Transmission Risk from Imported *Plasmodium vivax* Malaria in the China–Myanmar Border Region

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Malaria importation and local vector susceptibility to imported *Plasmodium vivax* infection are a continuing risk along the China–Myanmar border. Malaria transmission has been prevented in 3 border villages in Tengchong County, Yunnan Province, China, by use of active fever surveillance, integrated vector control measures, and intensified surveillance and response.

A sharp increase in imported malaria cases has made preventing reintroduction of malaria in China a major challenge (*1*). High importation risk from Myanmar, where malaria is endemic, and wide distribution and abundance of malaria vectors in the China–Myanmar border region sustain risk for secondary infections among local populations. Tengchong County in Yunnan Province, bordering Myanmar, reported the highest number of imported malaria cases during 2010–2014 in China. A recent field survey indicated secondary transmission from imported *Plasmodium vivax* malaria in this region (*2*). To inform malaria elimination efforts in the region, we assessed local vectorial capacity and evaluated risk for secondary infections arising from malaria importation.

The Study

Three villages (Manduo, Luoping, and Tuofeng; Figure) in Tengchong County, located in the westernmost part of Yunnan Province, were selected for study because of their 2011–2013 malaria incidence, ecologic features related to malaria transmission (i.e., altitude and proportion of land used for rice cultivation), and housing and economic status. The villages range in altitude from 1,276 to 1,893 meters and have 1,115 households and a population of 4,904 (detailed methods in online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/10/15-0679-Techapp1.pdf).

During 2011–2013, a total of 24 *P. vivax* malaria cases were reported from the study villages to the Chinese Information System for Disease Control and Prevention; all were classified as imported (online Technical Appendix), as determined by patients' travel history (*3*). All patients were adult men 18–49 years of age, and most worked in business or mining. Patients were radically cured with a regimen of chloroquine and primaquine, as recommended by national treatment guidelines. Malaria vulnerability or importation risk (i.e., incidence of imported malaria cases per 1,000 population per year) averaged 1.6 cases in the study area during 2011–2013 (online Technical Appendix Table 1). The average interval between symptom development and diagnosis of malaria was 2.4 days; average interval between diagnosis and treatment was 1.5 days (Table 1). All cases were reported within 1 day and investigated within 3 days (Table 1).

Health workers performing active fever surveillance during the 2013 transmission season (May–September) visited each house at 2-week intervals, conducted a total of 7,680 household interviews and 38,960 interviews with village residents, and collected 399 blood samples from persons with history of fever during the previous 2 weeks. A total of 268 (67.2%) samples were from local residents who reported no travel outside Tengchong County within the past 2 weeks; 131 were from mobile populations reporting travel. *P. vivax* isolates were detected by microscopy and PCR in 10 (7.6%) persons in the mobile population; no malaria infection was found in local persons (online Technical Appendix Table 2).

To estimate human biting rates, mosquitoes were collected in each study village every 2 weeks by using volunteer outdoor human-landing catches during May–September 2013 (online Technical Appendix). A total of 5,576 mosquitoes were caught; most (95%) were *Anopheles sinensis* mosquitoes. The average number of mosquitoes landing on a single person per night (human landing rate)

