

## Vector Bionomic Study in Kayah State and Kachin State

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Malaria is still one of the most important public health problems in Myanmar. National Malaria Control Programme (NMCP) has implemented malaria prevention and control activities based on national malaria control strategies. One of the strategies is selective and sustainable vector control measures with integrated vector management approach which includes distribution of Long Lasting Insecticide Treated Nets (LLINs), Insecticide Treated Nets (ITN) and Indoor Residual Spray (IRS) whenever and whatever necessary. As the country scale up distribution LLIN and ITN programme, insecticide selection pressure on the vector mosquito populations is increased. Susceptibility status of insecticides could depend on population migration and environmental changes and ecology factors in relation to human behavior. Therefore, malaria vector bionomic and insecticide susceptibility status study should be investigated in these malaria endemic areas. The present study was conducted in Kayah State and Kachin State. In Nankit Village, Kayah State, nineteen Anopheles species were collected and *An. minimus* was primary vector. In the whole night collection, peak biting time of *An. minimus* was 9 to 10 pm in first peak and 2 to 3 am in second peak. The highest density of *An. minimus* was observed in November. *An. minimus* was susceptible to 0.05% deltamethrin and 0.5% ectofenprox. In Tanphae and Ngwepyaw Sampya villages, Kachin State, more than 20 Anopheles species were collected and *An. minimus* and *An. dirus* were primary vectors in these areas. The density of the *An. minimus* was highest in June, and *An. maculatus* was also highest in June, but *An. annularis* which were higher density in December. Peak biting time of *An. minimus* was 20:00 to 21:00 and that of *An. maculatus* was 19:00 to 20:00. *An. kochi* was susceptible to 0.05% deltamethrin with 100% mortality.

*Keywords:* Anopheles, Biting habit, Susceptibility status, Deltamethrin

### INTRODUCTION

Malaria is still one of the important public health problems in Myanmar. National Malaria Control Programme (NMCP) has implemented malaria prevention and control activities based on national malaria control strategies. One of the strategies is selective and sustainable vector control measures with integrated vector management approach which includes distribution of LLINs, ITN and IRS whenever and whatever necessary.

As the country scale up distribution LLINs and ITN programme, insecticide selection pressure on the vector mosquito populations is increased. The success of any vector control program relies on knowledge of vector species and their bionomics which is very important to get update knowledge and information on primary and secondary

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vectors, distribution is related to environmental changes. Monitoring level of resistance is an essential tool in enabling to make a decision. Resistance to insecticides is concerned due to population migration and changes of environment and ecology factors in relation to human behavior.<sup>1, 2</sup> Thus, the vector bionomic and insecticide resistance susceptibility status survey should be investigated in this region.

## MATERIALS AND METHODS

### Localities and survey period

Vector bionomic survey was conducted in Nankit village, Phasaung Township, Kayah State during October, November and December, 2016. Nankit village is situated in latitude 18.89613°N and longitude 97.30016°E. Vector bionomic survey was also conducted in Tanphae and Ngwepyawe Sampya villages, Myitkyina Township, Kachin State during the period of March, June, September and December 2017. Tanphae village is situated in 25.71536°N, 97.49098°E, 633 feet above sea level and 27 miles far from Myitkyina and side of the Myitkyina-Putao highway road. Ngwepyawe Sampya village is about 14 miles far from Myitkyina, at the latitude 25.56707°N and longitude 97.35565°E with above sea level of 564 feet.

### Entomological parameters

Entomological survey include: human landing indoor and outdoor collection, animal bait net trap collection, light trap collection, morning resting indoor and outdoor hand collection, spray sheet collection, larval collection, susceptibility test, bio-assay test and sporozoite detection with ELISA test.<sup>3</sup>

## RESULTS

### Vector bionomic study (Nankit village, Phasaung Township, Kayah State)

The village is located at the forested and foothill area with slow running streams that is favorable for *An. minimus* breed. The

primary vectors such as *An. dirus*, *An. minimus*, and the secondary vectors and other 17 anopheles species were collected. The primary vector, *An. minimus* was collected by human landing indoor and outdoor collection and animal bait net trap collection (Table 1).

