Vector Bionomic and Efficacy of Insecticides in Malaria Endemic Areas of Chaung Zone Township, Mon State, Myanmar

Maung Maung Mya[1], Sein Thaung[1], Zarni Min Hein[1], Yee Yee Myint[1], Thu Zar Nyein Mu[1], Yan Naung Maung Maung[1] & Yan Sint[2]*

[1]Medical Entomology Research Division, Department of Medical Research
[2]VBDC Mon State, Ministry of Health and Sports

In malaria prevention and control, use of insecticide treated bed nets and application of focal residual spray at selected areas are important part of the programme in endemic regions. The study on vector bionomics and susceptibility of *Anopheles* (*An.*) mosquito vectors for malaria was studied at Kayinwinsein, Piankadar, and Kataungsein villages in Chaungzon Township, Mon State from April 2017 to March 2018. A total of 831 *Anopheles* mosquitoes belonging to 7 species were collected from the villages of Bilukyun, Chaungzon Township. The major vector *An. dirus* 24(2.89%) and secondary vectors *An. tessellatus* 40(4.81%) *An. vagus* 72(8.66%), *An. maculatus* 75(9.03%), *An. aconitus* 72(8.66%), *An. jamesii* 23(2.77%), *An. barbirostris* 326(39.23%) and *An. hyrcanus* 199(23.95%). Main vector *An. dirus* was collected in wet and cool season although in dry season *An. dirus* was disappear in all study villages due to high temperature and dryness of their breeding habitats. Main vector *An. dirus* was collected at 22:00-23:00 hour in biting outdoor collection in wet and cool seasons. All total of 146 domestic water wells (50, 50, 46) were searched 3 dips/well using WHO dipper and found that 12,8,12 wells were positive with *An. dirus* larvae in Kayinwinsein, Paingkadar and Kataungsein villages, respectively. *An. vagus* larvae were found in foot prints and small muddy water pools. All collected *An.* mosquitoes were found susceptible to Deltamethrin 0.05%, Permethrin 0.75% and Cyfuthrin 0.15%.

Keywords: Bionomic, Vector, Insecticide, *An. dirus*, Wells, Breeding

INTRODUCTION

Previously, malaria is one of the major public health problems in Myanmar although now it is still a main contributor to the morbidity and mortality in border and hard-to-reach areas in the country.[1] In malaria prevention and control, use of insecticide treated bed nets and application of focal residual spray at selected areas are important part of the programme in endemic regions. *An. dirus* and *An. minimus* are major vectors of malaria in forested and forested foot hill areas of the country and *An. annularis* is a local vector of malaria in Rakhine State. *Anopheles sundaicus* and *An. subpictus* are abundantly found in coastal areas of Rakhine, Mon and Tanintharyi Regions. The transmission of malaria is largely influenced by the abundance, survival and bionomics of the mosquito vectors.[2, 3] The main vector control tools, long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) at focal areas can be very effective in reducing malaria transmission. They are more effective against vectors which bite indoors (endophagic), late in the night (nocturnal) and which rest indoors after

*To whom correspondence should be addressed.
Tel: +95-9420155421
E-mail: dr.mgmgmya@gmail.com
DOI: https://doi.org//10.34299/mhsrj.009119

feeding (endophilic).[4] These characteristics are observed for the primary malaria vector like *An. minimus* and are contributing to the success of the malaria control programmes in this region. However, it might be possible that after widespread and long-term insecticide exposure, the feeding and resting behaviour of vectors may become much more variable. Furthermore, vector mosquitoes may develop tolerance and leading to Pyrethroid resistance in endemic areas.[5] At the same time, residual effectiveness of the Insecticide treated bed net (ITN) and IRS should be monitored periodically for judicious use of insecticides and sustainable vector control.[6] Updating and better understanding of the bionomics of vector mosquitoes are required together with monitoring of the insecticide susceptibility status and measuring of the residual effectiveness of insecticide treated surfaces at malaria endemic regions. It is essential to detect efficacy of currently used insecticides in the country and available information will be useful to complement the control programme. The present study planned to investigate the bionomics of malaria vectors and evaluate the efficacy of insecticides for strengthening of control strategies.