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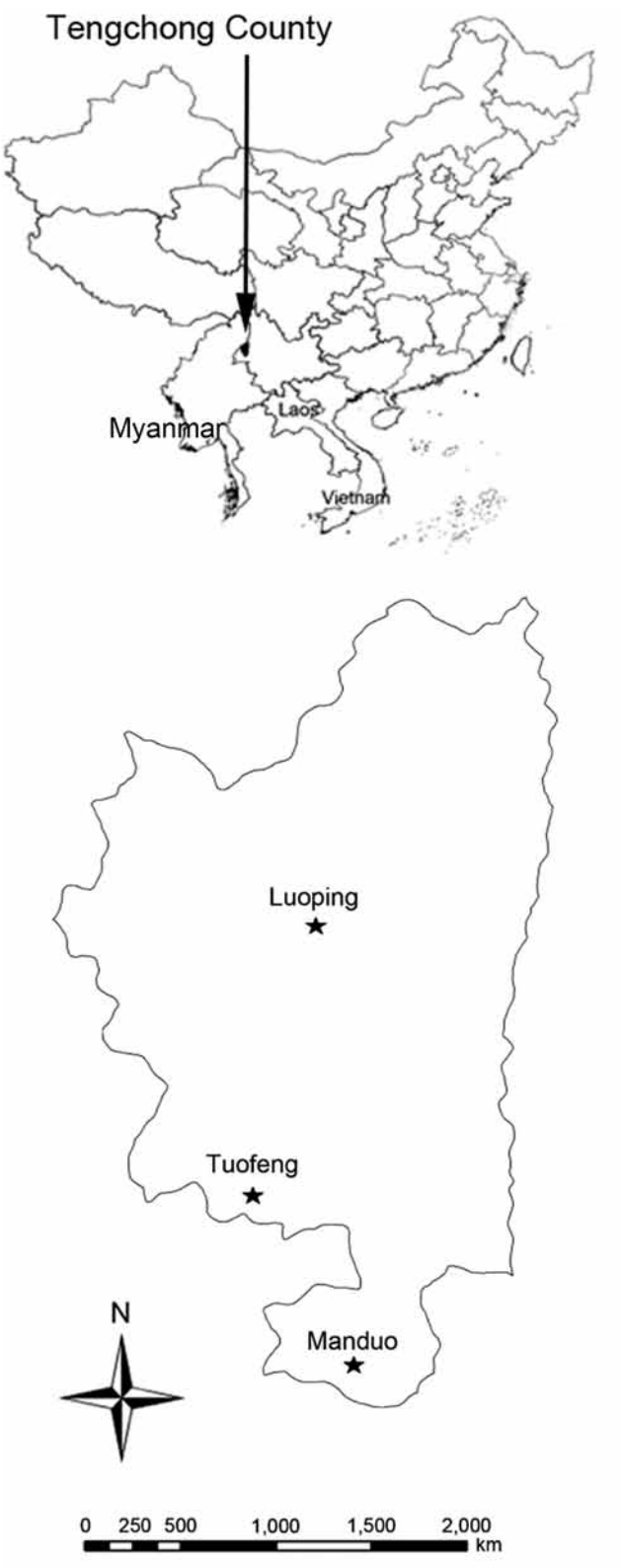

Figure. Location of 3 villages in Tengchong County, Yunnan Province, China, in which study of transmission risk from imported *Plasmodium vivax* malaria was conducted. Inset shows location of Tengchong County along the China–Myanmar border.

was 2.7 (Table 2; online Technical Appendix Table 3). The ratio of parous to nulliparous mosquitoes (multiparous ratio) of the *An. sinensis* mosquitoes tested for the villages of Manduo, Luoping, and Tuofeng was 0.52, 0.54, and 0.61, respectively (online Technical Appendix Table 4). The average human blood index (i.e., proportion of tested mosquitoes with ingested human blood) was 3.8% in the surveillance areas (Table 2; online Technical Appendix Table 5). Average vectorial capacity (i.e., expected number of new human infections from 1 infected person within 1 day, assuming all mosquitoes with sporozoites are potentially infective) of *An. sinensis* mosquitoes was 0.02 (Table 2), indicating that ≈50 cumulative days would be needed for transmission of malaria from an infected person to another person, assuming that all female mosquitoes biting malaria-infected persons become infected and transmit. The proportion of tested field-caught *An. sinensis* mosquitoes found to have ingested human blood (i.e., blood feeding rate) was 15.6% (31/199). The proportion of infected mosquitoes was 16.1% (5/31), the rate of susceptibility of local *An. sinensis* mosquitoes to imported *P. vivax* infection in this study.

Conclusions

An. sinensis mosquitoes were the main local vector for *P. vivax* and were widely distributed throughout this study area. The vector's susceptibility to imported *P. vivax* infection indicates potential for *P. vivax* malaria to be sustained in this region. Although a recent field survey showed secondary transmission from patients with imported *P. vivax* malaria in other villages in Tengchong County (*2*), we identified no secondary infections in the study villages.

