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RESEARCH ARTICLE

Using Respondent Driven Sampling to Identify Malaria Risks and Occupational Networks among Migrant Workers in Ranong, Thailand

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Abstract

Background

Ranong Province in southern Thailand is one of the primary entry points for migrants entering Thailand from Myanmar, and borders Kawthaung Township in Myanmar where artemisinin resistance in malaria parasites has been detected. Areas of high population movement could increase the risk of spread of artemisinin resistance in this region and beyond.

Methods

A respondent-driven sampling (RDS) methodology was used to compare migrant populations coming from Myanmar in urban (Site 1) vs. rural (Site 2) settings in Ranong, Thailand. The RDS methodology collected information on knowledge, attitudes, and practices for malaria, travel and occupational histories, as well as social network size and structure. Individuals enrolled were screened for malaria by microscopy, Real Time-PCR, and serology.

Results

A total of 619 participants were recruited in Ranong City and 623 participants in Kraburi, a rural sub-district. By PCR, a total of 14 (1.1%) samples were positive (2 P. falciparum in Site 1; 10 P. vivax, 1 Pf, and 1 P. malariae in Site 2). PCR analysis demonstrated an overall weighted prevalence of 0.5% (95% CI, 0–1.3%) in the urban site and 1.0% (95% CI, 0.5–1.7%) in the rural site for all parasite species. PCR positivity did not correlate with serological positivity; however, as expected there was a strong association between antibody...
prevalence and both age and exposure. Access to long-lasting insecticidal treated nets remains low despite relatively high reported traditional net use among these populations.

**Conclusions**

The low malaria prevalence, relatively smaller networks among migrants in rural settings, and limited frequency of travel to and from other areas of malaria transmission in Myanmar, suggest that the risk for the spread of artemisinin resistance from this area may be limited in these networks currently but may have implications for regional malaria elimination efforts.

**Introduction**

Historically, resistance to anti-malarial drugs emerged first in the Greater Mekong Sub-region (GMS) to chloroquine, sulfadoxine-pyrimethamine (SP), and mefloquine, and population movements were partially responsible for the spread of the resistant parasites to other countries and regions [1,2]. Since the confirmation of artemisinin resistance along the Thailand-Cambodian border in 2009 [3,4], there has been concern about the risks of spread of artemisinin resistance to neighboring countries and increasing parasite clearance times to artemisinins have now been reported in Kawthaung, Myanmar [5], a township that shares an international border with Ranong, Thailand.

Ranong Province in southern Thailand is known to be one of the primary points of entry for migrants entering Thailand from Myanmar. Ranong historically has had both a high incidence of malaria and a high proportion of migrants (approximately 50% of residents are from Myanmar) compared to other Thai provinces. From the routine surveillance system, the annual parasite incidence (API) trend reported from Ranong Province was 12.0, 7.1, and 9.9 per 1,000 population in 2010, 2011, and 2012, respectively. By comparison, the national API was reported to be below 1 per 1,000 during this period.

Understanding the movement of migrant and mobile populations is essential to curb the potential spread of the resistant parasites, but the characteristics of this group make them inherently difficult to study. They are thought to be highly mobile, often hidden, and difficult to track with routine surveillance and to target with health interventions. Current standard cross-sectional household survey methods are inadequate to obtain representative information on this hidden, transient population due to the absence of an appropriate sampling frame. In an attempt to address some of these methodological issues, respondent-driven sampling (RDS) approach was adapted [6,7] as a potential tool to access these hard-to-reach populations.

Respondent-driven sampling is a modified chain-referral or snowball sampling technique used to approximate more precise estimates from hidden populations and has been used to study HIV risk groups [8] despite some methodological limitations [9]. Results from this quantitative survey, complemented with other qualitative information, should enable the Ministry of Public Health and its partners to understand better the behaviors and migration patterns of these populations, leading to enhanced surveillance and case management, and more effective targeting of malaria control interventions and health messages among migrant workers.

The aim of this study was to determine the migratory patterns, occupational risk, healthcare-seeking and malaria prevention behaviors, network associations, and parasite infection/exposure among mobile and migrant populations along the Thai-Myanmar border in an area with known artemisinin-resistant malaria parasites and to provide a reasonable sampling
Respondent Driven Sampling among Myanmar Migrants

Methods

Site selection

Two sites (urban and rural) were selected in Ranong Province, a southern province in Thailand that historically has had high malaria incidence and serves as an entry point for migrants from Myanmar. Initial recruits (seeds) were selected from migrant worker populations of Ranong City (urban) and Kraburi Sub-district (rural). Since there was not expected to be inter-mixing between the two study sites, sample size calculations were obtained for each site and sites were analyzed separately. With a migrant population proportion of 50%, design effect of 1.5, 95% confidence level, and a non-response rate of 10%, a total sample size of 600 participants was required for each study site.

Project staff recruited six seeds from each selected site for diversity in gender, age, and occupation. In total, 16 seeds were required (6 seeds for Site 1 and 10 seeds for Site 2) to reach the specified sample size. For Site 2 (rural), initial seeds were not able to recruit sufficient participants in a timely manner due to the remoteness of some locations and mobile teams were used to reach the desired sample size.

