poi	23	M	36.6		+		+
Sanamxay	Som Poi	34	M	36.7				
Sanamxay	Som Poi	22	M	36.3				
Sanamxay	Som Poi	22	M	36.7				
Sanamxay	Som Poi	14	M	37.0				
Sanamxay	Som Poi	15	M	36.7				
Sanamxay	Som Poi	21	F	36.9				
Sanamxay	Som Poi	22	M	36.9			+	
Sanamxay	Som Poi	30	M	36.8				
Sanamxay	Som Poi	18	M	36.8				+
Sanamxay	Som Poi	24	M	37.1				
Sanamxay	Som Poi	30	M	36.5				
Sanamxay	Som Poi	29	M	36.6				
Sanamxay	Som Poi	42	F	36.8				
Sanamxay	Som Poi	16	M	36.2				
Sanamxay	Som Poi	17	M	37.0				
Sanamxay	Som Poi	41	M	36.3				
Sanamxay	Som Poi	13	M	36.7				
Sanamxay	Som Poi	23	M	36.6				
Sanamxay	Som Poi	46	M	36.8				
Sanamxay	Som Poi	24	M	36.4				
Sanamxay	Som Poi	36	M	36,7				
Sanamxay	Som Poi	57	M	36.7				
Sanamxay	Som Poi	33	M	36.9				
Sanamxay	Som Poi	20	M	36.8				
Sanamxay	Som Poi	15	M	36.9				
Sanamxay	Som Poi	35	M	36.9				

Results of Microscopy, RDT and Real-Time PCR

381 Samples

Microscopy: 3 +

RDT: 5 +

RT-PCR:

RDT: Rapid Diagnostic Test

P.f* : *P. falciparum

P.v* : *P. vivax

District	village	Age	Sex	Body tem.	Microscopy		RDT		Real-Time PCR	
					P.f	*P.v*	*P.f*	*P.v*	*P.f*	*P.v*
Saysetha	Hard Sun	60	M	36.6						+
Saysetha	Hard Sun	48	M	36.9						+
Phouvong	Vong Say	27	M	36.5	+		+		+	
Phouvong	Vong Say	26	M	36.0		+		+		+
Phouvong	Vong Say	35	M	36.4						+
Phouvong	Vong Say	29	F	36.9					+	
Phouvong	Vong Say	23	F	36.7						+
Phouvong	Vong Say	45	F	36.3						+
Phouvong	Vong Say	30	M	36.4						+
Phouvong	Vong Say	25	F	36.5					+	
Phouvong	Vong Say	24	M	36.6						+
Phouvong	Vong Say	47	M	37.1						+
Phouvong	Vong Say	41	M	36.7					+	
Phouvong	Vong Say	14	M	36.9						+
Sanamxay	Som Poi	22	M	36.9						+
Sanamxay	Som Poi	23	M	36.6		+		+		+
Sanamxay	Som Poi	34	M	36.7						+
Sanamxay	Som Poi	22	M	36.3						+
Sanamxay	Som Poi	22	M	36.7						+
Sanamxay	Som Poi	14	M	37.0						+
Sanamxay	Som Poi	15	M	36.7						+
Sanamxay	Som Poi	21	F	36.9						+
Sanamxay	Som Poi	22	M	36.9			+		+	
Sanamxay	Som Poi	30	M	36.8						+
Sanamxay	Som Poi	18	M	36.8				+		+
Sanamxay	Som Poi	24	M	37.1						+
Sanamxay	Som Poi	30	M	36.5						+
Sanamxay	Som Poi	29	M	36.6						+
Sanamxay	Som Poi	42	F	36.8						+
Sanamxay	Som Poi	16	M	36.2						+
Sanamxay	Som Poi	17	M	37.0						+
Sanamxay	Som Poi	41	M	36.3						+
Sanamxay	Som Poi	13	M	36.7						+
Sanamxay	Som Poi	23	M	36.6						+
Sanamxay	Som Poi	46	M	36.8						+
Sanamxay	Som Poi	24	M	36.4						+
Sanamxay	Som Poi	36	M	36,7						+
Sanamxay	Som Poi	57	M	36.7						+
Sanamxay	Som Poi	33	M	36.9						+
Sanamxay	Som Poi	20	M	36.8						+
Sanamxay	Som Poi	15	M	36.9						+
Sanamxay	Som Poi	35	M	36.9						+

Results of Microscopy, RDT and Real-Time PCR

381 Samples

Microscopy: 3 +

RDT: 5 +

RT-PCR: 42 +

***P.f*: 5 + *P.v*: 37 +**

Age: 29 years old (Ave.)

Male: 36

Female: 6

] ***P* < 0.05**

Body Temp.: 36.7°C (Ave.)

