Vector bionomics, potential vectors and insecticide efficacy in malaria endemic areas, Ye Township, Mon State Myanmar

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Abstract: Vector bionomic and evaluation of insecticide efficacy were carried out in Koe Mine, Man Aung and 14 Mine areas of Ye Township, Mon State from July 2018 to August 2019 for better understanding of the *Anopheles* species distribution current insecticide susceptibility status and potential vector of malaria. Mosquitoes were collected using cattle baited net and light traps and larval survey was conducted in water well using WHO dipper. Standard WHO insecticide susceptibility was employed using field collected specimens. Results revealed that all total of 684 and 89 *Anopheles* mosquitoes consisted of 9 and 5 specie were collected from Koe Mine and Man Aung villages. Main vector *An. minimus* (6.58% & 3.37%) and Secondary vector *An. maculatus* (52.34%&37.08%) was collected in high density, *An. philippinensis* and *An. jamesii* adult was observed low density in both areas. Other species of *An. willmori, An. annularis, An. kochi* and *An. aconitus* were collected in Koe Mine village. All collected *Anopheles* mosquitoes were found sporozoite negative. These mosquitoes were also collected in indoor, outdoor and cow shed morning resting collected in low density. Susceptibility status of all collected *Anopheles* were found susceptible to WHO recommended insecticides. Although main vector of *An. minius* and secondary vectors of *Anopheles* were available in the villages moreover there is also filarial vector *Culex quinquefasciatus* mosquitoes larvae were observed high density and co breeder with *An. minimus* larvae and also DHF vector *Aedes aegypti, Ae. albopictus* and a new species of *Ae. cogilli* were also breeding in some water wells. There is high risk for the transmission of malaria, filarial and DF/DHF in both study areas, if diseases carrier is available in the villages and need to proper control measure as use of insecticide treated bed nets and application of focal residual spray for adults as well as treatment of abate or larvivorus fishes in water wells for larvae control to prevent vector borne diseases transmission in these areas.

Key words: Vector, bionomics, breeding habit, domestic water wells, *An.minimus, Ae. aegypti, Ae.albopictus, Ae. cogilli, Cx. quinquefasciatus*

Introduction

Previously malaria is one of the major public health problems in Myanmar. Malaria cases and death reduced by 62% and 91% in 2015 compared to 2012. The incidence of malaria is fall down by 49% from 2012 to 2015 and no reported malaria outbreaks since 2012[1]. The malaria morbidity and mortality is still remaining in border and hard to reach areas. Primary and secondary vectors of malaria are still found in malaria free and endemic areas in the country. It is still a main contributor to the morbidity and mortality in some part of the country. 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. *An. sundaicus* and *An. subpictus* are abundantly found in coastal areas of Rakhine, Mon and Tanintharyi Regions. The transmission of

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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 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]. The operational criterion of resistance has usually been taken as the survival of 20% or more of the individuals tested at the currently known diagnostic concentrations using WHO test kits in field[7]. Therefore, 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. Present study planned to investigate the bionomics of malaria vectors and evaluate the efficacy of insecticides for strengthening of control strategies.

Rationale

Justification for the study site selection

Ye is a town in the southern end of Mon State. It is the principal town of the Ye Township of Mawlamyine District. Large and forested areas with over 40 villages are situated in the Township. The Township is the border area of the Thaninthayi Region and inhabited by 263624 people in 2721Km^2 areas. Population density is 96.90/km^2 as well as the economically it is most popular. The Township is situated in South part of the Thanbyuzayat Township, Mon State. Many creeks traverse the mainland and Ye river running across the Township as it drains into the Gulf of the Martaban and is surrounded by Thaninthayi hills in the east. The climate is tropical (24° to 30°C temperature) and humid (mean relative humidity of 78.5%) with abundant rainfall, supporting luxuriant and rich vegetation. In raining and cold seasons monthly new malaria cases were found in Ye Township and 1-2 malaria patients from Ye Township were admitted to Mawlamyaine General Hospital monthly (Personal communication).

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[8]. According to the ecological changes, distribution of malaria morbidity patterns and results from the micro-stratification, the high risk areas for the malaria was about 38.90% in the 1990 was reduced to 17.00% in 2013. And free areas in the country were increased from 8.60% in 1990 to 37% in 2013[9]. 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. Monthly malaria cases were found in Ye Township (Personal communication). There is a lack of data and information about the vectors, potential vectors and insecticide efficacy on *Anopheles* mosquito vectors in Ye Township, Mon State. Therefore an attempt has been made to determine the vector bionomics, potential vectors and insecticide efficacy of primary and secondary vectors of malaria in these areas.