The inclusion criteria for the study included: 1) not being a Thai citizen; 2) coming to find work or economic advantage, or with someone who is; 3) age of at least 18 years; 4) no prior participation in this survey; and 5) provision of informed consent prior to enrollment. Pregnant women were not excluded as they were referred to receive nationally recommended treatment if found to be positive for malaria.

Data collection and analysis

All enrolled participants were asked about their demographic background, migratory pattern, work history, health care seeking behavior including access and barriers to health messages, health status, and knowledge of malaria including its prevention. Data collection began on 1 May 2012 and ended on 15 July 2012 during the rainy season.

To minimize data collection errors, handheld personal digital assistants (HP iPAQ model HX 2007) with automatic data checks and skip patterns in the Myanmar language were used and the interviewers received training on the handling and use of these tools. Data were exported to Microsoft EXCEL. Following data cleaning and verification, analysis was performed using the Respondent-Driven Sampling Analysis Tool (RDSAT) Version 7.1.38 [15].
The data presented here are weighted based on reported individual network sizes using this tool.

**Biological screening**

In order to assess current and previous malaria exposure, participants were asked to provide a blood sample for microscopy and filter paper collection to assess for current malaria infection using PCR and previous malaria exposure using serology. Reading of thick and thin blood smears was performed by local malaria staff and microscopy positive individuals were provided with antimalarial treatment according to the Thailand National Treatment Policy. All malaria positive slides and 10% of negatives were re-read at the National Reference Laboratory in Bangkok, Thailand. For molecular analyses, standard pooled, Real-Time PCR (RT-PCR) assays were used to differentiate plasmodia infections in dried blood spots [16]. Universal Safety Precautions were used in the collection, transport, storage, and analysis of biological specimens.

For serology, dried blood spot samples were eluted and assayed against antigens for both *P. falciparum* and *P. vivax* using methodologies previously described [17]. Briefly, antibody levels were determined by ELISA in Immulon 96 well plates. Serum samples were added in duplicate at a concentration of 1/1000 for MSP-119 (*P. falciparum* and *P. vivax*), 1/1000 for MSP-2, 1/200 for CSP and 1/2000 for AMA (*P. falciparum* and *P. vivax*). Optical density was read at 492nm and antibodies reported as titers (as determined by standard on the plate).

**Ethical approval**

Approval was obtained by the Ethics Committee for Research in Human Subjects of the Department of Disease Control, Ministry of Public Health, Thailand (FWA 00013622) and the US Centers for Disease Control and Prevention. Prior to enrollment in the study, participants provided written informed consent in Myanmar language. The consent form and procedures were also approved by the Ethics Committee.

**Results**

**Demographics, occupational and travel history**

A total of 619 participants were recruited in Ranong City (Site 1) and 623 participants in Krabi sub-district (Site 2). Age distributions between the two sites were different, with proportionally younger participants in the rural site compared to the urban site (Table 1). The mean age of participants was 34.7 years (SD = 11.5) in the urban site (Site 1) compared to 30.8 years (SD = 11.0) in the rural site (Site 2). Gender distributions also differed significantly between the sites; more females were recruited in Site 1 (72%) than in Site 2 (42%). This could be due to the different types of work available in these two sites.

All participants were migrants from Myanmar. Migrants from other countries were not found in this study. Nearly all respondents in both sites were schooled in Myanmar (95%) and reported to be able to read Myanmar (>99%); although only 7% of migrants in Site 1 and none in Site 2 were able to read Thai. The most commonly used spoken languages among the respondents in both sites were Myanmar and Dawai. Nearly one-third of migrants in Site 1 were able to speak Thai. The majority of the respondents in both sites were long-term migrants classified as M1 (migrants living in Thailand for 6 months or more). Migrants in Site 1 reported having lived in Thailand on average 79.8 months compared to 61.6 months among migrants in Site 2. More than one-third of migrants in both sites had lived in Thailand for more than 5 years (Table 2).
The primary reason for participants to come to Thailand was for work; more than half were assisted by relatives already living in Thailand. Very few migrants used middlemen to broker their trip to Thailand suggesting that most travel arrangements were done on an individual basis. One-fifth (20%) of those surveyed in Site 2 reported that they were planning to move to another location (mostly back to Myanmar) suggesting a more transient population in the rural site compared to the urban site (5%). Up to 33% and 22% of migrants from Site 1 and Site 2, respectively, had ever returned back to Myanmar. This may be due to the high cost of travel to return to Myanmar—the cost per trip reported by participants ranged from 2,000 to 300,000 Kyats (equivalent to $2.50 to $375 USD at the time of the study).

