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Research Article

A Study of Malaria Prevalence among People in Conflict-Affected Areas and the Effectiveness of Leveraging Medical Laboratory Training

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Abstract

Aim: *The military coup in Myanmar in 2021 has exacerbated the challenges faced by conflict-affected regions, including heightened health risks such as malaria. The aim of this study is to assess the effectiveness of leveraging medical laboratory training in combating malaria within conflict zones since military coup, and to analyze whether improved diagnostic capabilities, treatment protocols, and preventive measures result in better outcomes for affected populations.*

Study design: *In response to the urgent need for enhanced epidemiological surveillance, a cross sectional descriptive study among febrile patients in local villages and townships of conflict-affected areas situated in Chin State, Sagaing Region and Karenni State was conducted between October to December 2023.*

Materials and Methods*: Capillary blood was collected via finger prick after receiving informed consent. Malaria infection was detected by Rapid Diagnostic Test kits and microscopy. A total number of 2147 patients were included in this study.*

Findings and results*: 5.49% (118) participants were positive for malaria parasites. Of all positive cases, 28% of samples (33) were positive for Plasmodium falciparum and 72% of all positive samples (72) showed Plasmodium vivax. 5 cases were found to be mixed infections: P. falciparum and P. vivax. There was higher incidence of malaria among male (94 cases, 79.66% of all positive samples) than female (24 cases, 20.33% of all positive samples). In this study, levels of parasitemia were calculated by microscopy, and the two species of malaria, P. falciparum and P. viviax, had been identified together with parasite count (parasite/ml). None of all positive samples for both species (P. falciparum and P. vivax) exceeded 0.3%. All the participants in this study showed typical signs and symptoms such as fever with chills and rigors. All were uncomplicated malaria patients. Malaria positive cases were found to be the highest in Chin State followed by Karenni and Sagaing Region. This research also investigates the impact of Humanitarian Lab Family which is a community service organization and its medical laboratory training platform in these areas, particularly focusing on malaria infection rates.*

Key words- *Malaria epidemiology, Laboratory training platform, Community based humanitarian aids*

1. Background

1.1. Malaria epidemiology

Malaria has been prevalent worldwide, causing significant morbidity and mortality among affected populations. It continues to be endemic in many tropical regions, and there is concern that climate change could result in its reemergence in areas currently free of the disease (1). Being a major cause of death in tropical and sub-tropical countries, malaria kills each year over 1 million people globally (2).

In 2022, nine countries in the South-East Asia Region contributed to about 2% of the burden of malaria globally (5.2 million cases). Most malaria cases in the Region were concentrated in India (66%) and about 94% of deaths were in India and Indonesia. Between 2019 and 2022, Myanmar saw a seven-fold increase in cases, from 78 000 to 584 000, fueled by political and social instability (3). In the Greater Mekong Subregion (GMS), Myanmar bears the greatest burden of malaria, representing a substantial public health challenge in the country. It holds the highest malaria burden among all countries in the Greater Mekong Sub-region (GMS), comprising approximately 70% of reported cases in the region (3,4). In Myanmar, 291 townships (out of 330) are malaria endemic according to 2018 WHO Myanmar newsletter special (5).

In 2022, the global malaria case incidence was 58.4 against a target of 26.2 cases per 1000 population at risk. Progress towards the 2025 milestone for case incidence is now 55% off track and, if this trajectory persists, the corresponding 2030 GTS target will be missed by a staggering 89% (6).

1.2. Malaria species and their pathogenesis

Malaria is caused by parasitic organisms belonging to the Plasmodium genus, which are conveyed into the bloodstream of human hosts by the bite of female anopheles mosquitoes. The species of protozoa that are responsible for malaria infections in humans are Plasmodium vivax, P. malariae, P. ovale, P. falciparum, and P. knowlesi. P. vivax and P. falciparum give the greatest threat to public health, with P. falciparum being more prevalent and causing the greatest morbidity and mortality (7).