Materials and Methods

Study area: Two villages as Koe Mine and Man Aung were selected in Ye Township, Mon State.

Study period: The study period was one year, stated from July 2018 to August 2019.

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Mosquito's population: all collected mosquitoes and larvae in different collection methods such as cattle bait and light traps for adult collection and WHO dipper.

Study areas

Koe Mine and Man Aung villages were selected for adult *Anopheles* mosquitoes and larvae collection because malaria cases occurred annually according to hospital report. In Koe Mine village a total of on about 2730 population were stayed in 380 houses and in Man Aung village a total of 1070 population were lived in130 houses. These villages are at least 10 kilo meter away from Ye Township. All most all of the villagers are worked in Rubber and battle nut plantation in hilly areas. Same were school teacher, health workers and government staff. Ye-Dawe road is across to the Koe Mane village and Man Aung village is 10 Km far away from Koe Mine village. And also want to know the distribution of malaria vector *Anopheles* mosquitoes in both villages.

Methodology

Data collection procedures

Entomological Survey:

Mosquito collection

Fixed mosquito catching stations were chosen in both selected villages. Indoor, outdoor light traps and animal bait catches were conducted in both areas in June. All catching stations were fixed in villages throughout the study periods.

Animal bite mosquito collection was done with big mosquito net (Kanda net=K net) and indoors and outdoors mosquito catching were done with CDC light traps method and mosquitoes were caught with WHO sucking tubes from 18:00 to 06:00 hours of the next day for 7days.

Indoor resting density: Mosquitoes resting place were searched in indoor and outdoor of the households and cowshed by the permission of householders in early in the morning and density was measured by the formula of **Resting Density** = total number of resting mosquitoes / total number of house checked. **Indoor resting density** = total number of resting mosquitoes indoor / total number of houses checked indoor.

Larval surveys

For identification of breeding sites larval surveys were conducted in and around three kilo-meters away from the study villages. Water pools, domestic wells, stream/creeks and all different types of water holding places were examined by 3 Dips /water holding place for larval detection[10]. In and around the villages, 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 different keys [11-13].

Insecticide susceptibility test

Insecticide susceptibility tests (WHO test kit):

Collected adult female Anopheles mosquitoes from the entomological survey were tested for measurement of insecticide susceptibility level using WHO test kits[7] and standard procedures. The efficacy of insecticides which are commonly used for malaria

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vector control in malaria endemic areas namely as DDT4%, Permethrin 0.75%, Cyfluthrin 0.15% and Deltamethrin 0.05% impregnated paper with WHO test kits were provided.

Procedure

5 to10 fields collected *Anopheles* mosquitoes were introduced in WHO insecticide impregnated paper attached plastic tube (WHO test kit) by sucking tube and exposed for 1 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 knockdown was measured after 60 minutes exposure and effective mortality was assessed after 24 hours exposure. Two replicate testing were done to confirm the susceptibility of mosquitoes. If the quantity of collected mosquitoes was not more than 10 while we used pool mosquitoes samples to test insecticides susceptibility.

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.,[14] for vector incrimination study.

Map of the study areas

Map of the study areas, adult *Anopheles* mosquito collection sites, (indoor, outdoor and animal bait) larva positive places and human settlement areas were drawn by using Global Positioning System GPS (GPSMap16 Garman, 18x-5HZ software interface application) method by expert person.

Data analysis:

Entomological data were analyzed and compared both study areas. Data entry, processing and analysis were carried out using Microsoft Excel software. Mosquitoes and larva density were determined in percentage. Insecticide susceptibility, potential vector were determine in percentage.