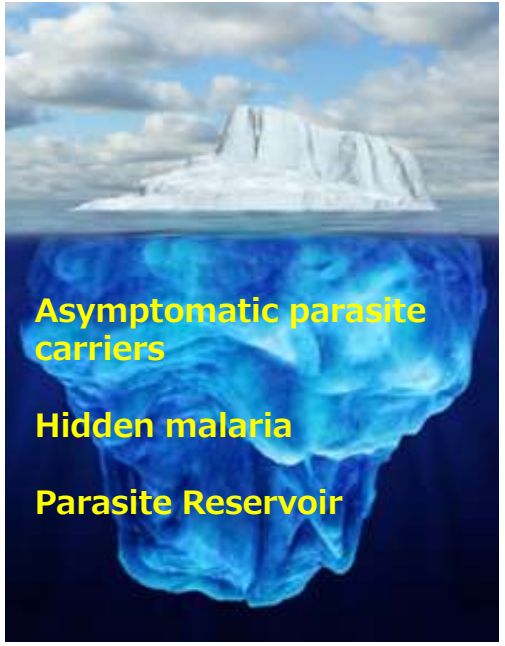
RDT: Rapid Diagnostic Test

P.f* : *P. falciparum

P.v* : *P. vivax

Asymptomatic individuals

- Unlikely to seek treatment
- Missed by passive surveillance
- Infectious to mosquitoes



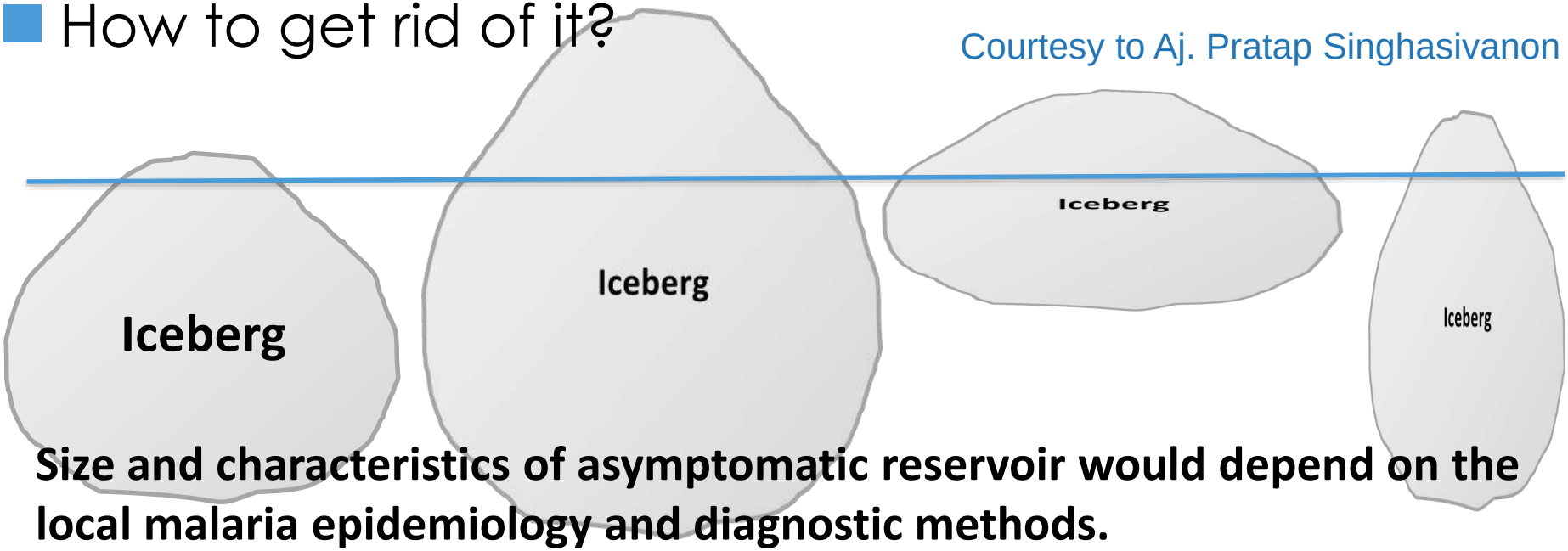
SILENT RESERVOIR OF TRANSMISSION

Iwagami M, Keomalaphet S, Khattignavong P, Soundala P, Lorphachan L, Matsumoto-Takahashi E, Strobel M, Reinharz D, Phommasansack M, Hongvanthong B, Brey PT, **Kano S**. The detection of cryptic Plasmodium infection among villagers in Attapeu province, Lao PDR. **PLoS Negl Trop Dis**. 2017 Dec 20;11(12):e0006148.

The problem of the asymptomatic reservoir

- How big is it?
- Does it cause disease?
- Does it contribute to transmission?
- Does it contribute to the resistant parasite pool?
- How to get rid of it?