In contrast to other human malaria strains, P. vivax can develop, albeit at a slower pace, in the vector at lower temperatures ranging from 16 to 18 °C, whereas P. falciparum requires temperatures above 18 °C for growth (8). Reactivation of P. vivax hypnozoites from the dormant stage of the parasite causes clinical relapses; hypnozoites are carried silently, with no symptoms, and humans can transport the parasite to new areas or areas where malaria transmission has been interrupted (9).

Plasmodium knowlesi, a malaria parasite primarily found in monkeys, gained significant attention following the emergence of numerous cases of human P. knowlesi infection reported in Sarawak, Malaysian Borneo, in 2004 (10).

1.3. Malaria workup within community

In Myanmar, 62 % of the population in the country lived in malaria-risk areas (21.4 % in high risk, 17.9 % in moderate risk, 22.4 % in low-risk areas) according to Myanmar national strategic plan 2010–2015 (11). According to that plan, the number of malaria cases reported by the National Malaria Control Programme (NMCP) are only confirmed cases where diagnostic services are available (12).

Evidence revealed, in settings with lower malaria incidence, expanding the range of services sought by the community and the malaria volunteers could provide, increased utilization of volunteer's services and enables more malaria testing in the community (13). Therefore, to maintain the Myanmar community-delivered model's effectiveness and popularity, Myanmar National Malaria Control Programme rolled out the Integrated Community Malaria Volunteer (ICMV) programme throughout Myanmar in 2017–18 (14).

1.4. Myanmar healthcare services after 2021 military regime

Before military coup in 2021, over 15,000 out of 67,285 villages in Myanmar (approximately 23%) had at least one malaria volunteer and able to use the malaria volunteer model, in conjunction with the government health facility-based model, to reduce the annual parasite incidence in several townships within recent decades.

Since the beginning of February 2021 military coup, Insecurity Insight has identified at least 1,087 attacks on Myanmar's health care system stating that at least 880 health workers had been arrested, 97 killed and 117 injured undermining health care providers' ability to maintain safe staffing levels to effectively meet patient needs; health facilities had been damaged over 180 times impacting the population's access to health care (14).

Humanitarian Lab Family has managed to determine the prevalence of malaria within conflict-affected populations. This involved conducting surveys and collecting data from affected regions to understand the extent of the malaria burden. Hence, this study shed light on how conflict influenced the proliferation and

control of malaria, delving into aspects like displacement, the collapse of healthcare systems, and restricted resource availability. These findings also revealed how these factors worsen the transmission of malaria.

By means of this study, Humanitarian Lab Family (HLF) has identified the challenges and barriers in implementing malaria control strategies in conflict-affected areas. Based on the findings, recommendations for policymakers, healthcare providers, and NGOs involved in malaria control efforts in conflict zones could be provided. Enhancing healthcare infrastructure involved strategies such as bolstering access to treatment and preventive measures, as well as enriching training programs for healthcare professionals.

Overall, the study would be to contribute to the understanding of malaria dynamics in conflict-affected areas and to provide evidence-based recommendations to mitigate the impact of malaria in these challenging contexts.

1.5. Malaria diagnosis by means of clinical presentations and diagnostic tools

Malaria patients present signs and symptoms such as high-grade fever with chills and rigor, profuse sweating, headache, abdominal pain, muscle pain, and complications due to respiratory distress, oliguria, black colored stool and urine, multiorgan failure and coma have been revealed (15).

Malaria rapid diagnostic tests, or RDTs, are useful test options when reliable microscopic diagnosis is not available. While malaria RDTs detect antigens from malaria parasites, a blood smear microscopy test must always confirm both positive and negative RDT results in a patient with suspected malaria. Despite these limitations, RDT's can provide results in less than 15 minutes (16). The accepted laboratory practice for malaria diagnosis is peripheral blood film examination stained with Giemsa, Wright's, or Field's stain (17).

1.6. Community service provision for malaria diagnosis

One of the key efforts to enhance the accurate diagnosis and treatment of malaria in Myanmar have involved initiatives led by the National Malaria Control Programme (NMCP) and various non-governmental organizations (NGOs). One key intervention has been the training and deployment of community health workers (CHWs) to rural areas, where the disease burden is highest. These CHWs support public healthcare workers by providing additional care and services (18).

