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Asymptomatic malaria infection at the China-Vietnam border: Knowledge and implications for the cross-border migrant population during the COVID-19 pandemic

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ABSTRACT

Background: Eliminating malaria along the China-Vietnam border remains one of the greatest challenges in China, especially during the coronavirus disease 2019 (COVID-19) pandemic, which has disrupted the continuity of malaria control and elimination programs. Understanding the factors associated with asymptomatic malaria infection will inform control interventions aimed at elimination of the disease among migrants from Vietnam working in China, who constitute an at-risk population.

Methods: From March 2018 to September 2019, 108 migrants from Vietnam working in Ningming County, Guangxi, were enrolled in this study. Each person was interviewed using a structured questionnaire. Blood samples were collected and sent for PCR detection and sequencing. The obtained sequences were analyzed using the BLAST program and DNAMAN software.

Results: The proportion of participants with malaria knowledge was low, with 19.4% (21/108) reporting knowledge about transmission, 23.2% (25/108) reporting knowledge about clinical symptoms, 7.4% (8/108) reporting awareness of the risk of death and 14.8% (16/108) reporting awareness of prevention methods. No significant difference in the malaria knowledge rate was found among occupational groups, except in the migrant worker group, whose knowledge rate was higher than those in the other occupational groups ($\chi^2 = 32.452$, $p < 0.001$). Although most of the participants (80.6%, 87/108) owned mosquito nets, only approximately half of the participants (49.1%, 53/108) reported using bed nets. The parasitological analysis revealed that 5.6% (6/108) of all the participants were positive for malaria, including 5 participants with *Plasmodium falciparum* and 1 participant with *Plasmodium vivax* malaria. There were no statistically significant differences in the positivity rates among the different age, sex, family-size, nationality, occupational, and behavior groups. The positivity rates in individuals who did not use mosquito nets, did not use mosquito coils, and did not install mosquito nets were 4.8% (1/21), 6.8% (3/44), and 3.6% (2/55), respectively.

Conclusion: Health education focused on high-risk populations, such as migrant workers and forest goers, should be strengthened. Verbal communication and information transmission via the internet, radio, and mobile phone platforms may be required during the COVID-19 pandemic. Further risk assessments and proactive case detection should also be performed in Ningming County and other border counties in Guangxi to detect active and asymptomatic infections in a timely manner and prevent re-establishment of the disease in these communities.

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1. Background

The malaria prevalence rates in border areas are often higher than those in other areas due to limited access to health services; a lack of treatment-seeking behavior among marginalized populations; difficulties in implementing prevention programs in hard-to-reach communities, often due to challenging terrain; and the constant movement of people across national boundaries [1]. China has eliminated malaria among its population, and no indigenous cases have been reported since 2017; accordingly, the World Health Organization (WHO) declared China malaria-free [2,3]. However, infections in border areas still pose a great challenge [4,5]. Guangxi Zhuang Autonomous Region (Guangxi) includes 8 counties along the Vietnam border and was once a high malaria-endemic area [6]. The malaria incidence rates in these 8 counties ranged from 125.58 to 605.77 per 10,000 individuals [7]. A continuous effort by the government and technical staff drastically reduced the incidence to 0.22 per 100,000 in 2010, and no local infection caused by *Plasmodium falciparum* has been reported since 1996. Ningming County is one of the 8 border counties and was once a malaria hyperendemic area, with 31,200 malaria cases per 10,000 individuals reported in 1953. *Plasmodium vivax* became the predominant species after the elimination of *P. falciparum* in 1988, and the elimination goal was achieved before 2000. However, imported malaria cases in Ningming County, similar to nationwide cases, have increased due to the frequent movement of migrants. Imported malaria caused by frequent migration remains the greatest challenge for border areas, as *Anopheles* mosquitoes are endemic in China. Blood tests performed between 2000 and 2010 revealed 7 positive cases among a total of 3439 migrant individuals, for a positivity rate of 0.20% [8]. The coronavirus disease 2019 (COVID-19) pandemic and the actions taken in response to the pandemic will have far-reaching effects on other diseases, poverty, and economic growth [9,10]. Considering the similarity of symptoms between malaria and COVID-19, clinicians may misdiagnose malaria as COVID-19 and vice versa. The lockdown and restriction of movement of health care providers due to the COVID-19 pandemic disturbed the continuity of malaria control and elimination programs, such as the distribution of seasonal malaria chemoprevention and insecticide-treated bed nets, which resulted in increases in the numbers of malaria cases and associated deaths [11,12]. Few publications have investigated and evaluated the malaria risk along the China-Vietnam border. Therefore, we administered a malaria knowledge survey and performed parasitological examinations among migrant workers to evaluate the risk and determine malaria-related needs among this population.

