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qPCR survey of submicroscopic malaria in Tha Song Yang District, Tak Province, Thailand

Background:

Plasmodium falciparum cases in Tha Song Yang district have increased in recent years. In particular one subdistrict (Mae Song) has experienced an increase in cases in Thai citizens and several sub-regions (moos) continue to have high ratios of *P. falciparum* to *Plasmodium vivax* (hereafter referred to as the "Pf/Pv ratio"). The Pf/Pv ratio is one indicator of the effectiveness of the malaria posts (MP). Given the potential existence of asymptomatic *P. falciparum* carriers contributing to the increase in cases in Mae Song Sub-district, the Tak Public Health Office approached the Shoklo Malaria Research Unit (SMRU) for technical help.

Methods:

The project began with a Geographic Information System (GIS) survey of the entire district, listing each hamlet, an estimate of the number of houses per hamlet, whether or not cell phones work in the hamlet and whether or not a malaria post (MP) was present and working in the hamlet. From this list, the largest villages were excluded (Mae Tan, Mae Salit Luang, and Ban Tha Song Yang) and a stratified simple random sample of 15 villages (shown in Figure 1 and listed in Table 1) was drawn. The strata were based on sub-district, ensuring that there were at least some survey villages in each sub-district. Villages were selected randomly using a random number generator and the R-cran statistical package. Random selection was chosen to avoid bias in the surveys. A power analysis was done in order to assess the sample sizes necessary to estimate the prevalence with 90% confidence and 10% precision.

Surveys were done between December 11 and 19, 2014 (exact dates listed in Table 1). Informed consent was collected from each participant according to Tak Public Health Office guidelines and forms. As surveys were completed, whole blood samples were sent to SMRU's qPCR lab to be analyzed for malaria infections. A first round of qPCR analysis was done to look for any malaria species and a second layer of nested PCR was conducted in order to identify the parasites species. The surveys were approved by the Tak Public Health Office Ethical Review Board.

Results:

The distribution of functioning MPs from the initial survey is illustrated in Figure 2. MPs were defined as functioning if they were staffed, had ACTs, and had diagnostic supplies (usually RDTs, but microscope is also possible). Most functioning MPs cluster near the main road and most areas with high Pf/Pv ratios occurred in areas with few or no MPs.

A total of 817 whole blood samples were collected from 15 different villages (Figure 1 and Table 1). Out of these samples, 27 were positive for malaria parasites (20 *Plasmodium*

vivax, 2 *Plasmodium falciparum*, and 5 undeterminate species). None of the patients exhibited symptoms during the survey. The age and sex of infected survey participants are given in Table 2. Given the low number of positive results (prevalence estimates given in Table 3 and mapped in Figure 3), no apparent age or sex patterns emerge. We find no evidence of malaria hotspots among the surveyed villages (highest overall prevalence is 10.3 per 100 people, with a confidence range of 4.0 – 21.2).

Comments:

These surveys undertaken by the teams of TAK PHO and TSY hospital with the technical assistance of SMRU show that most villages in the district are without functioning MPs. The consequences are that people with symptomatic malaria cannot get diagnosed and treated within 24-48 hours of fever, as recommended. This probably explains the resurgence of malaria in parts of the district. However the qPCR surveys did not detect a significant sub-microscopic reservoir of *P. falciparum* infections and therefore Targeted Malaria Elimination by mass treatment is not warranted at this stage. The most appropriate intervention is to ensure that all villages are equipped with a functioning MP, if possible before the start of the next rainy season.

Table 1: Age and Sex of qPCR positive individuals

Age Group	all malaria		P. vivax		P. falciparum		Plasm spp	
	male	female	male	female	male	female	male	female
0 - 4	0	0	0	0	0	0	0	0
5 - 14	1	1	1	1	0	0	0	0
15 plus	14	11	10	8	2	0	2	3
total	15	12	11	9	2	0	2	3