After the 2021 military regime, malaria treatment and control programs have been managed primarily by local and ethnic healthcare authorities at most of the remote areas through existing ethnic health organizations, CDM healthcare networks and local stakeholders. Some INGOs and local NGOs have been playing a supporting role in malaria control activities.

Humanitarian Lab Family (HLF) is one of the organizations trying to set up a systematic platform of medical laboratory technology services at remote and hard-to-reach areas including malaria control activities. Having programed malaria survey activities in order to encompass proper engagement and collaborating with ethnic and local healthcare network as well as local community, HLF programs deliver training of local young adults to volunteer based at local minilabs for the purpose of providing medical laboratory equipment including malaria antigen test kits as well as facilities.

2. Methods of study and materials used

This was a cross sectional descriptive study among febrile patients in local villages and townships of Chin State, Sagaing Region and Karenni State conducted between October to December 2023. After getting informed consent and proper history taking, blood samples were collected for laboratory examination. History taking included age, occupation, travel history, history of malaria and blood transfusion, pregnancy in female reproductive age patients, etc. Blood samples were collected before antimalarial treatment was initiated. For those presenting with recurrent febrile attack having diagnosed with malaria previously were also included.

2.1. Detailed methodology

Capillary blood was collected via finger prick and add into the well of antigen test-kit. Then put two drops of buffer solution and read the results appeared in test and control line within fifteen minutes. Detail methodology varies according to different commercial brands of RDT with different specifications.

All the test kits used in this survey study were of certain quality-controlled products with good sensitivity and specificity suitable for screening purposes. Table 1 shows RDTs of different brands used with their respective specifications together with positive cases identified at each local minilab.

This survey program applied two RDTs of different brands having high sensitivities and specificities. At each survey area, to confirm all positive results of 1st line RDT and all participants with clinical signs and

symptoms of malaria showing negative results with them, they were followed by 2nd line RDT with another brand. This favored participants with typical malaria cases presuming that those individuals were missed using with 1st line RDT.

Screening with rapid test kits was followed by blood film (thick film examination) to examine possible extracellular malaria parasites ensuring that there was no parasite after breakdown of the red blood cells. All the developmental stages of parasites were examined by local HLF minilab staff and positive identifications were checked and confirmed by HLF on- ground trainers, both online and in-person.

2.2. Detailed techniques and procedures of peripheral blood film examination:

Thick and thin blood films were prepared after rapid test, since thick film provides the greatest sensitivity for malaria screening while thin film for the best detection of morphology for parasite species identification. All HLF minilabs used Giemsa stains for parasite identification in blood film. The thick film was prepared from 1 to 2 drops of blood in a circle of 1.5 to 2.0 cm in diameter.

In comparison, the thin film was made using a single drop of blood that was spread in a layer such that the thickness decreased progressively toward the feathered edge. The thin film was fixed in absolute methanol and allowed to dry completely prior to staining. Then microscopic identification of malaria parasites and parasite percentage were calculated according to standard formula if the film was positive.

If venous blood was used for microscopic examinations, blood was collected in EDTA tube and read the result as soon as possible to avoid prolonged exposure to EDTA because anticoagulants might alter parasite morphology. Confirmation of malaria parasite for species identification and parasite morphology were done and recorded the results by HLF lab technicians under supervision of trainers.

3. Sampling and sample size determination:

The following formula was used for sample size determination:

$n = (z^2pq)/d^2$

$= (1.96)^2 (0.136)(0.864)/(0.05)^2$

$= 181$

Approximate 3sample size is 190 with assumption of drop out 5%. n = Minimum required sample size

z = Reliability coefficient =1.96 (95% CI, α=0.05)

p = Proportion of prevalence of malaria infection in four special regions of northern Myanmar near China (Wang and et.al, Malaria Journal 2014)

q = 1-p

d = absolute precision

In this study, the target population was the population living in crisis areas where HLF minilabs are located. The calculated sample size was 190. To minimize errors caused by different situations of these selected crisis areas, the samples were collected at each 11 HLF mini laboratories. Therefore, the final sample size was 2090 to reflect the difference between areas and population.