Table 1. Total number of Anopheles caught from Nankit village, Phasaung Township

Anopheles species	No. of mosquitoes caught (specieswise)					Total mosquitoes
	Human bait		CDC light trap		Cattle bait	
	In-door	Out-door	In-door	Out-door		
<i>An. minimus</i>	149	203	0	0	193	545
<i>An. annularis</i>	3	0	0	0	452	455
<i>An. maculatus</i>	0	5	0	0	483	488
<i>An. philippinensis</i>	0	6	0	0	521	527
Total	152	214	0	0	1649	2015

The feeding habit was both indoor and outdoor. Host preference was feeding on man (anthropophilic) and animal (zoophilic). The *An. minimus* biting density of human landing indoor collection is 0.915 per man-hour and outdoor is 0.86 per man-hour (Fig. 1).

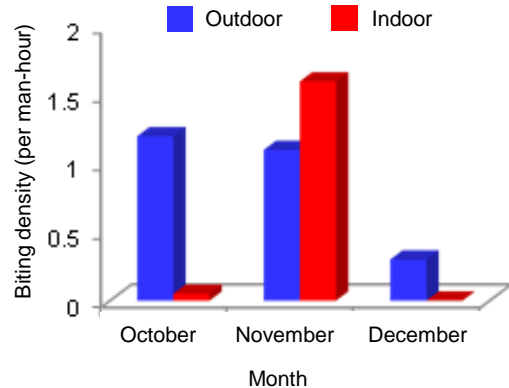


Fig. 1. Human landing outdoor and indoor density in Nankit village, Phasaung Township

In the whole night collection, peak biting time of *An. minimus* was about 21:00 to 22:00 in first peak and 2:00-3:00 in second peak of the night. By animal bait net trap collection, *An. minimus* and other anopheline species (19) were collected (Table 2). *An. minimus* were also collected in light trap collection. In monitoring the insecticide resistance, *An. minimus* was susceptible to

Table 2. Animal bait net trap collection, Nankit village, Kayah State, 2016

Anopheles Species	2016		
	October	November	December
<i>An. minimus</i>	16	60	117
<i>An. annularis</i>	114	273	65
<i>An. philippinensis</i>	219	237	65
<i>An. maculatus</i>	324	120	39
<i>An. aconitus</i>	0	23	0
<i>An. tessellatus</i>	7	12	2
<i>An. vagus</i>	28	36	9
<i>An. jamesi</i>	19	3	13
<i>An. peditaeniatus</i>	545	0	0
<i>An. hyrcanus</i>	0	471	13
<i>An. barbirostris</i>	11	33	9
<i>An. stephensi</i>	1	0	0
<i>An. splendidus</i>	1	0	0
<i>An. varuna</i>	9	0	0
<i>An. willmori</i>	4	3	0
<i>An. kochi</i>	0	9	7
<i>An. canditaeniatus</i>	1	0	0
<i>An. pallidus</i>	0	27	0
<i>An. kawari</i>	0	3	0

0.05% deltamethrin and 0.5% ectofenprox and *An. philippinensis* to 0.05% deltamethrin, 0.05% lambdacyhalothrin and 0.5% ectofenprox. *An. peditaeniatus* was resistant to 0.05% deltamethrin and 0.5% ectofenprox. *An. maculatus* was susceptible to 0.75% permethrin and *An. annularis* was susceptible to 4% malathion, respectively. Bio-assay test was conducted with Yorkool<sup>R</sup> impregnated bed net (date of production 2015) and 92% mortality of *An. minimus* and 96% mortality of *An. philippinensis* were observed. Human bait 18.16% and animal bait 81.8% were found. It might have been zoophylaxis effect in the village. There was reduction in number of cows and buffaloes in the village that can deviate the feeding of mosquitoes from animal to man.

*In Tanphae village, Myitkyina Township, Kachin State*

This village comprised a total of 67 houses with 168 population and the villagers earned their living by agricultures and forest-related works. In 2016, out of 807 blood smears taken by Rapid Diagnosis Test (RDT), 7 falciparum and 9 non-falciparum malaria were detected. The distribution of LLINs and Indoor residual spray has been conducted by NMCP since May 2016 for vector control.