Table 2: Survey village list and samples received

			village name					
Sample Collection Date	Sample Receiving Date	No. of Sample Received	Thai	English	estimated number of houses	MP in village?	subdistrict	Health center in charge
11-Dec-14	12-Dec-14	24	บ้านทีซวยคี	Baan Htee Suay Khee	50	no	แม่อุสุ	Re kha ti
11-Dec-14	12-Dec-14	59	บ้านตะวอซอ	Baan Ta Waw Saw	58	no	แม่อุสุ	Naung bua
11-Dec-14	12-Dec-14	61	บ้านเรกะติ	Baan Ray Ka Ti	98	no	แม่อุสุ	Re kha ti
11-Dec-14	12-Dec-14	57	บ้านเกร๊ะคี	Baan Krey Khee	50	yes	แม่วะหลวง	Kle Khee
17-Dec-14	18-Dec-14	59	บ้านแม่เหว่ยคริสต์	Baan Mae Wei Christ	60	yes	ท่าสองยาง	Mae wei
17-Dec-14	18-Dec-14	59	บ้านแม่อมยะ	Baan Ohm Ya	64	yes	ท่าสองยาง	Mae wei
17-Dec-14	18-Dec-14	59	บ้านแม่โปคี	Baan Mae Poe Khee	52	no	แม่วะหลวง	Mae wa laung
17-Dec-14	18-Dec-14	52	บ้านเซหนะเดอลู่	Baan Say Na Der Loo	26	no	แม่อุสุ	Se na de lou
17-Dec-14	18-Dec-14	52	บ้านพะดิ๊ซะคี	Baan Pa Di Za Khee	21	no	แม่วะหลวง	Sor krea ka
17-Dec-14	18-Dec-14	57	บ้านแม่โข๊ะ	Baan Mae Koh	47	no	แม่สอง	Mae song
18-Dec-14	18-Dec-14	59	บ้านห้วยแมงบุ้ง	Baan Huay Mang Bung	62	no	แม่ต๋าน	Thasongyang hospital
18-Dec-14	19-Dec-14	58	บ้านทีคุคีเหนือ	Baan Htee Khu Khee N	41	no	แม่สอง	Tee ku khee
18-Dec-14	19-Dec-14	52	บ้านบลาเด	Baan Bla Day	24	no	แม่สอง	Kleu de khee
19-Dec-14	19-Dec-14	52	บ้านทีหนีคี	Baan Htee Nue Khee	35	no	แม่หละ	Ka ma pha do
19-Dec-14	19-Dec-14	57	บ้านโบ๊ะโซะลู่	Baan Boh Soh Loo	39	no	แม่สอง	Wa doh kro
		817						

Table 3: Prevalence estimates by village and species

Village Name		# samples	qPCR positive			prevalence estimates			
			Pf	Pv	Plasm	Pf	Pv	Plasm	Total Prev
บ้านเซหนะเดอลู่	Baan Say Na Der Loo	52				0.00	0.00	0.00	0.00
บ้านทีหนึคี	Baan Htee Nue Khee	52				0.00	0.00	0.00	0.00
บ้านทีคุคีเหนือ	Baan Htee Khu Khee Nuar	58	2	3	1	3.45	5.17	1.72	10.34
บ้านแม่โขี๊ะ	Baan Mae Koh	57		3		0.00	5.26	0.00	5.26
บ้านเกร๊ะคี	Baan Krey Khee	57		1		0.00	1.75	0.00	1.75
บ้านทีซวยคี	Baan Htee Suay Khee	24				0.00	0.00	0.00	0.00
บ้านแม่โปคี	Baan Mae Poe Khee	59		1	1	0.00	1.69	1.69	3.39
บ้านห้วยแมงบุ้ง	Baan Huay Mang Bung	59		3	3	0.00	5.08	5.08	10.17
บ้านแม่อมยะ	Baan Ohm Ya	59				0.00	0.00	0.00	0.00
บ้านเรกะติ	Baan Ray Ka Ti	61		2		0.00	3.28	0.00	3.28
บ้านโบ๊ะโซะลู่	Baan Boh Soh Loo	57		5		0.00	8.77	0.00	8.77
บ้านตะวอซอ	Baan Ta Waw Saw	59		2		0.00	3.39	0.00	3.39