4. Findings of the study:

As shown in table 3, the total number of 2147 patients were included in this study. The youngest age was a nine-month-old baby boy, and the oldest participant was a 94-year-old lady. Male participants were 1435 and female were 712. Among them, 118 participants were positive for malaria parasites (5.49% of all cases) and 33 of them were positive for P. falciparum (28% of all positive samples). 72 of all samples were positive for P. vivax, (72% of all positive samples). 5 cases were found to be mixed infections: P. falciparum and P. vivax. There was no sample which was positive for other malaria species (P. ovale, P. malariae, P. knowlesi). Table 4 shows detailed description of species of malaria positive cases.

The study mainly focused on patients with fever at the time of testing and those with history of recurrent fever residing in the local area. Clinical presentation was included as diagnostic parameter as shown in table 2. Among patients suffering from fever for one to three consecutive days, after screening with RDT, positive cases were managed to be examined to look for malaria parasite using microscopy. This also helped to identify timely circadian of the malaria parasites (17,19). Participants with history of recurrent fever (fever off and on) were also included in this study to explore low level parasitemia in their blood.

Although some of the cases already were diagnosed as malaria by local medical centers previously and had been treated, they had persistent malaria symptoms like fever off and on, therefore they were involved in this

study. Patients presenting with other clinical symptoms such as myalgia, arthralgia, headache apart from fever were also put into this study.

5. Thin film examination in detail

In table 4, some of the parasite forms have been identified in the positive samples. Ring forms and trophozoites were mostly seen. The parasites burden was also counted in blood samples of positive RDT results and transferred to the data sheet prepared. Then, the level of parasitemia was revealed by calculating them and expressed in percentage (%).

The presentation of malaria is non-specific (i.e., headache, fever, chills, myalgia, nausea, vomiting, diarrhea, fatigue, abdominal pain, altered mentation), and no combination of signs or symptoms can accurately discriminate malaria from other causes of fever in an endemic area. However, malaria should be suspected in any patient presenting with fevers in an endemic area without other obvious cause, for timely diagnosis and intervention (20). On the other hand, a single negative smear does not rule out malaria as a potential diagnosis; multiple additional smears should be examined within a 36-hour timeframe (21). By doing so, patients presenting with symptoms other than typical symptomatic malaria can be revealed using microscopic examination if missed during previous episodes.

Parasite density and level of parasitemia were also calculated in all positive cases according to WHO guidelines. Parasite levels were assessed using thick blood films by enumerating the parasites per 200 white blood cells (WBCs). A slide was found to be negative if no asexual forms or gametocytes of Plasmodium were detected after examining 500 white blood cells (WBCs). Plasmodium species were identified on thin blood films by tallying the parasites per 5000 red blood cells (RBCs) (22).

6. Explanation:

As described in table 1, commercial test kit brands for finger-prick blood tests were different as the local mini laboratory units were situated in different regions of Myanmar. Logistics and transportation channels were also different from each other, and a single supplier could not provide the necessary medical laboratory equipment and staining facilities required for the survey programs to be able to reach target areas. The HLF project team managed to make proper procurement procedures by connecting with regional on-ground trainers, laboratory-in-charges as well as local medical representatives to facilitate the procurement.

Based on previous stool survey made during August 2023 to September 2023, trained HLF staff got improved and clinically more experienced along with community engagement, routine microscopic examination practice and data entry as well as practice of survey activities. This study also polished their performance under the guidance of online trainer pathologists, senior laboratory technicians and on-ground trainers.

Malaria parasite examination using microscopy was the best way to identify it to confirm diagnosis. Skill training is essential to accomplish the test methods as it requires certain experience related to the malaria units from time to time. HLF training programs, starting from basic medical laboratory practices to intensive quality training for specific modules of parasitology, were continuously upgrading minilab staff enabling them to deliver quality laboratory services to local vulnerable population.

On the contrary, despite positive samples being checked and confirmed by supervisors, on the other hand, there might still be samples missed during the survey due to shortcomings of the equipment and materials used: rapid test kits, microscopes, Giemsa stains, etc.