Differences were detected between Ranong city (Site 1) and Kraburi sub-district (Site 2) in terms of the occupational and residency profile of the migrants (Table 2). Migrants in the urban site were mostly associated with fisheries and those in the rural site predominantly worked on rubber plantations. Site 1 is more likely to have any type of work permit than Site 2, and more likely to have the more stable one-year permit (91%) compared to Site 2 (68%), where respondents were both less likely to have any kind of permit, and more likely to have only a temporary work visa. A majority of these migrants (72%) in Site 2 reported coming to work in Thailand because of the lack of work in Myanmar. Some migrants also cited other

| Table 1. RDS-weighted estimates of basic demographic characteristics of respondents by site. |
| --- |
| Site 1, Ranong City (N = 619) | Site 2, Kraburi (N = 623) |
| **n** | **%** | **95% CI** | **n** | **%** | **95% CI** |
| **Sex** | | | | | |
| Male | 177 | 28 | 23–33 | 361 | 58 | 54–64 |
| Female | 429 | 72 | 67–77 | 246 | 42 | 36–46 |
| **Age group (years)** | | | | | |
| 18–25 | 158 | 29 | 24–35 | 244 | 44 | 38–47 |
| 26–35 | 199 | 32 | 27–37 | 196 | 30 | 26–35 |
| 36–45 | 134 | 21 | 17–25 | 91 | 13 | 11–17 |
| 46–70 | 115 | 18 | 14–22 | 76 | 13 | 11–17 |
| **Mean (SD) Age** | | | | | |
| 34.7 (11.5) | | | | | |
| **Ethnic group** | | | | | |
| Dawai | 283 | 46 | 39–52 | 277 | 49 | 42–51 |
| Myanmar | 251 | 43 | 38–51 | 238 | 40 | 38–46 |
| Mon | 45 | 6 | 3–9 | 64 | 8 | 6–10 |
| Rhakine | 4 | 1 | 0–1.4 | 15 | 2 | 1–4 |
| Karen | 5 | 2 | 0–3 | 10 | 1 | 0–2 |
| **Able to speak** | | | | | |
| Myanmar | 468 | 77 | 73–82 | 387 | 63 | 60–68 |
| Dawai | 273 | 45 | 39–51 | 280 | 51 | 47–56 |
| Karen | 3 | 1 | 0–1.3 | 10 | 1 | 0–2 |
| Mon | 43 | 7 | 4–10 | 60 | 7 | 5–10 |
| Thai | 247 | 33 | 28–37 | - | - | - |
| **Able to read** | | | | | |
| Myanmar | 495 | 99 | 97–100 | 586 | 99 | 99–100 |
| Mon | 11 | - | - | 45 | 5 | 4–7 |
| Karen | 1 | 2 | 1–4 | 7 | 0.6 | 0–1 |
| Other | 23 | 4 | 2–6 | 1 | 0 | 0–1 |
| Thai | 32 | 7 | 4–11 | - | - | - |
| **Place of birth** | | | | | |
| Myanmar | 591 | 98 | 96–99 | 606 | 99 | 99–100 |
| Thailand | 11 | 2 | 1–4 | 1 | 0 | 0–0.5 |
| **Migrant status in Thailand** | | | | | |
| M1(>6 months) | 593 | 94 | 91–97 | 605 | 98 | 99–100 |
| M2(<6 months) | 15 | 6 | 3–9 | 2 | 0.3 | 0–1 |

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Table 2. RDS-weighted estimates of travel and occupational history by site.