Figure 1: qPCR survey villages in Tha Song Yang District

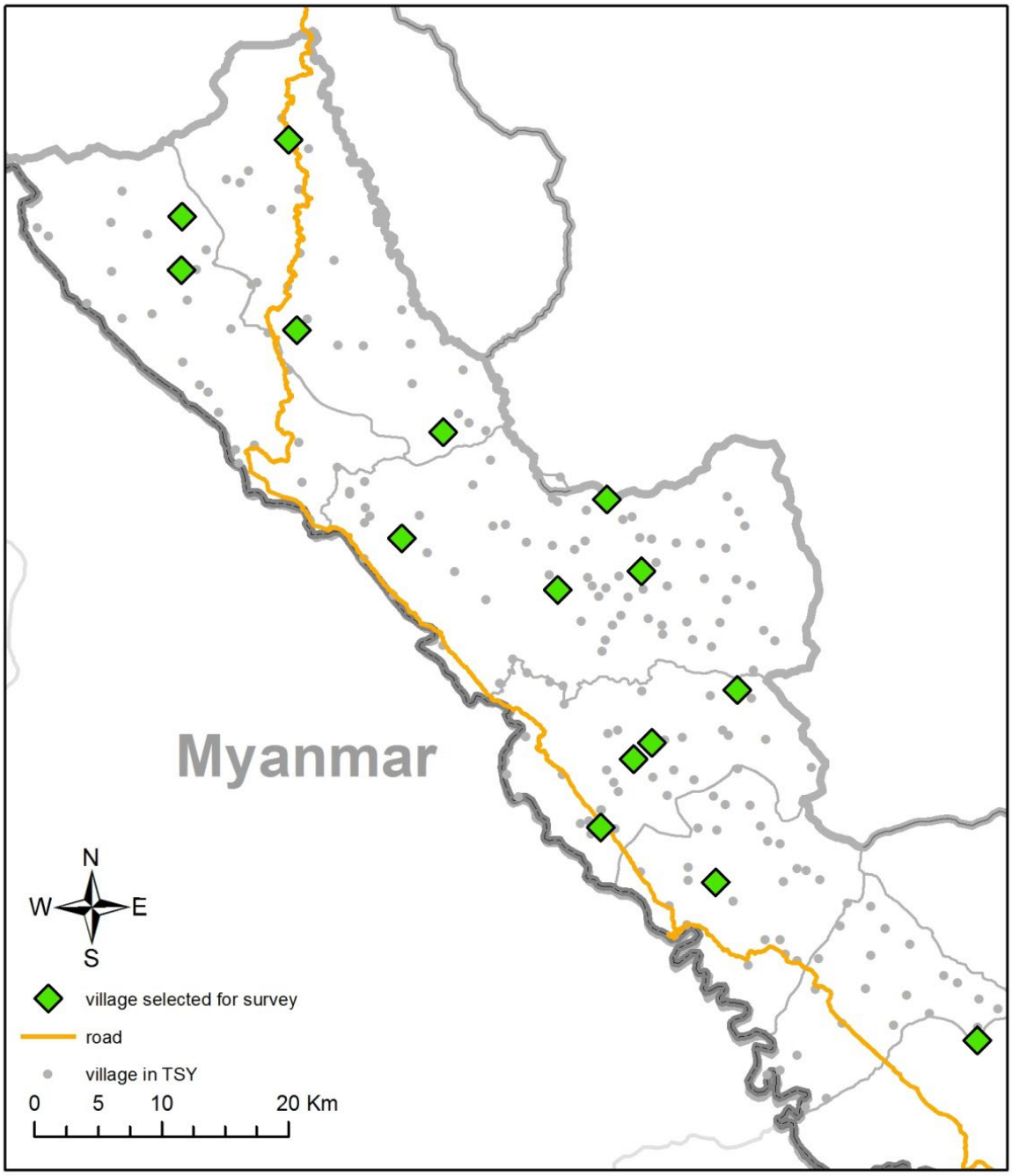

Figure 2: P. falciparum/P. vivax ratio by moo in Tha Song Yang district and MP status. Villages are indicated by points, black if they have no functioning MP and green if they have a functioning MP. The Pf/Pv ratio of a moo is indicated by color, with ≥ 1 in red, and higher ratios with a darker red color. Of the 10 Moos with high Pf/Pv ratios (≥1) in 2014, only 2 had functioning MPs.