7. Discussion

7.1. Geographical distribution

From this study, malaria positive cases were found to be the highest in Chin State among all study areas in the country. This finding was similar to the study of malaria incidence in Myanmar during 2005 to 2016 stating that Chin State had the The study concluded with the highest recorded malaria incidence and mortality rates, yet it suggests that socio-economic and geographical factors may offer a more plausible explanation for this observation than antimalarial resistance (23,24).

Among the positive cases in Chin State, most were mostly found to be at plane area compared to some hilly regions at Chin State where there were fewer mosquito breeding places. This showed that endemicity of malaria was related to geographical situations varying from each other within the country and was also consistent with the finding of The evolution of malaria risk mapping in the past decade has been shaped by advancements in data accessibility, computational capabilities, and methodological enhancements, enabling more refined and comprehensive assessments (25).

7.2. Species occurrence

There were only two malaria species identified in this study: P. falciparum (33 cases) and P. vivax (85 cases).

Number of patients having P. vivax was higher than P. falciparum in all study areas. Although WHO estimates that P. falciparum causes 75% of clinical malaria in Myanmar and P. vivax 25% (26,27), this finding was compatible with mass blood survey among 485 individuals from six villages in Kayah State during 2016 stating that prevalence of asymptomatic Plasmodium spp. infection was 2.3% (11/485) and Plasmodium vivax accounted for 72.7% (8/11), Plasmodium falciparum for 27.3% (3/11) of infections (23).

Again, cross-sectional surveys carried out in 3 seasons (March and April, July and November) and 2 sites (villages and IDP camps) of China-Myanmar border areas in 2015 also revealed that the proportions of Plasmodium vivax, Plasmodium falciparum and mixed-species infections were 89.6, 8.1 and 2.3%, respectively from 1680 finger-prick blood samples for asymptomatic parasite detection (28).

According to 2019 world malaria report, intensive malaria control and treatment efforts in Myanmar have reduced the prevalence of P. falciparum (29). This study was also consistent with the study of antimalarial drugs' efficacy and safety for uncomplicated P. falciparum and P. vivax in Myanmar during 2017 and 2019 stating that P. vivax have remained refractory to current interventions and has become the dominant parasite in some areas of the country (26).

7.3. Febrile pattern and diagnosis

There were 17 positive cases (14% of all positive samples) suffering from recurrent fever who had history of previous malaria in the study shown in table 2. Among them, 11 cases (64.7% of positive cases) were found to be due to P. vivax while the rest, 6 positive cases (35% of positive cases) were due to P. falciparum infection.

This finding was consistent with an open cohort study on epidemiological profiles of recurrent malaria episodes in an endemic area along Thailand-Myanmar border area during study among 7812 people during 2011-2017, revealing that 410 patients, representing 5.2% of the cohort, experienced 527 episodes of malaria. Among these cases, 340 episodes (64.5%) were attributed to P. vivax infection, while P. falciparum and mixed infections accounted for 181 (34.3%) and 6 (1.1%) episodes, respectively. Of the 410 malaria cases observed, 83 patients (20%) experienced multiple malaria episodes during the study period, ranging from 2 to 5 episodes. Approximately 80% (N = 67) of patients with multiple malaria episodes encountered recurrent episodes within one year following the primary infection (17).

7.3.1. Level of parasitemia and clinical presentations

Severe malaria cases can result from infections by either Plasmodium falciparum or Plasmodium vivax, although historically, severe and fatal outcomes have been primarily associated with infections caused by the former species Timely diagnosis and administration of antimalarial treatment can mitigate complications and prevent fatalities from this source. Although there is documented evidence linking the density of parasitemia with the prognosis of severe malaria patients, the severity of the disease may vary depending on the level of transmission intensity. Elevated levels of parasitemia represent a potential risk factor for complications and mortality associated with severe malaria (30).

The approximate numbers of parasites present in 1 µl of blood can be calculated by assuming that 1 µl of blood contains 5 x 106 RBC; therefore, a 1% parasitemia will contain 1 parasite/100 RBC or 50,000 parasites/µl of blood. Similarly, a 0.1% parasitemia will contain 5,000 parasites/µl of blood. In cases of non-falciparum malaria, parasitemia typically remains below 2%, whereas it often reaches significantly higher levels, surpassing 50%, in falciparum malaria. For non-immune individuals, severe illness is commonly associated with hyper parasitemia, defined as exceeding 5% parasitemia or having more than 250,000 parasites per microliter of blood (31).