An. sinensis mosquitoes are the most widely distributed malaria vector in China but have relatively low susceptibility to parasites compared with other malaria vectors (*4*). We confirmed that *An. sinensis* mosquitoes were susceptible to imported *P. vivax* infection; however, vectorial capacity of *An. sinensis* mosquitoes was lower than it was during the 1990s (0.05) in the same region (*5*) and much lower than reported in central China (0.3) in the 1980s (*6*). The reduced vectorial capacity in the study region since the 1990s is likely attributable to the change in the dominant malaria vector species. Historically, *An. kunmingensis* mosquitoes were the main malaria vector in Tengchong County and accounted for 77% of total malaria vector density; its vectorial capacity (0.3) was ≈10 times higher than that of *An. sinensis* mosquitoes (0.03) in the 1980s (*7*). Few *An. kunmingensis* mosquitoes (<5% of total malaria vectors) were captured in our study, likely because of extensive residual insecticide use and improved housing, which reduce contact with this mosquito and vectorial capacity. High coverage (>90%) of long-lasting insecticidal nets and indoor residual spraying

Table 1. Malaria case management and response in 3 villages in the China–Myanmar border region, 2011–2013

Village	No. cases	Days from illness onset to diagnosis	Days from diagnosis to treatment	Reported within 1 d, %	Investigated within 3 d, %	Febrile, screened within 7 d, no.	Additional cases identified, no.
Manduo	8	3.2	1.6	100	100	124	0
Luoping	11	1.8	1.5	100	100	224	0
Tuofeng	5	2.2	1.5	100	100	99	0
Total	24	2.4	1.5	100	100	447	0

has been achieved in southern China along the Myanmar border since implementation of the National Malaria Elimination Program in July 2010.

In early 2012, a real-time surveillance system and response strategy ("1-3-7") was rolled out nationally (*8*) and substantially improved timeliness of malaria surveillance and response activities. During 2011–2013, all malaria cases in the study area were reported within 1 day and investigated within 3 days, and screening of persons with fever was conducted within 7 days. Other countries reporting few days (range 3.0–8.2 days) before testing and treatment of imported malaria reported similar results and no subsequent secondary infections (*9–11*). In our study, additional testing by PCR for those reporting fever found no subpatent infections (i.e., slight infections with low parasitemia), a finding consistent with a recent review that showed no difference between PCR and microscopy for detecting parasites in symptomatic persons. However, a large proportion of *P. vivax* infections were subpatent in a cross-sectional survey of the general population in China (*12*).

Rigorous evaluation of malaria elimination programs is essential for continuously improving the programs, targeting limited resources, and maintaining financial and political support. We used robust entomologic and epidemiologic metrics to assess malaria elimination in a border region. Many national malaria control programs lack capacity to conduct entomologic surveillance to assess vectorial capacity. Although our study shows evidence of successful malaria elimination, additional validated metrics to ascertain success are needed. Recent efforts to compare the basic reproductive rate (total number of malaria cases derived from 1 infective case and distributed by mosquitoes in the absence of immunity) for imported versus local malaria cases provide a more nuanced and stable metric for measuring malaria elimination (*13*). Because this region has a unique ecology and distinct mosquito species composition, our findings need further validation to determine whether they can be extrapolated to other areas of China's border region (*14*).

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Table 2. Vectorial capacity of *Anopheles sinensis* mosquitoes in 3 villages in the China–Myanmar border region, May–September 2013*

Village	Human landing rate†	Human blood index‡	Mosquito biting habits§	Daily survival rate¶	Days of sporogonic development#	p^n**	Survival, d††	Receptivity‡‡
Manduo	1.2	0.04	0.02	0.8	12.4	0.1	5.4	0.01
Luoping	4.9	0.04	0.02	0.8	14.0	0.09	5.7	0.05
Tuofeng	2.0	0.03	0.01	0.9	23.3	0.04	7.2	0.01
Total	2.7	0.04	0.02	0.9	16.6	0.08	6.1	0.02