2. Methods

2.1. Study sites and sample collection

The study was performed between March 2018 and September 2019 in Ningming County, which is on the China-Vietnam border in Guangxi, using a cross-sectional design. Ningming County is home to 319,000 people and contains the longest border, at 212 km of southwestern Guangxi and Vietnam (Fig. 1). It has 1 national port and 3 county border exchange points; Vietnamese migrants frequently cross the border into China, with some migrants working in Ningming in the morning and returning to their home in Vietnam in the evening. Historically, several vector species were found in Ningming, including *Anopheles sinensis*, *Anopheles minimus*, and *Anopheles jeyporiensis*. Before the commencement of the study, small meetings were held with Vietnamese migrants to provide a clear explanation of the study objectives and their involvement. The selection of the participants was universal and based on the availability and willingness of the people. Verbal consent was obtained from the participants. Finally, 108 Vietnamese migrants were selected for inclusion.

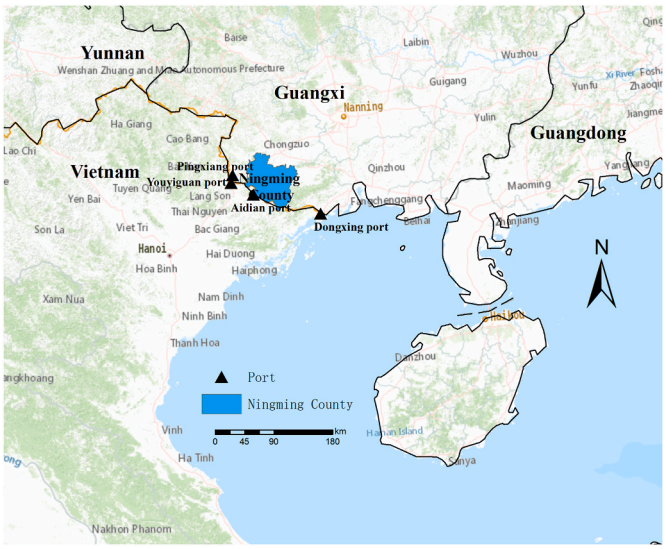

Fig. 1. The study site in Guangxi Zhuang Autonomous Region, China. The study site (Ningming County) is labeled in blue. All ports (Pingxiang, Youyiguan, Aidian, and Dongxing) for entry of the migrant population are labeled with black triangles. The map was created in ArcGIS 10.1.

2.2. Questionnaire

A questionnaire was used to obtain participant information, such as sex, ethnicity, occupation, travel history, knowledge about malaria and knowledge about malaria prevention. The National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (NIPD) designed the questionnaires and obtained informed consent. The first part of the questionnaire included questions about demographic characteristics, and the second part included questions about the participant's attitude towards and understanding of malaria transmission, perception of its cause, recognition of signs and symptoms, and knowledge about preventive measures and practices. The questionnaire was translated into Vietnamese before administration (Additional File 1 shows the questionnaire in English).

2.3. Parasite identification and genotyping

A total of 108 blood samples were collected from March 2018 and September 2019 and examined at enrollment. Approximately 100 μl of blood was obtained from a finger prick, spotted on a piece of 3 MM Whatman filter paper (GE Healthcare, Boston, MA, USA) and allowed to air dry. Each of the samples was labeled with a study number and stored at −20 °C until extraction. The genomic DNA from approximately 20 μl of each dried blood sample was extracted using a QIAamp DNA blood kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. Malaria parasite species were confirmed using nested PCR analysis of the 18S rRNA gene. The PCR products were separated by electrophoresis on a 1.5% agarose gel and subjected to Sanger sequencing (Shanghai BioTechnologies Co., Ltd., Shanghai, China).