Rationale

Bilu Kyun is an island of Chaungzon Township is a large island area with 60 villages on the Island in Mon State. The island is roughly the size of Singapore, and inhabited by 200,000 people and economically popular island. The island is situated in West part of the Mawlamyaing Township, Mon State. The island is surrounded by Mudama Sea and it is connected by bridge to Mawlamying Township. Many creeks traverse the mainland with tributaries of varying lengths and during high tide, water from the sea penetrates creating swamps and marshes, which nurture the thriving mangrove. The topography of the islands is relatively flat Island and abounds in evergreen forests, rice fields and rubber plants. Rubber plantations provide a living for many and the locals also maintain a remarkable range of traditional cottage industries. The climate is tropical (24°C to 30°C temperature) and humid (mean relative humidity of 78.5%) with abundant rainfall, supporting a luxuriant and rich vegetation. Mon State is a malaria endemic area and primary and secondary vectors are abundantly present in this area[2] and larvae of main vector *An. dirus* are abundantly found in domestic water wells.[7]

According to the ecological changes, distribution of malaria morbidity patterns and control of malaria using prompt treatment and distribution of LLINs nets in high-risk areas revealed that the malaria was about 38.90% in the 1990 was reduced to 17% in 2013. And free areas in the country were increased from 8.60% in 1990 to 37% in 2013.[8] Large numbers of native and migrant workers are working in rubber plantation and handmade factory's and contracted sites and they have needed to protect from vector borne diseases. And also, there is a lack of data and information about the vectors and potential vectors and insecticide efficacy of malaria in Bilu Kyun, Chaungzon Township, Mon State. Therefore, an attempt has been made to determine the vector bionomics and insecticide efficacy of primary and secondary vectors of malaria in these areas.

MATERIALS AND METHODS

Study area

Kayinwinsein, Piankadar, and Kataungsein villages were selected in Bilu Kyun, Chaungzon Township, Mon State.

Study period

April 2017 to March 2018

Mosquito's population

It was determined all collected mosquitoes with different collection methods.

Selection criteria

Three villages which were malaria cases occurred annually and the population was about 200. These villages were randomly selected and at least 10 kilometers away from each other.

Entomological Survey

Mosquito collection

Fixed mosquito catching stations were chosen in both selected villages. Indoor, outdoor light traps and animal bait K Net (330 x 330 x 180cm) big mosquito net trap catches were conducted seasonally (June, Hot season), (August, Wet season), (December, Cool season). All catching was done in fixed stations throughout the study as follow: Animal bite, both indoors and outdoors light traps catching were done with WHO sucking tubes from 18:00 to 06:00 hours of the next day for 7 days.

Larval surveys

For identification of breeding sites larval survey was conducted in and around three kilometers away from the study villages. Dam, canal and irrigation, domestic wells, stream/creeks and all different types of water holding places were examined for larvae detection by 3 dips/water holding place.[9] In the rainy season, all water pockets, coconut shells, discarded tins and utensils bamboo stumps including foot print of animals were examined. The captured larvae and pupae were put in labeled plastic bags and brought back to the laboratory for species identification and colonization.

Identification of adult *Anopheles* mosquitoes

Collected adult *Anopheles* mosquitoes and adult emerged from larva survey were identified by species according to Peyton and Harrison[10], Reid[11] and Myo Paing[12].

Insecticide susceptibility test

Insecticide susceptibility tests (WHO test kit)

Adult female *Anopheles* mosquitoes collected from the entomological survey were tested for measurement of insecticide susceptibility level using WHO test kits[7] and standard procedures. The efficacy of insecticides as DDT 4%, Permethrin 0.75%, Cyfluthrin 0.15% and Deltamethrin 0.05% were measured.