*Vectorial capacity, expected number of new human infections from 1 infected person within 1 day, assuming all mosquitoes with sporozoites are potentially infective. HBI, human blood index (proportion of tested mosquitoes having ingested human blood.
†Average number of mosquitoes landing on a single person per night (*ma*).
‡Proportion of tested mosquitoes having ingested human blood.
§Human blood index divided by days needed to complete gonotrophic cycle (cycle of taking a blood meal and laying eggs).
¶Probability (*p*) of a mosquito surviving 1 whole day.
#Time (*n*) needed for parasites to complete development from ingested gametocytes during blood meal to sporozoites in salivary glands, when parasites are transmissible to humans.
**Fraction of infected mosquitoes after duration of sporogony.
††Duration of vector's life, in days, after surviving the extrinsic incubation period, calculated as the negative logarithmic reciprocal of the daily survival rate: $1/-\ln(p)$.
‡‡Expressed by the vectorial capacity index: $ma^2(p^n/-\ln(p))$.

References

1. Cotter C, Sturrock HJ, Hsiang MS, Liu J, Phillips AA, Hwang J, et al. The changing epidemiology of malaria elimination: new strategies for new challenges. Lancet. 2013;382:900–11. http://dx.doi.org/10.1016/S0140-6736(13)60310-4
2. Yi-zhong D, Sheng-guo L, Xian-hu K, Shou-qing Y, Xiao-dong S. A point-like outbreak caused by secondary transmission from imported malaria vivax case. Int J Med Parasit Dis. 2013;40:57–9.
3. Chinese Centers for Disease Control and Prevention. Technical scheme of malaria elimination. 2011 [cited 2011 Sep 2]. http://www.chinacdc.cn/jkzt/crb/nj/jszl_2223/201109/U020110906536170416565.pdf
4. Rueda LMZT, Ma YJ, Gao Q, Zhu GD, Khuntirat B, Sattabongkot J, et al. Updated distribution records of the *Anopheles* (*Anopheles*) *hyrcanus* species-group (Diptera: Culicidae) in China. Zootaxa. 2007;1407:43–55.
5. Qu CZST, Wang MY. Vectorial capacity of malaria transmission of *Anopheles sinensis* in Zhengzhou in nature [in Chinese]. J Henan Med Univ. 2000;35:394–6.
6. Qian H, Tang L, Cheng Y, Yang B. Preliminary estimation of malaria transmission potential in areas where *Anopheles sinensis* is the only vector [in Chinese]. Chinese Journal of Parasitology and Parasitic Diseases. 1994;12:265–7.
7. Zhang GC, Dong XS. W XZ, Lu YR. Quantitative study on transmission of malaria by *An. Kunmingensis* [in Chinese]. Chinese Journal of Parasitology and Parasitic Diseases. 1989;7:100–2.
8. Cao J, Sturrock HJ, Cotter C, Zhou S, Zhou H, Liu Y, et al. Communicating and monitoring surveillance and response activities for malaria elimination: China's "1-3-7" strategy. PLoS Med. 2014;11:e1001642. http://dx.doi.org/10.1371/journal.pmed.1001642
9. Romi R, Sabatinelli G, Majori G. Could malaria reappear in Italy? Emerg Infect Dis. 2001;7:915–9. http://dx.doi.org/10.3201/eid0706.010601
10. Askling HH, Ekdahl K, Janzon R, Henric Braconier J, Bronner U, Hellgren U, et al. Travellers returning to Sweden with *falciparum* malaria: pre-travel advice, behaviour, chemoprophylaxis and diagnostic delay. Scand J Infect Dis. 2005;37:760–5. http://dx.doi.org/10.1080/00365540510044120
11. Chalumeau M, Holvoet L, Cheron G, Minodier P, Foix-L'Helias L, Ovetchkine P, et al. Delay in diagnosis of imported *Plasmodium falciparum* malaria in children. Eur J Clin Microbiol Infect Dis. 2006;25:186–9. http://dx.doi.org/10.1007/s10096-006-0105-3
12. Cheng Q, Cunningham J, Gatton ML. Systematic review of sub-microscopic *P. vivax* infections: prevalence and determining factors. PLoS Negl Trop Dis. 2015;9:e3413. http://dx.doi.org/10.1371/journal.pntd.0003413
13. Churcher TS, Cohen JM, Novotny J, Ntshalintshali N, Kunene S, Cauchemez S. Measuring the path toward malaria elimination. Science. 2014;344:1230–2. http://dx.doi.org/10.1126/science.1251449
14. Yu G, Yan G, Zhang N, Zhong D, Wang Y, He Z, et al. The *Anopheles* community and the role of *Anopheles minimus* on malaria transmission on the China–Myanmar border. Parasit Vectors. 2013;6:264. http://dx.doi.org/10.1186/1756-3305-6-264