2.4. Data analysis

Asymptomatic malaria infection was defined as malarial parasitemia of any density in the absence of fever or other acute symptoms in individuals who have not received recent antimalarial treatment. Sequences were analyzed using the BLAST program (http://blast.ncbi.nlm.nih.gov/). Multiple nucleotide sequence alignment and analysis was performed using the DNAMAN software editor (https://www.lynnon.com/pc/framepc.html). A map showing the study sites was created in ArcGIS 10.1 (Environmental Systems Research Institute, Inc.). Statistical

analyses were performed using R software (version 4.0.2, R Foundation for Statistical Computing, Vienna, Austria). The chi-squared test was used to compare categorical variables between groups. A P value < 0.05 was considered statistically significant.

2.5. Ethical consideration

The study was reviewed and approved by the ethics committee of NIPD (No. 2019008).

3. Results

3.1. Demographic study

A total of 108 migrants who travelled to Guangxi from Vietnam between March 2018 and September 2019 were enrolled in this study. All participants were Vietnamese; 52.8% were male (n = 57), and 47.2% were female (n = 51). Most participants were aged 20–30 (36.1%) and 30–40 (40.7%) years. The average age of the participants was 32 years and ranged from 16 to 54 years. Most participants were migrant workers (50.9%) and farmers (37.0%). Eighty-five people crossed the border Vietnam to China only 1 time (78.7%), but the range of the number of border crossings was 0–6. Twenty-six people (24.1%) stayed in China for less than 1 week, 50 people (46.3%) stayed for 1 month, 14 people (13.0%) stayed 1–6 months, and 5 people (4.6%) stayed longer than 6 months. Most of the migrants went to Guangxi (80.6%), and a small number worked in Guangdong (5.6%) (Table 1).

3.2. Malaria knowledge and prevention behaviors

A survey of malaria knowledge among all the participants found that the proportion of participants with knowledge about malaria transmission was 19.4% (n = 21), and that of participants with knowledge about malaria symptoms was 23.2% (n = 25). The proportion of participants with awareness of the risk of death from malaria was 7.4% (n = 8), and that of participants with awareness of prevention methods was 14.8% (n = 16). No significant difference was found among the occupational groups, except in the migrant worker group, whose knowledge rate was higher than those in the other occupational groups, including farmers and plant workers ($\chi^2 = 32.452$, $p < 0.001$) (Table 2). Regarding prevention and control methods, 80.6% (n = 87) of the participants had mosquito nets in their homes, and 58.3% (n = 63) had screen doors and windows installed. A total of 73.2% (n = 79) of the participants reported 2 people sleeping under the bed net at night, and 7.4% (n = 8) reported 1 person. Approximately half of the respondents (49.1%, n = 53) used bed nets. A small proportion (7.4%, n = 8) of the participants reported sleeping outside in summer.

Table 1
Demographics of and positive infection rates in participants.

General	Participants		Positive for infection		χ^2	*P*
	N	%	n	%		
Sex						
Male	57	52.78	4	7.02	0.492	0.483
Female	51	47.22	2	3.92		
Age						
−20	8	7.41	0	0.00	1.486	0.476
20-30	39	36.11	2	5.13		
30-40	44	40.74	2	4.55		
50-	17	15.74	2	11.76		
Family size						
0-4	68	62.96	4	5.88	1.001	0.606
4-	39	36.11	2	5.13		
Nationality						
Jing	33	30.56	4	12.12	0.486	0.496
Han	35	32.41	1	2.86		
Other	30	27.78	1	3.33		
Occupation						
Farmer	40	37.04	2	5.00	0.153	0.926
Worker	55	50.92	4	7.55		
Other	13	12.04	0	0.00		

3.3. Malaria parasitological analysis

Of the 108 participants, 5.6% (n = 6) tested positive for malaria, including 5 patients with *P. falciparum* and 1 patient with *P. vivax* malaria. The positivity rate was 7.0% in males ($P > 0.05$) and 3.9% in females. There were no statistically significant differences in the positivity rates among the age, sex, family-size, nationality or occupational groups (Table 1). No statistically significant differences in positivity rates were observed for the number of outbound visits, time of overseas stay, location of entry or exit or level of malaria knowledge ($P > 0.05$). Malaria knowledge about transmission, symptoms, and prevention measures was low among the positive patients (9.52%, 8.00%, and 12.50%, respectively). The positivity rates among individuals who did not use mosquito nets, did not have mosquito nets installed, and did not use mosquito coils at home were 4.8% (1/21), 6.8% (3/44), and 3.6% (2/55), respectively. The positivity rate among individuals who slept outside was 0.0%, but the differences in the positivity rates between the different sleeping behaviors were not statistically significant ($P > 0.05$) (Table 3).