Table 3. Total number of Anopheles caught from Tanphae village, Myitkyina Township, Kachin State, 2017

Anopheles species	No of mosquitoes caught (specieswise)					Total mosquitoes
	Human bait		CDC light trap		Cattle bait	
	In-door	Out-door	In-door	Out-door		
<i>An. minimus</i>	0	33	4	2	100	139
<i>An. maculatus</i>	1	47	14	4	93	159
<i>An. aconitus</i>	0	18	0	0	64	82
<i>An. annularis</i>	1	62	0	3	55	121
<i>An. candidiensis</i>	0	18	10	2	2	32
<i>An. philippinensis</i>	2	36	1	0	68	107
<i>An. hyrcanus</i>	33	0	1	6	119	159
<i>An. jamesi</i>	0	4	1	0	5	10
<i>An. tessellatus</i>	0	2	0	0	2	4
<i>An. vagus</i>	1	21	0	0	8	30
<i>An. barbirostris</i>	0	2	0	4	54	60
<i>An. kochi</i>	0	6	1	0	90	97
<i>An. varuna</i>	2	0	0	0	100	102
<i>An. willmori</i>	0	1	0	0	4	5
<i>An. peditaeniatus</i>	0	2	0	0	0	2
<i>An. splendidus</i>	0	33	6	4	58	101
<i>An. subpictus</i>	0	8	0	0	2	10
<i>An. tessellatus</i>	0	2	0	0	0	2
<i>An. nigerrimus</i>	0	1	0	0	0	1
<i>An. nitidus</i>	0	1	0	0	0	1
<i>An. karwari</i>	0	1	0	0	0	1
<i>An. pseudojamesi</i>	0	1	0	0	4	5
<i>An. sinensis</i>	0	1	0	0	0	1
<i>An. jeyporiensis</i>	0	1	0	0	32	33
<i>An. agropus</i>	0	0	0	0	4	4
Total	40	301	38	25	864	1268

A total of 25 Anopheles species were caught from Tanphae village (Table 3). Biting habit of the Anopheles vector was assessed by human bait and cattle bait. Zoophilic nature of both vector species was observed with 72% of *An. minimus* vs 58% of *An. maculatus*. Peak biting time of *An. minimus* was 20:00 to 21:00 and *An. maculatus* was 19:00-20:00. The behavior and biting pattern of the vectors seemed to be related to the night-time activities of the villagers. Annual man biting rates were higher in *An. minimus*, *An. maculatus* and *An. annularis*, ie, 174.84, 264.26 and 199.66, respectively. Annual man biting rate was highest in *An. maculatus* followed by *An. annularis* and *An. minimus* (Table 4). Animal bait net trap collection: The anopheline species were tested for insecticide resistance with insecticide impregnated paper and bioassay test for LLIN efficacy. For bioassay test,

LLIN (permanet for one and half year, no wash) showed effective 100% mortality with *An. minimus* and LLIN (permanet for one and half year, no wash) and LLIN (Yorkkool for two years with no wash) were effective 80% and 89% mortality with *An. maculatus*, LLIN (Yorkkool for two years with no wash) was effective 80% with *An. kochi*. Monitoring of insecticide resistance with insecticide impregnated paper was carried out and *An. kochi* was susceptible to 0.05% deltamethrin with 100% mortality.

Table 4. Seasonal man biting rate (MBR) of different Anopheles species in Tanphae village, Myitkyina Township, Kachin State, 2017

Anopheles species	March	June	Sep-tember	De-cember	MBR (annual)
<i>An. minimus</i>	0.17	0.244	0	0.07	174.84
<i>An. dirus</i>	0	0	0.03	0	10.95
<i>An. maculatus</i>	0.09	0.344	0.075	0.22	264.26
<i>An. annularis</i>	0.01	0.077	0.15	0.31	199.66
<i>An. culicifacies</i>	0	0.119	0	0	43.44
<i>An. aconitus</i>	0.04	0	0	0.1	51.71
<i>An. varuna</i>	0.01	0.006	0	0.02	14.56
<i>An. hyrcanus</i>	0.01	0.105	0.11	0.18	149.24
<i>An. pseudojamesi</i>	0.01	0	0	0	5.07
<i>An. willmori</i>	0.01	0.018	0	0	11.64
<i>An. splendidus</i>	0.18	0.225	0.01	0.05	169.73
<i>An. kochi</i>	0.01	0.077	0.03	0.03	55.07
<i>An. jamesi</i>	0	0.131	0	0.06	69.72
<i>An. candidienseis</i>	0	0.131	0.065	0.06	93.44
<i>An. tessellatus</i>	0	0.006	0	0	2.19
<i>An. barbirostris</i>	0	0.042	0.01	0.02	26.28
<i>An. peditaeniatus</i>	0.01	0	0.01	0	8.72
<i>An. vagus</i>	0	0	0.096	0	35.04
<i>An. philippinensis</i>	0	0	0.126	0.13	93.44
<i>An. subpictus</i>	0	0	0.1	0	36.50
<i>An. tessellatus</i>	0	0	0.02	0	7.30
<i>An. nigerimus</i>	0	0	0.01	0	3.65
<i>An. indiensis</i>	0	0	0.01	0	3.65
<i>An. karwari</i>	0	0	0.01	0	3.65
<i>An. sinensis</i>	0	0	0	0.02	7.30
<i>An. jeyporiensis</i>	0	0	0	0.02	7.30

Annual man biting rate was highest in *An. maculatus* followed by *An. minimus*.