|                                | Site 1, Ranong City (N = 619) |          | Site 2, Kraburi (N = 623) |          |
|--------------------------------|-------------------------------|----------|---------------------------|----------|
|                                | n    | %    | 95% CI                    | n    | %    | 95% CI |
| Months in Thailand             |      |      |                          |      |      |        |
| 1–5                            | 15   | 6    | 3–9                       | 2    | 0    | 0–1    |
| 6–12                           | 48   | 10   | 7–14                      | 117  | 16   | 13–20  |
| 13–60                          | 260  | 48   | 43–53                     | 292  | 50   | 46–55  |
| > 60                           | 265  | 36   | 31–41                     | 192  | 34   | 28–37  |
| Plans to move to another place | Yes  | 34   | 5                          | 100  | 20   | 15–23  |
| Ever returned to Myanmar       | Yes  | 229  | 33 28–38                   | 146  | 22   | 19–29  |
|                                | No   | 373  | 67 62–72                   | 454  | 78   | 71–81  |
| Frequency of return            |      |      |                            |      |      |        |
| >1x/month                      | 6    | 10   | 2–18                      | 3    | 9    | 1–20   |
| >2x/year                       | 2    | 5    | 0–9                       | 1    | 1    | 1–2    |
| 1–2x/year                      | 13   | 15   | 5–27                      | 34   | 44   | 32–62  |
| 1x/2–3 yrs                     | 35   | 33   | 22–52                     | 5    | 41   | 27–55  |
| 1x/5 yrs                       | 21   | 24   | 15–41                     | 1    | 4    | 1–6    |
| Have work permit               | Yes  | 342  | 91 87–95                   | 401  | 68   | 65–75  |
|                                | No   | 28   | 9                          | 139  | 32   | 26–35  |
| Type of permit                 |      |      |                            |      |      |        |
| 1 year                         | 109  | 40   | 32–47                     | 65   | 22   | 16–27  |
| Temp passport/visa             | 208  | 58   | 52–66                     | 241  | 78   | 73–84  |
| Border pass                    | 7    | 2    | 1–4                       | -    | -    | -      |
| Why come to Thailand           |      |      |                            |      |      |        |
| No work in MYR                 | 89   | 22   | 17–27                     | 384  | 72   | 66–76  |
| Jobs irregular                 | 79   | 22   | 18–29                     | 236  | 39   | 35–45  |
| Get paid more                  | 250  | 63   | 57–70                     | 145  | 27   | 23–33  |
| Born here                      | 4    | 1    | 0–2                       | 6    | 2    | 1–3    |
| Persecution                    | 1    | 1    | 0–1                       | 1    | 0.4  | 0–0.6  |
| Benefit from employer          |      |      |                            |      |      |        |
| Housing                        | 35   | 10   | 6–13                      | 542  | 99   | 99–100 |
| Water                          | 4    | 4    | 0–2                       | 462  | 87   | 82–91  |
| Land to farm                   | -    | -    | -                         | 13   | 4    | 2–6    |
| Food                           | 3    | 1    | 0–2                       | 10   | 2    | 1–3    |
| Health insurance               | 227  | 58   | 50–63                     | 3    | 1    | 0–2    |
| Previous Industry              |      |      |                            |      |      |        |
| Agriculture                    | 1    | -    | -                         | 71   | 13   | 9–16   |
| Rubber plantation              | 4    | 1    | 0–3                       | 436  | 79   | 75–83  |
| Domestic work                  | 23   | 6    | 3–10                      | 17   | 3    | 2–5    |
| Construction                   | 63   | 15   | 11–20                     | 20   | 5    | 3–8    |
| Factory                        | 37   | 8    | 5–12                      | 2    | 1    | 0–1.2  |
| Fishery                        | 232  | 63   | 55–70                     | -    | -    | -      |
| Other                          | 13   | 4    | 2–8                       | 4    | 2    | 0–3    |
| Duration                       |      |      |                            |      |      |        |
| 1–5 months                     | 1    | 2    | -                         | 1    | 0    | 0–1    |
| 6–12 months                    | 26   | 19   | -                         | 71   | 20   | 17–27  |
| 13–36 months                   | 60   | 41   | -                         | 101  | 29   | 23–33  |
| >36 months                     | 64   | 38   | -                         | 205  | 51   | 45–55  |
| Industry currently working     |      |      |                            |      |      |        |
| Rubber plantation              | -    | -    | -                         | 441  | 77   | 72–82  |
| Palm plantation                | -    | -    | -                         | 67   | 14   | 9–16   |
| Domestic work                  | 14   | 5    | 2–8                       | 14   | 2    | 1–3    |
| Wood                           | 47   | 10   | 7–15                      | 25   | 5    | 3–9    |
| Fishery                        | 218  | 61   | 54–69                     | -    | -    | -      |
| Factory                        | 25   | 7    | 4–10                      | -    | -    | -      |
| Other                          | 28   | 7    | 4–10                      | 2    | 0    | 0–2    |

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benefits received from their employers, including the provision of housing and health insurance.

**Knowledge, treatment-seeking, and health messages**

Knowledge about malaria and how it is transmitted was quite high among the migrants in both sites (Table 3). While knowledge about the symptoms of malaria was generally acceptable with most respondents citing fever and chills to be associated with malaria, fevers as a sign of malaria were more often cited in urban Site 1 compared to rural Site 2. Consistent with malaria incidence data, very few respondents or family members had experienced a fever within the past 3 months in both sites.

Only one third (33%) and 19% of migrants in Site 1 and Site 2, respectively, reported having heard a health message within past 3 months. Among those who received health messages in Site 1, the majority reported receiving those messages through health education by health workers (66%) and brochures (68%). In Site 2, the most common channels were through radio (76%) and health education by health workers (71%). Respondents in Site 1 preferred migrant volunteers (64%) and brochures (47%); whereas those in Site 2 preferred getting their information about health through health workers at facilities (57%) and the radio (49%). Malaria-related messages were more commonly heard in the rural Site 2 compared to the urban Site 1, where most health messages were about HIV/AIDS, STDs, and TB.

Treatment-seeking behaviors for fever among migrant populations were generally good. Half of the respondents in Site 1 and 87% in Site 2 reported having gone to a public government hospital for their last episode of fever (Table 3). In both sites, respondents did not report going to a malaria post for treatment of fever. A further 12% of respondents in Site 1 did not do anything for their fever; while 11% in Site 2 acknowledged purchasing drugs and treatments from local pharmacies. The main factors for choosing where to go for treatment of fever included proximity, better quality, and acceptance of health insurance.

**Malaria prevention**

Ownership of at least one mosquito net was high in both sites (94% in Site 1 and 83% in Site 2) (Table 4). Among those who did not own a mosquito net, the most common reason cited was that they were not available or were too expensive.

Two-thirds of respondents in Site 1 preferred to use conventional untreated nets; whereas an overwhelming majority (82%) of respondents in Site 2 preferred to use long lasting insecticidal treated nets (LLINs). Although not specifically probed, this difference could be due to the repellency effect of the LLIN.

Migrant workers in the urban site generally were employed during the day, while those in the rural site were generally working at night, mostly in the rubber plantations. Nearly all migrant workers in Site 2 reported having slept the previous night in housing structures or sleeping areas provided by their employers and having slept under a mosquito net. However, most of these mosquito nets were not LLINs even though there was a strong preference for LLINs and most mosquito nets had been purchased from the shops or market in both sites.