4. Discussion

The transmission of infectious diseases, such as malaria and COVID-19, across borders poses a major obstacle to achieving and maintaining the elimination of communicable diseases [12,13]. Our study detected 6 asymptomatic infections, accounting for 5.6% of the migrant population from Vietnam, in this study. Unlike the China-Myanmar border, which poses a substantial challenge for malaria elimination in Yunnan Province due to the high prevalence of *P. vivax* and *P. falciparum* in northern Myanmar [14,15], the China-Vietnam border seems to be "forgotten" in terms of malaria prevention because of the low incidence of malaria in northern Vietnam. The incidence rates in two border counties on the China-Myanmar border, Jingxi and Longzhou counties, decreased to 4.0 and 4.27 per 100,000 in the late 20th century, respectively, and no indigenous cases occurred during the early 21st century [16,17]. Hai Phong, located in northern Vietnam, had an average positive predictive value of 0.10% in 2010–2014 [18]. This value was similar in the Guangxi-Vietnam border area and the Yunnan-Vietnam border area. For example, the annual malaria positivity rate was 358.62 per 1000 in Hekou County in Yunnan Province, which is recognized as a malaria hyperendemic area, on the Yunnan-Vietnam border but decreased to 0.18 per 1000 in 2008. In 2015, Hekou became the first county to achieve malaria elimination along its border with Vietnam [19].

Despite achieving the goal of malaria elimination in the border counties of Guangxi [7], the frequently mobile population poses some challenges. First, how can authorities detect asymptomatic infections in a timely manner? Detection of asymptomatic infections is crucial for malaria control interventions on both sides of the border. In Vietnam, the high-risk migrant population has been proposed to be forest goers, who may live in forest border regions, have little knowledge about malaria and have limited access to preventive and therapeutic services [20,21]. As malaria transmission has declined in Vietnam, the high prevalence rates of asymptomatic and submicroscopic infections have become the main challenges [22–25]. Asymptomatic infected individuals generally do not seek treatment and have a low parasite density that is undetectable on microscopy examination. Therefore, parasites persist in these individuals from one season to the next, maintaining local transmission [26]. Asymptomatic infections have been reported in central and southern Vietnam, but our study found that residents in northern Vietnam are also at risk for asymptomatic infections. Second, the susceptibility of *P. falciparum* to artemisinin-based combination therapy (ACT) and *P. vivax* to chloroquine has declined in

Table 2
Participant awareness of factors contributing to malaria transmission, hazards, control, prevention and symptoms.

General	Participants	Awareness of malaria transmission		Awareness of malaria hazards		Awareness of malaria control and prevention measures		Awareness of malaria symptoms	
	N	n	%	n	%	n	%	n	%
Sex									
Male	57	13	22.81	5	8.77	11	19.3	15	26.32
Female	51	8	15.69	3	5.88	5	9.8	10	19.61
Age									
−20	8	0	0.00	0	0.00	0	0.00	0	0.00
20-30	39	11	28.21	2	5.13	8	20.51	13	33.33
30-40	44	7	15.91	3	6.82	6	13.64	8	18.18
40-	17	3	17.65	3	17.65	2	11.76	4	23.53
Family size									
0-4	68	12	17.65	4	5.88	8	11.76	14	20.59
4-	39	9	22.50	4	10.00	8	20.00	11	27.50
Nationality									
Jing	33	8	18.18	4	9.09	5	11.36	11	25.00
Han	35	6	16.67	3	8.33	4	11.11	6	16.67
Other	30	7	25.00	1	3.57	7	25.00	8	28.57
Occupation									
Farmer	40	3	7.50	2	5.00	1	2.50	2	5.00
Worker	55	17	30.91	5	9.09	15	27.27	22	40.00
Other	13	1	7.69	1	7.69	0	0.00	1	7.69

Table 3
Differences in positive infection rates among groups with different malaria-related behaviors, attitudes, and practices.