Procedure

Ten fields collected *Anopheles* mosquitoes were introduced in WHO insecticide impregnated paper attached plastic tube (WHO test kit) by sucking tube and exposed for an hour. After one hour of exposing the mosquitoes were then removed from the plastic tubes and placed in clean plastic tubes without paper with 10% glucose-soaked cotton and moisture were maintained by water soak dump towel. Percentage of knock-down was measured after 60 minutes exposure and mortality was assessed after 24 hours' exposure. Two replicates testing were done to confirm the susceptibility of mosquitoes.

Incrimination of vector

Head and Thorax of vector mosquitoes were dissected for *Plasmodium* sporozoites. Enzyme Linked Immunosorbent Assay (ELISA) test for circumsporozoite antigen detection was supplemented according to Wirtz, *et al*,[13] for vector incrimination study.

Meteorological data

Meteorological data as rainfall, Relative Humidity (RH) and Temperature (ºC) were obtained from TMEP Township weather station of Bilu Kyun Chaungzon Township, Mon State.

Data analysis

Seasonal entomological data was analyzed and compared both study areas. Data entry, processing and analysis was carried out using SPSS software applying Student '*t*' test and ANOVA and Microsoft Excel (Analysis package) software and appropriate statistical analysis was applied as necessary.

RESULTS

Table 1 shows that a total of 831 *Anopheles* mosquitoes belonging to 7 species were collected from three villages of Bilu Kyun Chaungzon Township, Mon State.

The major vector *An. dirus* 2.89%(24) and secondary vectors *An. tessellatus* 4.81%(40) *An. vagus* 8.66%(72), *An. maculatus* 9.03% (75), *An. aconitus* 8.66% (72), *An. jamesii* 2.77%(23), *An. barbirostris* 39.23%(326) and *An. hyrcanus* 23.95%(199). Highest number of *Anopheles* mosquitoes were collected in rainy season (339) followed by cold season and lowest was observed in dry season (169).

Table 1. Seasonal distribution of *Anopheles* (*An.*) mosquitoes in three villages

Anopheles species	Dry season		Rainy season		Cold season		Total	
	No. C	%	No. C	%	No. C	%	No. C	%
An. dirus	0	0	13	3.83	11	3.41	24	2.89
An. tessellatus	7	4.14	18	5.31	15	4.64	40	4.81
An. vagus	16	9.47	29	8.55	27	8.36	72	8.66
An. maculatus	18	10.65	26	7.67	31	9.60	75	9.03
An. aconitus	15	8.88	28	8.26	29	8.98	72	8.66
An. jamesis	3	1.78	11	3.24	9	2.79	23	2.77
An. barbirostris	74	43.79	135	39.82	117	36.22	326	39.23
An. hyricanus	36	21.30	79	23.30	84	26.01	199	23.95
Total	169	100	339	100	323	100	831	100

No. C=Number of collected

Main vector *An. dirus* was collected only in rainy and cold seasons. *An. barbirostris* and *An. hyrcanus* were found high density in all season i.e., 43.79%, 21.30% in dry, 39.82%, 23.30% in rainy and 36.22%, 26.01% in cold seasons, respectively. Remaining secondary vectors were collected in all seasons. Table 2 shows that high density of *Anopheles* mosquitoes were collected from Kayinwinsein village 288(34.66%) and Piankadar village 289(34.78%) followed by Kataungsein village (30.57%). The collected mosquitoes from studied villages were found same species of *Anopheles*. Although main vector *An. dirus* was found lowest density i.e., 1.74%, 3.46%

Table 2. Total collected mosquitoes from three villages

Species	Villages						Sporozoites detection by ELISA method	
	Kayinwinsein		Piankadar		Kataungsein			
	No. C	%	No. C	%	No. C	%	No. C	% positive
An. dirus	5	1.74	10	3.46	9	3.54	24	1*Pf* (4.16%)
An. tessellatus	9	3.13	19	6.57	12	4.72	40	0
An. vagus	27	9.38	23	7.96	22	8.66	72	0
An. maculatus	23	7.99	31	10.73	21	8.27	75	0
An. aconitus	18	6.25	28	9.69	26	10.24	72	0
An. jamesis	14	4.86	2	0.69	7	2.76	23	0
An.barbirostris	125	43.40	100	34.60	101	39.76	326	0
An. hyricanus	67	23.26	76	26.30	56	22.05	199	0
Total	288(34.66%)	100	289(34.78%)	100	254(30.57%)	100	831	1*Pf* (0.12%)