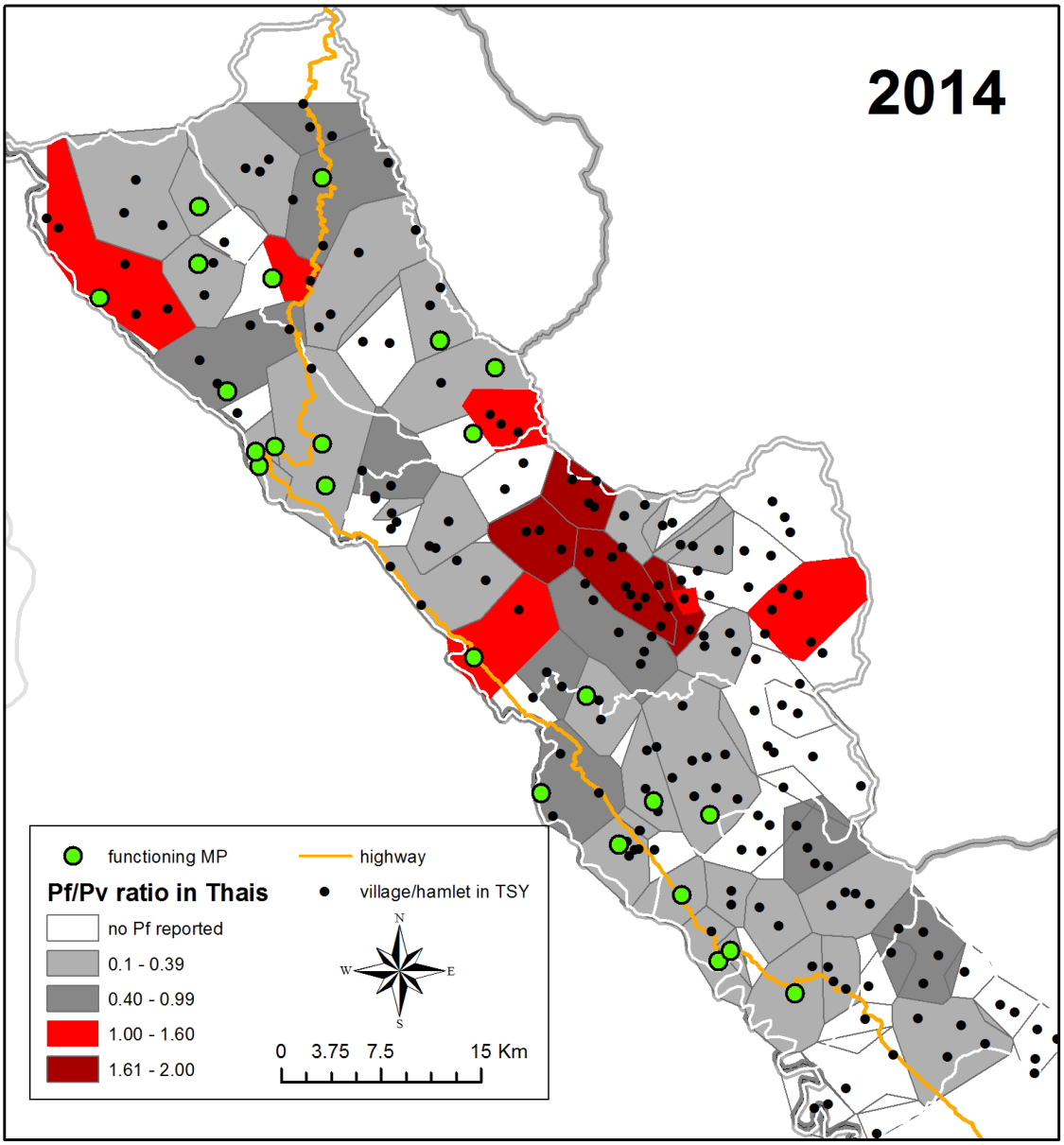

Figure 3: Total malaria prevalence (regardless of species) by survey site. Villages with qPCR detected malaria are named in the map. The actual break-down by species is given in Table 3.