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Technical Appendix

Methods

Study Areas

The 3 villages (Manduo, Luoping, and Tuofeng) in our study have an altitude range of 1,276–1,893 meters and a total of 1,115 households with a population of 4,904. This area belongs to a tropical rainy climate and can be divided into a rainy season from July–September and a dry season from October–May. The proportion of the terraced rice fields in the study area was 20%–30% of total land in use. The villages have many different ethnic groups: Han, Dai, and Lisu (dominant groups) and Tibetans, Bai, Aini, and persons with Burmese ancestry. The villages are largely undeveloped areas in the border region, and many people frequently cross back and forth into Myanmar for employment in the logging, mining, and farming industries.

Ethical Approval

Approval for the study was obtained from the ethical committee of the National Institute of Parasitic Diseases, China Centers for Disease Control and Prevention (World Health Organization Collaborating Centre for Malaria, Schistosomiasis and Filariasis), Shanghai, China, and written informed consent was obtained from participants. No specific permissions were required, and field studies involved no endangered or protected species.

Malaria Surveillance Data

Routine malaria surveillance data during 2011–2013 from the study villages were obtained from the Chinese Information System for Disease Control and Prevention. All passively detected malaria cases were classified as local or imported on the basis of travel history. Imported malaria was defined by the following 3 criteria that must be simultaneously met: 1) diagnostically confirmed malaria; 2) travel history to malaria-endemic areas outside China during the malaria transmission season; and 3) onset of malaria <1 month after returning to China (*1*). Since the launch of the National Malaria Elimination Program in July 2010, each case of malaria is required to be classified as local or imported, and the source of infection for each case should be investigated within 3 days according to the above criteria.

Study Participants

To monitor for malaria infection among mobile and local populations in the study areas, active case detection was implemented and targeted all populations through household visits every 2 weeks during the 2013 transmission season (May–September). Any persons, local or mobile, with history of fever in the past 2 weeks were tested for malaria. Local populations were defined as those residents with no travel history outside Tengchong County within the past 2 weeks; mobile populations reported travel.

Blood Sample and Detection

Thick and thin blood films for parasitologic diagnosis were taken from all persons who reported a history of fever in the past 2 weeks in the study areas. Blood films were stained by the standard Giemsa method, usually within 48 hours, but always within 96 hours. Examinations were performed by a senior technician from the local Provincial Center for Disease Control and Prevention and then checked by national experts for quality assurance of findings. All discordant results were reviewed together and then confirmed by PCR. Examination of >200 high-power oil immersion fields was required to verify a negative blood film. Dried blood spot samples obtained by filling 2 delineated circles (≈0.5 inch in radius) on a filter paper were thoroughly dried and kept refrigerated at 4°C before their delivery to the national laboratory for PCR processing. In this study, we applied a novel high-throughput PCR, Capture and Ligation Amplification PCR method (CLIP-PCR) (*2*), to detect the 18S rRNA of *Plasmodium* spp. in all dried blood spots. To accelerate the diagnostic efficiency and reduce costs, samples were tested with a matrix pooling strategy (*3*). At least 1 positive control and 1 negative control were included in each assay, and each sample was tested in duplicate.