No. C=Number of collected, T No. C= Total number of collected, *Pf= Plasmodium falciparum, An.=Anopheles*

Table 3. Breeding habitats of *Anopheles* mosquitoes in three villages

Breeding habited	Villages						Species of larvae (n)
	Kayinwinsein		Piankadar		Kataungsein		
	Examined	Positive	Examined	Positive	Examined	Positive	
Domestic water wells	50	12	50	8	46	12	199 *An. dirus* 321 *Cx. quinquefasciatus*
Pond	8	2	0	0	0	0	6 *An. Maculatus,* 12 *An barbirostris*
Foot prints	14	2	17	2	12	1	28 *An. vagus*
Water pools	16	7	10	3	12	4	36 *An. maculatus, 12 An. jamesii,* 21 *An.aconitus*

and 3.54% in Kayinwinsein, Piankadar and Kataungsein villages, respectively, *Anopheles vagus* was found highest in Kayinwinsein 9.38%, *An. aconitus* was highest in Kataungsein (10.24%), |*An. maculatus* was highest in Piankadar village (10.73%) and *An. jamesis* was highest in all villages followed by Kayinwinsein village (4.86%). Non-malaria vector *An. barbirostris* was found highest density followed by *An. hyrcanus* in villages *An. dirus* was found *Pf* Sporozoites positive in pool serum (4.16%).

Table 4. Susceptibility status of collected *Anopheles* mosquitoes against WHO recommended insecticides

Species*	WHO insecticide impregnated paper	Susceptibility states
An. dirus *An. tessellatus* *An. vagus* *An. maculatus* *An. aconitus* *An. jamesii* *An. barbirostric* *An. hyrcanus*	Deltamethrin 0.05% Permethrin 0.75% Cyfluthrin 0.15% DDT4%	Susceptibility (90-100% mortality)

* =No of mosquitoes tested, 10 each

Table 3 shows that highest number of wells were positive in Kayinwinsein and Kataaungsein villages followed by Piankadar village with *An. dirus* larvae. *Anopheles maculatus*, *An. barbirostris*, *An. vagus, An. jamesii,* and *An. aconitus* larvae were collected from pond footprints and water pools. *Cx. quinquefasciatus* larvae were found co breeder of *An. dirus* in water wells.

Table 4 shows that all collected *Anopheles* mosquitoes were found susceptible to WHO recommended Deltamethrin 0.05%, Permethrin 0.75%, Cyphyuthrin 0.15% and DDT4% of WHO impregnated papers were effectively killed the collected *Anopheles* mosquitoes.

DISCUSSION

Previously Mon state is a malaria endemic area in Myanmar and malaria transmission is high in border areas, rubber plantation sites, hard-to-reach and forested hilly areas.[14] Kayinwinsein, Piankadar and Kataungsein villages are situated in Bilu island Township, Mawlamyaing District in Mon State.

Total of 169,339 and 323 *Anopheles* mosquitoes from Bilu Island were collected in dry, rainy and cold season, respectively. High number of *An. dirus* was collected in rainy season followed by cold season and dry season in both Katine Htit and Kine Taw villages. Secondary vector of *An. vagus*, *An. maculatus*, *An. aconitus* were also collected in all season. Same result has been found by other researcher in Kayin State, mentioned that main vector *An. minimus* and *An. dirus* adult and larvae were collected from Katine Htit and Kine Taw villages in rainy season and secondary vector *An. culicifacies* were collected in highest number followed by main vector *An. minimus* in both Katine Htit and Kine Taw villages. *Anopheles dirus* was observed in rainy season and cold season in both areas.[15]

Other studies revealed that *An. culicifacies* were abundantly collected in cold season in Thabwewa village, in Oktwin Township, Bago Region, Sedawgyi village, Madaya Township Mandalay and Myothit village, Myolulin Township, Magwe Region.[16-18]