In this study, levels of parasitemia were calculated by microscopy, and the two species of malaria, P. falciparum and P. viviax, had been identified together with parasite count (parasite/ml). None of all positive samples for both species (P. falciparum and P. vivax) exceeded 0.3%. All the participants in this study showed typical signs and symptoms such as fever with chills and rigors. All were OPD cases: in other words, uncomplicated malaria patients.

Similarly, this finding of parasite density was compatible with that conducted in tertiary health-care center in North India. Among 100 cases of falciparum malaria stating that in the uncomplicated malaria group, all patients exhibited a parasite density below 5%, whereas the majority of patients in the complicated malaria group had a parasite density exceeding 5%, with a statistically significant difference observed between the two groups. Cases with higher parasite density showed a significantly elevated incidence of cerebral malaria, with a mortality rate of 58.33% observed in these instances. These findings indicate an association between parasite density and the development of complications, as well as unfavorable clinical outcomes in P. falciparum malaria. Such insights could guide treatment strategies and imply that a threshold parasite density of 5% may serve as a valuable indicator (31).

In contrast, this finding did not necessarily mean that lower levels of parasite density were not observed in severe cases of malaria. According to the study conducted in Colombia, a retrospective, observational and descriptive one during the period from 2014 to 2017, a total of 2,352 confirmed cases of severe malaria were documented in the departments most affected by this condition in Colombia, from regions of low and unstable malaria transmission. Interestingly, the observed parasite density levels in individuals with severe malaria were found to be lower than the thresholds officially established. Consequently, it appears that the criterion of parasite density is not particularly relevant for defining severe cases in Colombia, and it should not be relied upon to make clinical decisions regarding the severity of the disease (32).

7.4. Malaria and gender preference

There was higher incidence of malaria among male individuals (94 cases, 79.66% of all positive samples) than female (24 cases, 20.33% of all positive samples) during this study. This significant gender bias of malaria incidence was compatible with the findings in the study among individuals accessing healthcare services through the public sector in malaria endemic townships of Ayeyarwady Region, Myanmar upon key factors associated with malaria infection: highlighting that certain groups were found to be at higher risk, including males and individuals working in forested areas particularly in logging and rubber plantation industries. The study recruited a total of 119 cases and 1744 controls from 41 public facilities, with an average age of 31.3 years and 63.7% being male (33).

8. What are missing gaps amid crises?

8.1. Competencies of newly trained HLF laboratory workforce

According to this study, a new prevalence of malaria infection was identified and highlighted that there are many missing gaps requiring useful diagnostic tools to get definite diagnosis after the military coup in Myanmar. Microscopic examination was the gold standard to test malaria. Trained HLF staff initiated the process of epidemiological study of malaria in their local areas in terms of survey programs using antigen test by RDT followed by microscopic examination.

In order to cover more areas for medical laboratory service delivery, HLF is continuing to provide technical support for other remote regions wherever the project areas are extendable. Because of current political situation in Myanmar, advanced investigations like polymerase chain reaction (PCR) and DNA tests to detect

mutant strain are not accessible. To integrate with malaria eradication strategies in the country, HLF aims to keep up with regular and advanced training programs. To achieve these activities, HLF must overcome challenging facts which include recruitment of skillful medical laboratory workforce along with supporting training facilities. In this survey period, financial aid and time limitation were the main constraint of HLF as the project team had to run the whole survey program with limited financial resources in a particular, short period of time.

Due to limitations, this study did not cover the whole population of Myanmar. To control malaria transmission, to get early diagnosis and definitive treatment and to deliver preventive measures especially at endemic areas are all equally important. As HLF has been providing proper training programs and laboratory materials through appropriate projects, it would be more advantageous and effective if it came to collaborate with other partner organizations which provide technical support as well as medications, insecticide treated nets for malaria control.