#### Ngwepyawe Sampya village

As of 2017, the total population was 1799 and the occupation of the people were forest related workers, selling and buying of goods and commodities. In 2016, 69 malaria blood smears were taken and 2 *Pf* positives were found. Indoor residual spray with Fondana (alphacypermethrin) has been conducted by

NMCP since 2014. By human landing outdoor collection at about 22:00-23:00, more density was collected at the later part of the night than the early part of the night at about 19:00-21:00. It is closely related to the night movement and sleeping pattern of the local people, because the locality is also urbanized and with electricity at night. By human landing outdoor collection *An. minimus* was 0.05 m/hr, *An. maculatus* was 0.03 m/hr, *An. aconitus* was 0.04 m/hr, *An. splendidus* was 0.1 m/hr, *An. peditaeniatus* was 0.01 m/hr and *An. kochi* was 0.01 m/hr. By animal bait net trap collection, *An. minimus* and 9 *Anopheles* species were collected. *An. minimus*, *An. maculatus* and *An. splendidus* were collected in light trap indoor collection and *An. hyrcanus* and *Culex* species were collected in light trap outdoor collection. *An. minimus* larvae (0.73 per dips) were collected in small pools. *An. hyrcanus* of anopheles specie, was tested for insecticide resistant and found that it was highly resistant to 0.05% deltamethrin with mortality of 15%.

Human bait of 31.86% and animal bait of 68.13% were found. It might have been zoophylaxis effect in the village. There was reduction in number of cows and buffaloes in the village, that could deviate the feeding of mosquitoes from animal to man.

#### ELISA for circumsporozoite protein for vector Anophelines from Kayah State and Kachin State

The ELISA test for circumsporozoite protein was conducted. From Phasaung Township, Kayah State and Myitkyina Township, Kachin State, *An. minimus*, *An. maculatus*, *An. annularia*, *An. aconitus*, *An. philippinensis*, *An. tessellatus* and *An. hyrcanus* of the vector anopheline species were tested with ELISA. Out of 850 mosquitoes samples collected from field, five were found positive in Kachin State. The *Pf* positives of *An. minimus* and *An. philippinensis* were found in Ngwepyaw Sanpya village.

## DISCUSSION

Entomological surveillance in sentinel sites was conducted annually by Entomology team of the NMCP. Both in Phasaung Township, Kayah State and Myitkyina Township, Kachin State, primary vectors of *An. minimus*, *An. dirus* and other secondary vectors were observed in entomological survey. Entomological surveillance and vector control is an important component in malaria elimination programme. In Kayah State, the primary vectors of *An. dirus*, *An. minimus*, and 17 anopheles species were observed. The *An. minimus* biting density of human landing indoor collection is higher than outdoor. The peak biting time of *An. minimus* was 21:00 to 22:00 in first peak and 2-3 am in second peak. *An. minimus*, *An. maculatus* and *An. philippinensis* were susceptible to pyrethroid and *An. annularis* was susceptible to organophosphate. According to the efficacy of the local LLINs, WHO optimal effective was observed.

In Kachin State, *An. minimus* was observed in all entomological collection methods. Peak biting time of *An. minimus* was 20:00 to 21:00 and *An. maculatus* was 19:00-20:00. *An. kochi* is susceptible to pyrethroid. Biting habit of the Anopheles vector was assessed by human bait and cattle bait. Zoophilic nature of both vector species was observed as 72% of *An. minimus* vs. 58% of *An. maculatus*. There was reduction in number of cows and buffaloes in the village that could deviate the feeding of mosquitoes from animal to man. According to the efficacy of the local LLINs, WHO optimal effective was also observed. According to

ELISA test, five positives were found in Kachin State.

### *Competing interests*

The authors declare that they have no competing interests.

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