Entomological Surveillance

To estimate human biting rates, mosquitoes were collected in each study village every 2 weeks by using volunteer outdoor human-landing catches during May–September 2013, according to methods recommended by the World Health Organization (*4*). All mosquitoes that landed on a volunteer were assumed to have bitten and were collected and identified by their morphologic features (*5*). Three villages were sampled simultaneously, and mosquito collectors were rotated among the 3 study sites to eliminate bias. The mosquito-biting rate was estimated by the number of certain species of mosquitoes caught per person per night in each village. To estimate a human blood index, blood-engorged *Anopheles* spp. were collected from different types of mosquito resting places (i.e., the bush, empty cow-sheds, small warehouses, and concrete bunkers) by using a US Centers for Disease Control and Prevention backpack aspirator (John W. Hock Co.,

Gainesville, Florida, USA); blood meals were identified by PCR (*6*). The human blood index was estimated by applying the unweighted mean of a selected portion of all collected samples collected from different types of mosquito resting places (*7*). A survey to determine multiparous mosquito ratios was conducted in the villages, where mosquitoes were dissected daily to determine their parity, following methods reported by Dong et al. (*8*). Half of the female mosquitoes collected were randomly selected and dissected after each collection. To estimate vector competence, female anopheline mosquitoes collected from the study areas were reared at 27°C (± 1°C) and 70%–80% relative humidity and were provided with a 10% (weight/volume) sucrose solution. Adult female mosquitoes were starved for 6 hours before infection by membrane feeding assay, as previously described (*2*). The mosquitoes were allowed to feed for 45 minutes on *P. vivax*–infected whole blood meals. Mosquitoes that had not fed on blood were removed within 24 hours. Mosquitoes were then maintained at 26°C and 70% (± 5%) relative humidity and were provided with a 10% sucrose solution. To estimate infectivity, the midguts of the vectors were dissected on day 7 after the blood feeding and stained with 0.1% (weight/volume) mercurochrome in phosphate-buffered saline; oocyst numbers per midgut were examined by microscopy.

Estimation of receptivity

The receptivity index or vectorial capacity (VC = $ma2[Pn/-\ln P]$) is defined as the expected number of new infections per infective case per day (provided all mosquitoes with sporozoites are potentially infective); vectorial capacity was interpreted by Garret-Jones as the product of the man-biting rate (ma), biting habits (a), and longevity factor ($P^n/-\ln P$) (*7*). Mosquito biting habits (a) are defined as the human blood index divided by the length of the gonotrophic cycle, on the basis of previous research from China. The daily survival rate (P) was calculated according to Qian et al (*8*). We calculated life expectancy values for *P. vivax*–infected *Anopheles* spp. by using methods outlined in a similar study (n = 105/[T-14.5]). In the equation, 105 is the number of days needed for the *P. vivax* sporozoite to mature in the *Anopheles* mosquito at the threshold

temperature of 14.5°C, and T is the local average atmosphere temperature during May–September 2013 (*5*).

References

1. Chinese Centers for Disease Control and Prevention. Technical scheme of malaria elimination [in Chinese]. 2011 [cited 2011 Sep 2]. http://www.chinacdc.cn/jkzt/crb/nj/jszl_2223/201109/U020110906536170416565.pdf

2. Cheng Z, Wang D, Tian X, Sun Y, Sun X, Xiao N, et al. Capture and Ligation Probe-PCR (CLIP-PCR) for Molecular Screening, with application to active malaria surveillance for elimination. Clin Chem. 2015;61:821–8. PubMed http://dx.doi.org/10.1373/clinchem.2014.237115

3. Smith DM, May SJ, Perez-Santiago J, Strain MC, Ignacio CC, Haubrich RH, et al. The use of pooled viral load testing to identify antiretroviral treatment failure. AIDS. 2009;23:2151–8. PubMed http://dx.doi.org/10.1097/QAD.0b013e3283313ca9

4. World Health Organization. Manual on practical entomology in malaria. part II: methods and techniques. Geneva: The Organization; 1975.

5. Ministry of Health Disease Prevention and Control Bureau. Handbook for malaria control and prevention. Beijing: People's Hygiene Publishing House Press; 2007.