**Malaria prevalence**

Although the prevalence of malaria detected during the period of the survey was found to be very low in both sites, there was marginal statistical association (p = 0.054) between having traveled back to Myanmar in the past 12 months and serological positivity among respondents in Site 1, but not statistically different among respondents in Site 2 (p = 0.216) using multi-variate logistic regression analysis. By double-read microscopy, only one (1) *P. vivax* positive case
Table 3. RDS-weighted estimates of malaria knowledge, exposure to health messages, and treatment-seeking behavior by site.

|                                | Site 1, Ranong City (N = 619) | Site 2, Kraburi (N = 623) |
|--------------------------------|-------------------------------|---------------------------|
|                                | n    | %   | 95% CI | n    | %   | 95% CI |
| **Malaria transmission**       |      |     |        |      |     |        |
| Mosquito                       | 368  | 74  | 69–79  | 407  | 94  | 91–96  |
| River water                    | 85   | 16  | 13–20  | 126  | 35  | 31–42  |
| Rain                           | 22   | 5   | 3–7    | 134  | 23  | 19–27  |
| Do not know                    | 66   | 14  | 10–18  | 45   | 9   | 7–12   |
| Other insect                   | 10   | 2   | 1–4    | 3    | 1   | 0–2    |
| Eating bananas                 | 12   | 3   | 2–6    | 2    | 0   | 0–2    |
| Forest                         | 86   | 16  | 12–20  | -    | -   |        |
| **Malaria symptoms**           |      |     |        |      |     |        |
| Chills                          | 310  | 58  | 53–63  | 302  | 74  | 69–78  |
| Fever                          | 371  | 75  | 71–80  | 153  | 33  | 28–38  |
| No appetite                     | 18   | 4   | 2–7    | 11   | 24  | 19–28  |
| Sweat                          | 26   | 5   | 3–7    | 42   | 8   | 6–11   |
| Cough                          | 9    | 1   | 0–2    | 19   | 4   | 2–6    |
| Headache                       | 134  | 27  | 23–34  | 97   | 24  | 19–29  |
| Do not know                    | 87   | 18  | 14–22  | -    | -   |        |
| **Heard health message in last 3 months** |      |     |        |      |     |        |
| Yes                            | 210  | 33  | 29–38  | 102  | 19  | 16–23  |
| **Format of health message received** |      |     |        |      |     |        |
| Radio                          | 2    | 2   | 0–5    | 19   | 76  | 69–96  |
| Health education               | 76   | 66  | 46–73  | 17   | 71  | 52–86  |
| Interpersonal                  | 9    | 7   | 2–15   | 7    | 23  | 6–42   |
| TV                             | 13   | 18  | 10–36  | 2    | 10  | 0–61   |
| Brochure                       | 64   | 68  | 60–81  | 2    | 11  | 0–16   |
| Billboards                     | 40   | 41  | 27–51  | 1    | 3   | 0–11   |
| **Preferred format for health messages** |      |     |        |      |     |        |
| Health worker                  | 105  | 15  | 11–18  | 297  | 57  | 54–63  |
| Radio                          | 12   | 1   | 0–2    | 320  | 49  | 45–54  |
| Interpersonal                  | 14   | 3   | 1–5    | 181  | 35  | 32–41  |
| Migrant volunteers             | 422  | 64  | 59–68  | 53   | 7   | 5–10   |
| TV                             | 77   | 16  | 12–20  | 20   | 4   | 3–6    |
| Billboard                      | 152  | 26  | 21–30  | 1    | 3   | 2–4    |
| Brochures                      | 284  | 47  | 42–53  | 2    | 1   | 0–2    |
| **Treatment seeking for last illness** |      |     |        |      |     |        |
| Gov't Hospital                 | 323  | 50  | 44–55  | 548  | 87  | -      |
| Private Hospital               | 98   | 16  | 12–20  | -    | -   | -      |
| Malaria Post                   | -    | -   | -      | 1    | 0   | 0–1    |
| NGO                            | 76   | 11  | 8–15   | 1    | 0   | 0–1    |
| Pharmacy                       | 42   | 6   | 4–9    | 43   | 11  | -      |
| Market/Shop                    | 10   | 3   | 1–6    | 6    | 1   | 0–1.3  |
| CHW                            | 1    | 0.2 | 0–1    | 4    | 0   | 0–1    |
| Self-treatment                 | 8    | 2   | 0–3    | 1    | 0   | 0–1    |
| Nothing/nowhere                | 47   | 12  | 8–16   | -    | -   | -      |
| **Country where treatment was sought** |      |     |        |      |     |        |
| Thailand                       | 486  | 99  | 97–100 | 536  | 89  | 86–92  |
| Myanmar                        | 8    | 1   | 0–3    | 64   | 11  | 8–14   |
| **Why chose that location?**   |      |     |        |      |     |        |
| Closest                        | 243  | 52  | 46–57  | 516  | 84  | 82–88  |
| Better quality                 | 130  | 28  | 23–33  | 94   | 17  | 13–21  |
| Less expensive                 | 49   | 12  | 8–16   | 9    | 3   | 0–5    |
| Treated better                 | 23   | 5   | 2–7    | 99   | 13  | 11–17  |
| Health insurance               | 229  | 42  | 37–47  | 15   | 3   | 2–5    |
| Have translator                | 68   | 14  | 11–18  | 13   | 3   | 1–4    |