Behaviors, attitudes, and practices	Participants		Positive for infection		χ^2	*P*
	N	%	n	%		
Number of customs visits per year in the last 3 years?						
1	85	78.70	6	7.06	1.719	0.423
≥2	23	21.30	0	0.00		
Length of stay in China?						
One week	26	24.07	1	3.85		
One month	50	46.30	4	8.00	0.377	0.828
A month to half one year	14	12.96	1	7.14		
Half one year to one year	5	4.63	0	0.00		
Destination of entry and exit?						
Guangxi	87	80.56	4	4.60	1.599	0.450
Guangzhou	6	5.56	1	16.67		
Vietnam	15	13.89	1	6.67		
Knowledge about malaria						
How is malaria transmitted?						
Correct	21	19.44	2	9.52	0.782	0.376
Incorrect or did not know	87	80.56	4	4.60		
What are the main symptoms of malaria?						
Correct	25	23.15	2	8.00	0.370	0.540
Incorrect or did not know	83	76.85	4	4.82		
Is malaria a direct threat to life if untreated?						
Correct	8	7.41	1	12.50	0.794	0.373
Incorrect or did not know	100	92.59	5	5.00		
How can malaria be prevented?						
Correct	16	14.81	2	12.50	1.726	0.189
Incorrect or did not know	92	85.19	4	4.35		
Behavior to prevent malaria						
Do you have mosquito nets at home?						
No	21	19.44	1	4.76	0.031	0.860
Yes	87	80.56	5	5.75		
Have you installed screens on doors and windows?						
No	44	40.74	3	6.82	0.207	0.649
Yes	63	58.33	3	4.76		
How many people sleep under mosquito nets?						
0	21	19.44	2	9.52	0.136	0.712
1	8	7.41	0	6.90		
2	79	73.15	4	5.06		
Have you used mosquito coils?						
No	55	50.93	2	3.64	0.787	0.375
Yes	53	49.07	4	7.55		
Do you sleep outside in the summer?						
No	98	90.74	6	6.12	0.519	0.471
Yes	8	7.41	0	0.00		

Vietnam [27,28]. The risk of the spread of antimalarial drug resistance across the border is high due to the importation of multidrug-resistant malaria by the migrant population [29]. The emergence of Kelch 13 mutations associated with increased ring survival rates and parasite clearance delay have been reported on the China-Myanmar border [30–33]. Although there is little evidence suggesting the emergence of an ACT-resistant *P. falciparum* strain along the China-Vietnam border, more attention should be given to monitoring of the pathogen population and evaluating the potential emergence of ACT resistance. *P. vivax* is an important human malaria parasite that is endemic to Asia [34], and resistance of *P. vivax* was still a challenge for malaria elimiantion in this region. Third, the malaria knowledge rate was low among the migrant population in our study. Notably, border residents, especially young adults and women, had little malaria knowledge [35,36]. Only 19.4% of the surveyed population in our study knew that malaria is transmitted through mosquito bites, and 23.2% of them had awareness of malaria symptoms.

This study has some limitations. First, not all of the questionnaires in the survey were completed by the participants, which may be due to the use of only the English language version. Second, the study focused on the migrant population along the China-Vietnam border. Only 1 of the 8 border counties in China was selected, and the participant sample size was small. The results obtained from this study may not be representative of the other counties along the China-Vietnam border. Most of the participants were low-income labor workers and did not represent the entire migrant population. These biases may explain the relatively high infection rate.

5. Conclusions

In summary, this study indicated poor malaria knowledge among the migrant population on the China-Vietnam border and the presence of asymptomatic infections, which may increase the risk of malaria re-establishment in the post-elimination stage in Guangxi. The findings of this study indicate that health education focusing on high-risk populations, such as migrant workers and forest goers, should be strengthened. In areas such as Guangxi, where literacy and language may be a barrier, health education based on verbal communication via the internet, radio, and mobile phone platforms may be required during the COVID-19 pandemic. Further proactive case detection should be performed in Ningming County and other border counties in Guangxi to detect active and asymptomatic infections that may promote the re-establishment of malaria.

Ethics approval and consent to participate

This study was reviewed and approved by the ethical committee of the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (NIPD, China CDC, No. 2019008).

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Abbreviations

Not applicable.

CRediT authorship contribution statement

Hong Tu: Writing, Methodology, Software. **Jun Feng:** Conceptualization, Writing – review & editing. **Kangming Lin:** Investigation, Field survey and Investigation, All authors have read and agreed to the published version of the manuscript. **Wang Peiyu:** Investigation, Field survey and Investigation, All authors have read and agreed to the published version of the manuscript. **Xiang Shaomi:** Investigation, Field survey and Investigation, All authors have read and agreed to the published version of the manuscript. **Luo Lingyun:** Investigation, Field survey and Investigation, All authors have read and agreed to the published version of the manuscript. **Li Jian:** Investigation, Field survey and Investigation, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2022.102307.

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