Although in the present study *An. minimus* and *An. culicifacies* were absent in all study villages in Bilu island, it may be due to breeding sources were not suitable for above mentioned species. Study carried out in different areas reported that *An. dirus* and *An. minimus* are the major vectors of malaria although *An. culicifacies, An. vagus, An maculatus, An. annularis* and *An. philippinensis* are the secondary or suspected vectors in Myanmar.[2]

In the present study, main malaria vector *An. dirus* was found moderately distributed in all villages. *Anopheles dirus* larvae were observed in domestic water wells in all study areas with co-breeder of *Cx. quinquefasciatus* larvae. Other researchers observed that *An. vagus, An. maculatus, An. annularis, An. tesselatus, An. barbirostic* and *An. hyrcanus* larvae were abundantly collected in domestic wells, slow running streams, sand pools and water pools in the creek bank and creek around the villages are the breeding sites of above *Anopheles* mosquitoes.[7, 15] *Anopheles minimus* were

found in high numbers in forest fringe foot hill and plain areas in the country.[2] *Anopheles dirus* are found in deep forested hilly areas.[19] *Anopheles dirus* larvae were collected in water well and *An. minimus* was collected in sand pools and slowly running water from the bank of the Younsalin creek in both Katine Htit and Kine Tow villages.[15]

Monitoring of insecticide susceptibility status of some *Anopheles* mosquitoes which were collected by animal bait and adult emerged from larval survey from Kayinwinsein, Piankadar and Kataungsein were found susceptible to WHO recommended Deltamethrin 0.05%, Permethrin 0.75%, Cyphyuthrin 0.15% and DDT4%. *Anopheles annularis* from Rakhine State was resistance to DDT 4%, and the mosquitoes are local vector of malaria.[2]

Same susceptibility results has been found against *Anophelines* in Oktwin Township of Bago Region and Mudon Township, Mon State revealed that *An. dirus, An. minimus, An. culicifacies, An. maculatus* and *An. annularis* were found to be susceptible to Icon, deltamethrin 0.025%, and permethrin.[20]

In conclusion, main vector *An. dirus* adult and larvae were found abundantly present in domestic water wells and secondary vectors such as *An. maculatus, An. vagus, An. aconitus, An. tesselatus* and *An. jamesii* adult and larvae were found in these Kayinwinsein, Piankadar and Kataungsein villages in Bilu Island, Chaung Zone Township, showing a risk of malaria transmission in these villages near future although all collected mosquitoes were susceptible to WHO recommended insecticides- DDT 4%, Deltamethrin 0.05%, Permethrin 0.75% and Cyfluthrin 0.15%.

Expected output and applicability

This study provided further information on risk of malaria transmission in these malaria endemic areas and it would support the vector borne diseases control programme in Myanmar.

ACKNOWLEDGEMENT

This study was supported by the grant from Department of Medical Research, Ministry of Health and Sports.

REFERENCES

1. National Elimination Plan (2016-2030) vision Myanmar free from malaria by 2030. Goals interrupt transmission & eliminate indigenous malaria by 2030.
2. Khin Maung Kyi. Malaria vectors in Burma *Anopheles balabacensis balabacensis* Baisas, 1936. *Union of Burma Journal of Life Sciences* 1970; 3(3): 217-225.
3. Maung Maung Mya, Myat Phone Kyaw, Sein Thaung, Tin Tin Aung & Yan Naung Maung Maung. Vector bionomics and potential vectors of malaria in Kamamaung Township, Phapun District, Kayin State. *Programme and Abstracts of the 44th Myanmar Health Research Congress*; 2016 Jan 5-9; Yangon, Myanmar. P. 77.
4. Yukich JO, Lengeler C, Tediosi F, Brown N, Mulligan JA, Chavasse D, *et al*. Costs and consequences of large-scale vector control for malaria. *Malaria Journal* 2008; 7: 258.
5. N' Guessan R, Corbel V, Akogbeto M & Rowland M. Reduced efficacy of insecticide-reated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerging Infectious Diseases* 2007; 13(2): 199-206.
6. Corbel V, Chabi J, Dabiré Roch K, Etang J, Nwane P, Pigeon O, *et al*. Field efficacy of a new mosaic long-lasting mosquito net (PermaNet® 3.0) against pyrethroid resistant malaria vectors: a multi centre study in Western and Central Africa. *Malaria Journal* 2010; 9: 113.
7. W Tun Lin, Myo Paing & Zaw Myint. A modification of the WHO dipping procedure for well-breeding *Anopheles* in Burma. *Tropical Biomedicine* 1988; 5:51-55.
8. Annual public health statistics, Ministry of Health. Health in Myanmar 2014.
9. World Health Organization. Manual on practical entomology in malaria part II. Geneva: WHO; 1975.