8.2. How far does the survey program need to be extended?

This study also helped to upgrade skills of HLF lab technicians not only providing primary health care services for local community but also collecting effective data for malaria control program by means of identifying most infected malaria species in targeted population. In the future, advanced training and technologies in malaria diagnosis such as DNA testing to detect mutant strains of malaria parasite and test for drug resistance malaria species will be upgraded by HLF together with malaria vaccination programs.

9. Conclusion

In conclusion, malaria is a life-threatening disease if early diagnosis and treatment were not timely provided. High prevalence of the disease during conflicts situation makes malaria a serious health problem for the country. HLF values to be a part of the healthcare service providers at remote areas in Myanmar to control malaria transmission.

Table.1 Different brands of Malaria Rapid test kit used in survey program

HLF Minilab No	Malaria RDT Name	Species/Pan	Purpose (1st Line/ 2nd Line)	Accuracy		Overall positive cases
				Sensitivity	Specificity	
001	One step	Pf/Pv	1st line	99.03%	99%	4
	Abbot	Pf/Pv	2nd line	95.5%	99.5%	4
002	Malcard(JM)	Species	1st Line	100	99.5	0
	First response	Species	2nd Line	100	100	0
003	Malcard(JM)	Species	1st Line	100	99.5	2
	First response	Species	2nd Line	100	100	6
004	Malcard(JM)	Species	1st Line	100	99.5	0
	First response	Species	2nd Line	100	100	0
005	Malcard(JM)	Species	1st Line	100	99.5	3
	First response	Species	2nd Line	100	100	2
006	Standard Q	Pf/Pan	1st line	99.58%	100%	20
	Diagnos	Pf/Pan	2nd line	99.0%	97.0%	20
007	Malcard(JM)	Species	1st Line	100	99.5	6
	First response	Species	2nd line	100	100	7
008	Malcard(JM)	Species	1st Line	100	99.5	0
	First response	Species	2nd line	100	100	2
009	Malcard(JM)	Species	1st Line	100	99.5	24
	First response	Species	2nd line	100	100	17
010	Malcard(JM)	Species	1st Line	100	99.5	0
	First response	Species	2nd line	100	100	0
011	Standard Q	Pf/Pan	1st line	99.58%	100%	17
	Diagnos	Pf/Pan	2nd line	99.0%	97.0%	17

Species = P.f (*Plasmodium falciparum*)/ P.v (*Plasmodium vivax*)/ P.m (*Plasmodium malaria*)/ P.o/ (*Plasmodium ovale*)

Table.2 Sociodemographic information of malaria in Chin, Sagaing and Karenni State (n = 2147)

Variable	Number	Percentage %
Gender		
Male	94	79.66
Female	24	20.33
Pregnant women	0	0
Clinical Presentation		
Current symptoms (fever, C&R, myalgia, arthralgia)	102	86
History of recurrent fever/previous malaria	16	14
Geographical Area		
Sagaing Region	4	3.39
Chin State	77	65.25
Karenni State	37	31.35

Table 3 Positive cases in study areas according to Geographical data (n = 2147)

HLF minilab No:	Village/Township	State	Positive cases/all samples	*P.f*	*P.v*	*P.o*	*P.m*	*P.k*
001	Myaung	Sagaing region	4/151	-	4	-	-	-
002	Lungler village, Than Tlang	Chin Stae	0/200	-	0	-	-	-
003	Surkhur village, Hakha	Chin State	8/200	3	5	-	-	-
004	Khuabung village, Than Tlang	Chin State	8/200	3	5	-	-	-
005	Tikir village, ThanTlang	Chin State	5/200	3	2	-	-	-
006	Cebu village	Karenni State	20/200	3	17	-	-	-
007	Mual Bem village, Tedim	Chin State	13/200	5	8	-	-	-
008	Hnaring village,Thantlang	Chin State	2/200	1	1	-	-	-
009	Camp Victoria, Falam/ ThanTlang	Chin State	41/200	9	32	-	-	-
010	Hrawng Vun village, Hakha	Chin State	0/200	0	0	-	-	-
011	Pekon, Karenni State	Chin State	17/200	6	11	-	-	-
Total			118/2147	33	85	-	-	-