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### Table 4. RDS-weighted estimates of malaria prevention by site.

|                                            | Site 1, Ranong City (N = 619) | Site 2, Kraburi (N = 623) |
|--------------------------------------------|--------------------------------|----------------------------|
|                                            | n    | %   | 95% CI | n    | %   | 95% CI |
| Number of mosquito nets owned              |      |     |        |      |     |        |
| 0                                          | 31   | 6   | 3–8    | 108  | 17  | 13–20  |
| 1                                          | 247  | 39  | 35–44  | 460  | 76  | 75–82  |
| 2                                          | 235  | 38  | 33–44  | 25   | 4   | 2–6    |
| 3                                          | 81   | 15  | 11–18  | 8    | 1   | 0–2    |
| 4                                          | 8    | 2   | 1–4    | 2    | -   | -      |
| 5 or more                                  | 3    | 1   | 0–1    | 1    | -   | -      |
| Owned at least one mosquito net             |      |     |        |      |     |        |
| Yes                                        | 575  | 94  | 2–97   | 499  | 83  | 80–87  |
| Type of net preferred                      |      |     |        |      |     |        |
| Conventional                               | 288  | 59  | 54–65  | 76   | 20  | 16–25  |
| LLIN                                       | 237  | 41  | 35–46  | 333  | 82  | 76–86  |
| Hammock net                                | 1    | 0   | 0–1    | -    | -   | -      |
| Usual working time                         |      |     |        |      |     |        |
| Day                                        | 444  | 85  | 80–88  | 78   | 20  | 16–25  |
| Night                                      | 5    | 1   | 0–2    | 327  | 79  | 74–83  |
| Slept under a net last night (among net owners) |      |     |        |      |     |        |
| Yes                                        | 476  | 90  | 87–93  | 404  | 99  | 97–100 |
| Slept under an LLIN                        |      |     |        |      |     |        |
| Yes                                        | 3    | 1   | 0–1    | 8    | 2   | 1–5    |
| Net obtained from?                         |      |     |        |      |     |        |
| **Free**                                   |      |     |        |      |     |        |
| Employer                                   | 4    | 1   | 0–2    | 16   | 4   | 2–5    |
| Health facility                            | -    | -   | -      | 8    | 3   | 1–5    |
| CHW                                        | -    | -   | -      | 6    | 1   | 0–2    |
| Family/friends                             | 42   | 11  | 7–15   | -    | -   | -      |
| **Bought**                                 |      |     |        |      |     |        |
| Employer                                   | 4    | 2   | 1–5    | 2    | 1   | 0–1    |
| Health facility                            | 1    | 0   | 0–1    | 6    | 1   | 0–3    |
| CHW                                        | 1    | 1   | 0–1    | 2    | 1   | 0–1    |
| Family/friends                             | 8    | 2   | 1–4    | -    | -   | -      |
| Market/Shop                                | 374  | 84  | 79–88  | 367  | 93  | 90–95  |

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### Table 5. Summary of individuals with positive blood film, PCR, and/or serology.

| Individual | Site   | Age | Sex | Ethnicity | Travel to Myanmar | Blood Slide       | PCR               | Serology          |
|------------|--------|-----|-----|-----------|--------------------|-------------------|-------------------|-------------------|
| 1          | 1      | 28  | F   | Myanmar   | Yes                | Negative          | P. falciparum, P. vivax | Negative          |
| 2          | 1      | 25  | F   | Dawai     | No                 | Negative          | P. falciparum     | Negative          |
| 3          | 2      | 19  | F   | Dawai     | No                 | Negative          | P. vivax          | Negative          |
| 4          | 2      | 36  | F   | Dawai     | No                 | Negative          | P. vivax          | Negative          |
| 5          | 2      | 38  | F   | Mon       | No                 | Negative          | P. vivax          | P. falciparum, P. vivax |
| 6          | 2      | 30  | M   | Myanmar   | No                 | Negative          | P. vivax          | P. vivax          |
| 7          | 2      | 30  | M   | Myanmar   | No                 | Negative          | P. vivax          | Negative          |
| 8          | 2      | 27  | F   | Myanmar   | Yes                | Negative          | P. vivax          | P. falciparum, P. vivax |
| 9          | 2      | 40  | M   | Dawai     | No                 | Negative          | P. vivax          | P. vivax          |
| 10         | 2      | 30  | F   | Dawai     | No                 | Negative          | P. malariae       | P. falciparum, P. vivax |
| 11         | 2      | 48  | F   | Dawai     | Yes                | P. vivax          | Negative          | Negative          |
| 12         | 2      | 18  | F   | Myanmar   | No                 | Negative          | P. vivax          | Negative          |
| 13         | 2      | 41  | M   | Myanmar   | No                 | Negative          | P. falciparum     | P. falciparum, P. vivax |
| 14         | 2      | 21  | M   | Myanmar   | No                 | Negative          | P. vivax          | P. vivax          |
| 15         | 2      | 27  | F   | Dawai     | No                 | Negative          | P. vivax          | Negative          |

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was detected in an individual from Site 2. This individual was a 48-year-old female from the Dawai ethnic group, who had lived in Thailand for 3 years, and had traveled back to Myanmar once or twice per year. However, this individual was found to be both PCR and serologically confirmed negative, which might suggest the possibility of a false positive slide reading despite double cross-checking.