10. Harrison BA. Medical Entomology Studies: XIII. The Myzomyia series of *Anopheles* (*Cellia*) in Thailand, with emphasis on intra-interspecific variation (Diptera:Culicdae). *Contributions of the American Entomological Institute* 1980; 17(4):1-195.
11. Raid JA. Two Forms of *Anopheles philippinensis* Malaya. *Journal of Medical Entomology* 1967; 4(2): 175-179.
12. Myo Paing, Thi Thi Naing, Sein Min & Zaw Myint. *Anopheles* mosquitoes of Myanmar. III. *Anopheles (Cellia) philippinensis* Ludlow 1902 & *Anopheles (cellia) nivipes* Thebald 1903 in Myanmar and their differentiating characters. *Myanmar Health Sciences Research Journal* 1990; 2(1): 32-38.
13. Wirtz RA, Burkot TR, Graves PM & Andre RG. Field evaluation of Enzyme Link Immunosorbent Assay for *Plasmodium vivax* sporozoites in mosquitoes (Diptera: Culicidae) in Papua New Guinea. *Journal of Medical Entomology* 1987; 24(4): 433-437.
14. Maung Maung Mya, Sein Thaung, Nyan Sint, Yee Yee Myint, Sai Zaw Min Oo, Pae Phyo Kyaw, *et al*. Vector bionomics, potential vectors and insecticide efficacy in malaria endemic areas, Ye Township, Mon State Myanmar. *Scientific Research Journal* 2020; 8(7): 31-43.
15. Maung Maung Mya, Myat Phone Kyaw, Sein Thaung, Tin Tin Aung & Yan Naung Maung Maung. Potential vectors of malaria in Kamamaung, Myanmar and their bionomic. *Indian Journal of Entomology* 2018; 80(4):1261-1268. doi: 10.5958/ 0974-8172. 00245.6.
16. W Tun Lin, Myat Myat Thu, Sein Maung Than & Maung Maung Mya. Hyper-endemic malaria in a forested hilly Myanmar village. *Journal of American Mosquitoes Control Association* 1995; 11(4): 401-407.
17. Maung Maung Mya, Pe Than Htun, Sein Min & Saxena R.K. Cytotaxonomical Studies of *Anopheles culicifacies* complex and its malaria vectorial capacity in Myanmar. *International Journal of Contemporary Research in Engineering and Technology* 2012; 2(2): 17-26.
18. Maung Maung Mya, Phyo Wai Win, Aye Mya Thanda, Thiha, Maung Maung Gyi, Myin Zu Min, *et al*. Breeding habit and habitat of *Anopheles* mosquitoes in forest fringe and plain areas in Myanmar. *International Journal of Educational Research and Studies* 2019; 1(1): 30-37.
19. Maung Maung Mya, Myat Phone Kyaw, Sein Thaung, Tin Tin Aung & Yan Naung Maung Maung. Occurrence of *Anopheles* mosquitoes, potential vector, sibling species and susceptibility in malaria endemic areas of Kamamaung Township, Kayin State. *Myanmar Health Sciences Research Journal* 2017; 29(2):165-166.
20. Ministry of Health and Sports, Department of Medical Research. Entomology Section. In: *Annual report 2019*. Department of Medical Research, 2018; 88-98.