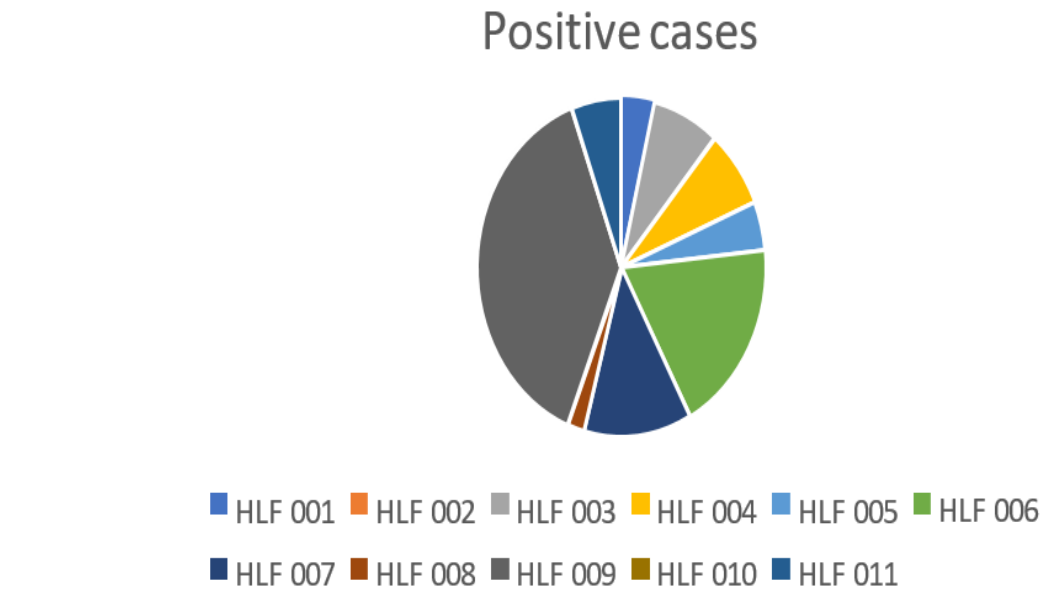

Table 4 Malaria species in study areas (n = 2147)

No	Malaria Species	Number	Percentage (%)
1.	*P.falciparum*	33	28
2.	*P.vivax*	85	72
3.	*P.ovale*	-	-
4.	*P.malariae*	-	-
5.	*P.knowlesi*	-	-

10. Abbreviations

CDM Civil Disobedience Movement

HLF Humanitarian Lab Family

MNCP Myanmar National Malaria Control Programme

RDT Rapid Diagnostic Test kit

NGO Non-Government Organization

INGO International Non-Government Organization

WHO World Health Organization

ICMV Integrated Community Malaria Volunteer

11. Human Subjects (Risks & Benefits)

Research materials were sourced from living human subjects in the form of blood samples, with each subject identifiable individually, including other medical records or data related to malaria infestations. These materials or data have been obtained specifically for research purposes. Participants with symptomatic carriers and patients were identified as potential subjects through some type of record (e.g., medical records, patient charts, registries for logbooks, school records).

12. Contractual Agreements

Collaboration with local healthcare networks and stakeholders, ethnic authorities according to regional healthcare guidelines was made to facilitate engagement with local community at each study area.

13. Facilities and Equipment

Most of the facilities and equipment to be used in the survey have already been supplied by previous HLF mini laboratory training program. Additional consumables; glass slides, sample containers, disposable gloves, 3-layered masks, chlorine power, normal saline, rapid antigen test kits and survey tools; logbooks and stationery were provided by the budget from survey project fund. Allowances related to survey activities for minilab office running have been covered. Indirect costs for travel allowance, mobile data usage and fuels to run microscopic examinations were also included.

14. Data availability

The datasets analyzed during the current study are not publicly available due to institutional regulation but are available from the corresponding author on reasonable request.

15. Conflicts Of Interest

The authors declare that they have no competing interests.

16. Confidentiality

The privacy and confidentiality of the subjects are well-protected to the rights as well as welfare of vulnerable individuals.

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