6. Cheng Q, Cunningham J, Gatton ML. Systematic review of sub-microscopic P. vivax infections: prevalence and determining factors. PLoS Negl Trop Dis. 2015;9:e3413. PubMed http://dx.doi.org/10.1371/journal.pntd.0003413

7. Garrett-Jones C. Prognosis for interruption of malaria transmission through assessment of the mosquito's vectorial capacity. Nature. 1964;204:1173–5. PubMed http://dx.doi.org/10.1038/2041173a0

8. Qian H, Tang L, Cheng Y, Yang B. Preliminary estimation of malaria transmission potential in areas where *Anopheles sinensis* is the only vector [in Chinese]. Zhongguo Ji Sheng Chong Xue Yu Ji

Sheng Chong Bing Za Zhi [Chinese Journal of Parasitology and Parasitic Diseases]. 1994;12:265–7. PubMed

Technical Appendix Table 1. Number of malaria cases reported in 3 villages and annual parasite incidence rates, classified as imported or local cases, China–Myanmar border region, 2011–2013*

Village	Altitude, m	Population	Malaria cases by year, no. (API)							
			2011		2012		2013		2011–2013	
			Local	Imported	Local	Imported	Local	Imported	Local	Imported
Manduo	1,276	1,079	0	2 (1.9)	0	3 (2.8)	0	3 (2.8)	0	8 (2.5)
Luoping	1,752	2,249	0	6 (2.7)	0	2 (0.9)	0	3 (1.3)	0	11 (1.6)
Tuofeng	1,893	1,576	0	2 (1.3)	0	1 (0.6)	0	2 (1.3)	0	5 (1.1)
Total		4,904	0	10 (2.0)	0	6 (1.2)	0	8 (1.6)	0	24 (1.6)

*API, Annual parasite incidence (cases/1,000 persons/year). Source: Chinese Information System for Disease Control and Prevention.

Technical Appendix Table 2. Number of persons tested for malaria and those testing positive in 3 villages during the malaria transmission season, by local and mobile populations and testing method, China–Myanmar border region, 2013*

Village	Mobile Population			Local Population		
	No. tested	Method of Testing		No. tested	Method of Testing	
		Microscopy, no. + (%)	PCR, no. + (%)		Microscopy, no. + (%)	PCR, no. + (%)
Manduo	28	3 (10.7)	3 (10.7)	70	0 (0.0)	0 (0.0)
Luoping	69	5 (7.3)	5 (7.3)	152	0 (0.0)	0 (0.0)
Tuofeng	34	2 (5.9)	2 (5.9)	46	0 (0.0)	0 (0.0)
Total	131	10 (7.6)	10 (7.6)	268	0 (0.0)	0 (0.0)

*Testing resulted from active fever surveillance. Malaria transmission season in study area is May–September; no. +, number testing positive.

Technical Appendix Table 3. Average monthly human-landing rate *of Anopheles sinensis* mosquitoes in 3 villages during the malaria transmission season, China–Myanmar border region, 2013*

Village	May	June	July	August	September	Village Average
Manduo	0.7	1.2	3.5	0.7	0.0	1.2
Luoping	2.0	5.3	8.0	5.0	4.3	4.9
Tuofeng	1.7	4.3	2.6	1.6	0.0	2.0
Monthly Average	1.5	3.6	4.7	2.4	1.4	2.7

*Human landing rate = number of mosquitoes landing on a single person per night. Malaria transmission season in the study area is May–September.

Technical Appendix Table 4. Parity of *Anopheles sinensis* mosquitoes collected in 3 villages during the malaria transmission season, China–Myanmar border region, 2013*

Village	Test mosquitoes	Parous	Nulliparous	Parity Ratio	X^2 test among groups
Manduo	128	66	62	0.5	$X^2 = 6.23$
Luoping	136	74	62	0.5	p-value = 0.10
Tuofeng	140	92	48	0.7	
Total average	135	77	57	0.6	

*Malaria transmission season in the study area is May–September.

Technical Appendix Table 5. Human blood–meal Identification of *Anopheles sinensis* mosquitoes in 3 villages during the malaria transmission season, China–Myanmar border region, 2013*

Village	Engorged mosquitoes tested, no.	Mosquitoes containing human blood, no. (%)	X^2 test among groups
Manduo	74	3 (4.1)	$X^2 = 0.14$
Luoping	71	3 (4.2)	p-value = 0.9
Tuofeng	65	2 (3.1)	
Total	210	8 (3.8)	

*Malaria transmission season in the study area is May–September.