By PCR, 14 samples were found to be positive (2 individuals in Site 1 and 12 individuals in Site 2) (Table 5). The majority of cases were *P. vivax* (10/14) with two *P. falciparum*, one mixed *P. falciparum* and *P. vivax*, and one *P. malariae* infections. PCR analysis demonstrated an overall weighted prevalence of 0.5% (95% CI, 0–1.3%) in the urban site (Ranong City) and 1.0% (95% CI, 0.5–1.7%) in the rural site (Kraburi) for both parasite species—although the predominant species was *P. vivax*, which presents a limited risk for spread of artemisinin resistance and to date has only been identified in *P. falciparum*.

There were significant differences in serological profiles between the two sites (Table 6). The population in Site 2, a rural site with proximity to the forest fringes, had higher serological reactivity to *P. falciparum* (24%) and *P. vivax* (15%) antigens, compared to the urban site (4% *P. falciparum* and 3% *P. vivax*). Serological reactivity to either *P. falciparum* or *P. vivax* antigens among the population in Site 2 was 31% compared to 7% in Site 1. Particularly in Site 2, there was a clear association between increasing seroprevalence and age strongly suggesting that seroprevalence may be cumulatively representing current and past exposure (Fig 1). Furthermore, serological response appeared higher to each combined antigen implying differential responsiveness and supporting the use of multiple antigens for serological screening in low transmission settings. Seroconversion rates represent the rate at which the population becomes antibody positive to specific malarial antigens. Assuming a constant rate of reversion, measurable differences in exposure to both *P. falciparum* and *P. vivax* between the two study populations can be observed (Fig 2).

Table 6. RDS-weighted estimates comparing biological results by site.

|                      | Site 1, Ranong City (N = 619) | Site 2, Kraburi (N = 623) |
|----------------------|-------------------------------|----------------------------|
|                      | n % 95% CI                    | n % 95% CI                  |
| **PCR** (P. falciparum or P. vivax) | Positive 2 0.5 0–1.3 | 12 1 0.5–1.7 |
|                      | Negative 606 99.5 98.7–100  | 595 99 98–100               |
| **Serological profile** | *P. falciparum* 26 4 2–6 | 157 24 21–29 |
|                      | *P. vivax* 16 3 1–5           | 118 15 13–18                |
|                      | Either *P. falciparum* or *P. vivax* 37 7 4–9 | 201 31 28–37 |

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Fig 1. Mean of seropositivity to any tested antigens for *P. falciparum* (left) and *P. vivax* (right) by age groups.

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Network associations

One of the key aspects of the RDS methodology was the determination of network patterns among the respondents. In Site 1, most of the recruiters were either friends (47%) or neighbors (46%) of the respondent; whereas, in Site 2, a majority of the recruiters were identified as employers (Table 7). The majority of respondents in both sites had relatively modest network sizes. That is, each respondent in Site 1, on average, reported having seen or interacted with 17 other migrants during the past week; whereas, the average was 20 migrants per week for Site 2. Personal interest in this research was cited as the most common reason for joining the study in both sites. Analysis of migrants in Site 1 who have been in Thailand for 6 months or more (M1) compared to those who have been in Thailand for less than 6 months (M2) shows that M1 migrants with an average network size of 10.4 tended to be more homophilous (Hx = 0.552)–that is, they preferred to associate more within their own established networks. On the other hand, M2 migrants with a smaller average network size of 4.4, exhibited strong heterophily (Hx = -1.0) (or tendency to not associate within their own networks).

Discussion

Respondent-driven sampling has been used for nearly 20 years for the sampling of hard-to-reach populations such as intravenous drug users and commercial sex workers [18,19]. This methodology has matured to serve as the basis for the surveillance of HIV/AIDS and other biological markers [20]. More recently, RDS was adaptively used along the Thailand-Cambodia border to study migrant populations [10,11] that could contribute to spreading artemisinin resistant malaria parasites throughout the GMS. Despite the advantages of being able to sample from such hidden, hard-to-reach populations, there are challenges with sampling errors, statistical inferences, and wide confidence intervals that should be kept in mind when interpreting data from RDS [21]. This innovative sampling technique aims to provide stable estimates for populations lacking adequate sampling frames, but is not without its drawbacks. Biases can be
introduced from the high variance of estimates and fairly narrow confidence intervals obtained from the RDS methodology. Discussed in detail elsewhere [9], these issues should be considered in the interpretation of results. Despite these statistical limitations, however, the operational application of RDS as a means for data collection and biological screening of hard-to-reach populations could make this an attractive tool for national programs seeking representative evaluation data.