Courtesy to Aj. Pratap Singhasivanon

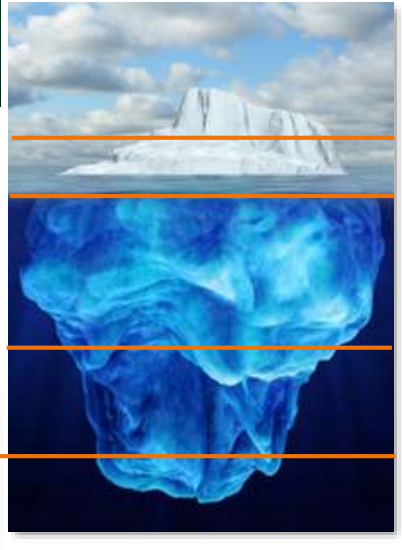


Size and characteristics of asymptomatic reservoir would depend on the local malaria epidemiology and diagnostic methods.

How much malaria can we detect?

RDT

Microscopy



100,000/mL

50,000/mL

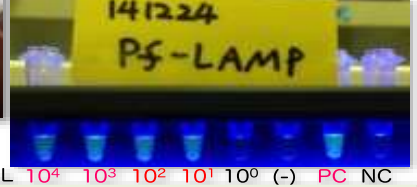
PCR

20,000/mL

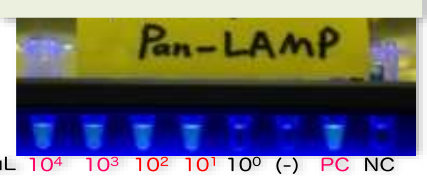
1,000/mL

Loopamp Malaria Detection kit
(LAMP)

EIKEN CHEMICAL CO.,LTD.



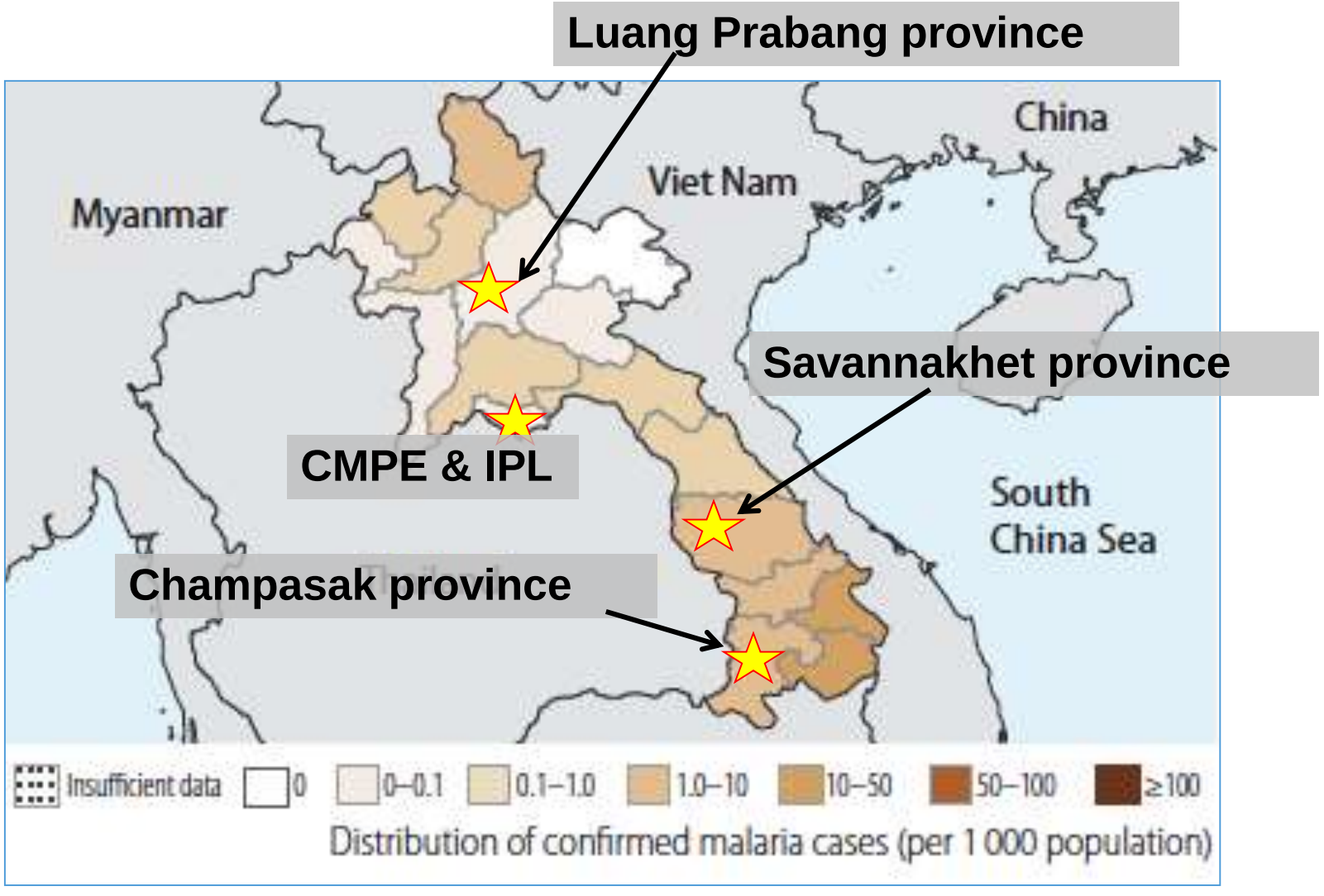
原虫数/30 μL 10^4 10^3 10^2 10^1 10^0 (-) PC NC



原虫数/30 μL 10^4 10^3 10^2 10^1 10^0 (-) PC NC

NCGM

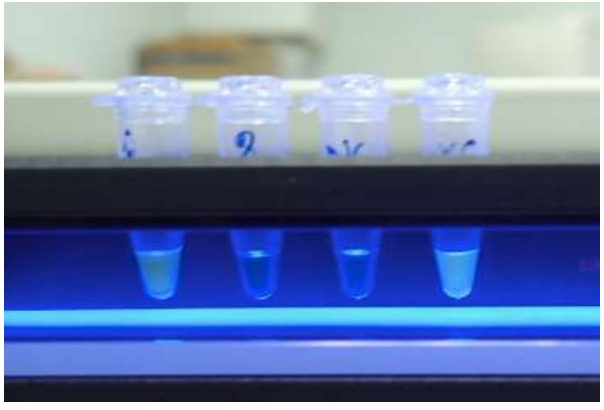
Field implementation of Malaria LAMP method



Implementation of malaria DNA diagnosis method (LAMP) in 2016








Division of Malariology, Parasitology and Entomology
Savannakhet Provincial Health Office

Luang Prabang

OBJECTIVE

Field Evaluation of PURE-LAMP

- Study Area: **Haiti**
- Endemic (*P. falciparum*)
- Low transmission
- Elimination targeted (2020)
- Chloroquine (60 years)
- Point-of-care diagnosis: RDT and microscopy

Loopamp PURE DNA Extraction kit

Vincent JP, Komaki-Yasuda K, Existe AV, Boncy J, **Kano S**. No *Plasmodium falciparum* chloroquine fesistance gransporter and artemisinin resistance mutations, Haiti. Emerg Infect Dis. **2018** Nov; 24(11): 2124-2126.

BACKGROUND

Previous Research CONCLUSION

- PURE-LAMP is sensitive on clinical dried blood spots
- Useful for diagnosis of imported malaria
- PURE-LAMP might be a valuable alternative to PCR on the field for detection of submicroscopic infection

Vincent JP, Komaki-Yasuda K, Iwagami M, Kawai S, **Kano S**. Malar J. 2018 Oct 22;17(1):373.

Study Sites


HOPITAL DE LA COMMUNAUTE
DANE HARIENNE


CUBA
ATLANTIC OCEAN
Windward Passage
(Canal de Vieux)
HAITI
Port-de-Paix
Cap-Haïtien
NORD-OUEST
NORD
NORD-EST
Gonaïves
ARTIBONITE
Golfe de la Gonâve
Saint-Marc
CENTRE
DOMINICAN REPUBLIC
Ile de la Gonâve
Jérémie
GRANDE-ANSE
NIPPES
SUD
Les Cayes
PORT-AU-PRINCE
OUEST
Pétionville
SUD-EST
Jacmel
H i s p a n i o l a
CARIBBEAN SEA

LABORATOIRE NATIONAL

The problem of the asymptomatic reservoir

- How big is it? • • • Bigger than previously thought
- Does it cause disease? • • • Probably
- Does it contribute to transmission? • • • Almost certainly
- Does it contribute to the resistant parasite pool? • • • Maybe yes
- How to get rid of it? • • • Mass survey by ultrasensitive diagnostic methods **such as LAMP will help**?? MDA?? Dedicated prompt diagnosis and proper treatment will melt the iceberg?

Conclusion:

Description of the asymptomatic reservoir has changed fundamentally our insights in malaria epidemiology.

This has huge implications for the strategies regarding malaria elimination in the endemic countries or regions.

The parasite reservoir must be tackled in order to eliminate the disease.

Courtesy to Aj. Pratap Singhasivanon

Thus, we need LAMP to “Test and Treat”

NCGM

150TH ANNIVERSARY