Accessing migrant populations is intrinsically difficult for public health programs, particularly those in the GMS, where borders are porous and often where illicit forest-related activities occur. Furthermore, migrant populations who are unregistered and without work permits or have entered the country illegally often will avoid government facilities for fear of being caught, which makes accessing these populations that much more difficult. The majority of migrant workers in both study sites have work permits and were likely more amendable to enrolling in this study. Developing innovative and effective methods of reaching migrant populations was identified as a priority for responding to artemisinin resistance [22], and ultimately for the elimination of malaria [23].

To our knowledge, this is the first use of RDS to collect biological specimens for malaria from cross-border migrant populations. More than 1,200 migrants from Myanmar who are living and working in urban and rural sites in Thailand were screened using standard
microscopy, q-PCR, and serological assays for the detection of malaria infection, previous exposure, and assessed for their access to malaria prevention and treatment services. Malaria prevalence detected through microscopy yielded only one *P. vivax* malaria case, raising the question whether microscopy alone is sufficient in such low transmission settings.

To ascertain previous malaria infection, serology has been proposed as a useful way for estimating exposure to malaria parasites in low transmission settings [24–26]. Serological analyses using antigen-specific assays for both *P. falciparum* and *P. vivax* yielded an interesting distribution of seropositivity when comparing between the urban and rural sites. Sero-reactivity to *P. falciparum* antigens ranged from 4% (2%-6%) in Site 1 compared to 24% (21%-29%) in Site 2, suggesting not surprisingly, significantly higher risks of *P. falciparum* infection in those residing in the rural site compared to the urban site. Similar patterns of sero-reactivity to *P. vivax* antigens were also observed between the two sites. These results suggest that the rural site, although having only one parasitemic individual identified through gold-standard microscopy at the time of this cross sectional survey, may still experience substantial seasonal malaria transmission as evidenced by the prevalence of antibodies in these relatively stationary migrant populations. Additional investigations may be needed to determine whether these individuals were exposed and infected in Myanmar or in Thailand, though the data suggest that cross border movement occurs less frequently in this population than previously expected. Furthermore, in Site 1, the two malaria cases detectable by PCR were *P. falciparum* and serologically negative; whereas in Site 2, most of the PCR positive cases were *P. vivax* with a much more diverse serological profile, suggesting greater exposure to malaria antigens over time.

Most migrants in Ranong Province are long-term residents and have not traveled frequently back to Myanmar. According to the definition used in Thailand, these migrants would be considered M1 migrants (those who have lived in Thailand for 6 months or more). Migrants in both sites had access to and reported use of mosquito nets; however, the vast majority of these were conventional, untreated mosquito nets that were purchased in the markets. The current net culture that exists among these migrant populations is encouraging, and strategies (e.g., employer-based distribution) should be considered to replace and/or convert these conventional nets into more effective LLINs that could have greater impact on the reduction of malaria transmission. Furthermore, three-quarters of those in Site 2 were rubber tappers and alternative outdoor personal protection methods (e.g., insecticide-treated clothing) should also need to be considered.

Examination of a group’s tendency to associate within or outside one’s own networks, average network sizes, and social network analyses offer insights into the potential use of influential individuals to promote health and treatment-seeking behaviors. This study highlights that the network sizes of migrants in urban Ranong are larger and more homophilous (i.e., tendency to associate within their own established networks) compared to migrants in the rural setting, who tend to have smaller network sizes and stronger affiliations with their employers. Using RDS to better understand the social and occupational networks of migrants in different settings could help malaria programs better target delivery of malaria services such as distribution of LLINs, health education and promotion, or even routine screening and treatment of migrant populations. The benefits of identifying migrants who otherwise would not have been reached through routine public services, and providing health care services for these individuals are worth considering. Furthermore, analysis of networks and mobility patterns through the use of RDS can contribute valuable epidemiological and service utilization data from these hard-to-reach populations which is increasingly critical for malaria surveillance in the context of elimination.

There were challenges and limitations that may have affected the outcome of this study. Firstly, relatively high transportation costs and long travel distances to the enrollment site may
have affected recruitment of participants in the more rural site. Measures were put in place to mitigate these barriers, including moving the recruitment sites closer to the migrants wherever possible. Secondly, there may be a risk of convenience sampling occurring if coupons were not distributed through networks. Therefore, the selection of seeds is critical [27] and should be reflective of the target population profile. It should be noted that the migrant workers in this study were relatively accessible and may not be representative of the migrant population in Ranong. Thirdly, as with cross-sectional surveys, measuring parasitemia through the use of RDS only provides a snapshot of the prevalence and longitudinal monitoring may be needed to better understand malaria transmission trends. Lastly, formative research must inform the appropriateness of the use of RDS or snowball sampling to where social networks exist within the population of interest.

The low prevalence of malaria parasitemia, relatively homogenous networks among these migrants, and limited frequency of travel to and from other areas of malaria transmission, specifically Myanmar, all suggest limited potential for spread of artemisinin resistance along the Kawthaung-Ranong corridor through these populations but the frequency and extent of population mobility in this region can be variable. Described here, RDS can be a potential programmatic tool to obtain reasonably representative estimates among migrant populations, to better understand different migrant networks and behaviors, and ultimately to improve access to and delivery of malaria services to these hard-to-reach populations and their associated networks.

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