Results

Table 1: Occurrence of adult *Anopheles* mosquitoes in Koe Mine and Man Aung village Ye Township

Sr.No.	Species	Koe Mine village			Man Aung village		
		Collected mosquitoes	Density %	Sporozoite positivity	Collected mosquitoes	Density %	Sporozoite positivity
1	*An. minimus*	45	6.58	Neg	3	3.37	Neg
2	*An. maculatus*	358	52.34	Neg	33	37.08	Neg
3	*An. jamesii*	27	3.95	Neg	27	30.34	Neg
4	*An. willmori*	33	4.83	Neg	0	0	Neg
5	*An. annularis*	47	6.87	Neg	0	0	Neg
6	*An. kochi*	37	5.41	Neg	0	0	Neg
7	*An. aconitus*	19	2.78	Neg	0	0	Neg
8	*An. philippinensis*	31	4.53	Neg	17	19.10	Neg
9	*An. barbirostris*	81	11.84	Neg	9	10.12	Neg
10		684	100	Neg	89	100	Neg

Table 1. shows that total of 684 *Anopheles* mosquitoes consist of 9 *Anopheles* species and 89 adult *Anopheles* mosquitoes consist of 5 *Anopheles* species were collected from both Kow Mine and Man Aung villages. Main vector of *An. minimus* were collected in both Koe mine 45(6.58%) and Man Aung village 3 (3.37%). In Koe Mine village highest density of *An. maculatus* 52.34% were collected followed by *An. barbirostris* 11.84% lowest was observed *An. aconitus* 2.78%. In Man Aung village highest density of *An. maculatus*

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37.08% were collected followed by *An. jamesii* 30.34% and the lowest density was found *An. minimus* 3.37%. Vector incrimination study found that all collected head and thorax of *Anopheles* mosquitoes were found sporozoite negative.

Table 2. Morning resting mosquito collection in indoor, outdoor and cowshed in Koe Mine and Man Aung villages in Ye Township

Sr. No.		Koe Mine village (5 days morning resting collection)			Man Aung village (5 days morning resting collection)		
		Indoor	Outdoor	Cow shed	Indoor	Outdoor	Cow shed
1	*An. minimus*	2±0.54	1±0.45	5±0.71	1±0.45	0±0.00	2±0.54
	An. maculatus	6±0.45	2±0.54	13±1.14	4±0.84	4±0.84	12±1.14
	An. kochi	2±0.54	1±0.45	5±0.71	0	0	0
	An.jamesii	0	0	0	3±0.55	3±0.89	6±0.84
	An. philippinensis	0	0	3±0.71	0	0	1±0.71
	An. annularis	5±0.71	1±0.45	4±0.84	3±0.89	1±0.45	3±1.34
	Culex	19±1.64	3±0.89	37±1.94	19±1.09	9±0.1.30	54±2.78
	Total	34±7.05	8±0.89	67±13.15	30±7.35	17±3.51	78±20.47

Table 2. shows that highest number of mosquitoes were collected from cowshed in both areas i.e. 67±13.15 and 78±20.47 followed by 34±7.05 and 30±7.35 from indoor and lowest were observed in outdoor of both Koe Mine and Man Aung villages. Of this six species of *Anopheles* mosquitoes were collected. The highest number of *An. maculatus* was collected 13±1.14 in koe mine and 12±1.14 from Man Aung villages in cowshed morning resting collection. Main vector *An. minimus* was collected higher 5±0.71 and 2±0.54 from cowshed than indoor 2±0.54 and 1±0.45 resting morning collection in both Koe Mine and Man Aung villages. *Culex* mosquitoes were found higher in all indoor (19±1.64, 19±1.09) outdoor (3±0.89, 9±0.1.30) and cowsheds(37±1.94, 54±2.78) in both areas.

Table 3. Breeding habitats of different mosquito species in Koe Mine, Man Aung and 14 Mine (Ma Hlew Taung villages in Ye Township Mon State

Sr. No.	Collected mosquito larvae	Koe Mine			Man Aung			14 mine (Ma Hlew Taung)		
		Total examined well	Larva positive well & %		Total examined well	Larva positive well & %		Total examined place in creek	Larva positive place & %	
		72	49	68.05	25	19	76.00	31	13	41.93
2	*An. minimus*		6	12.25		3	15.79	3	3	23.08
3	*Cx.quinquefasciatus*		40	81.63		11	57.90		0	0
4	*Ae.aegypti+Ae.albopictus*		0	0		1	5.26		0	0
	Ae.cogilli		0	0		1	5.26		0	0
5	*Cx.quinq+An.mini)*		3	6.12		2	10.53		0	0
	Cx.quinq+An.mini+Taxo)		0	0		1	5.26		0	0
6	*An.maculatus*		0	0		0	0	5	10	76.92
	Total	72	49	100	25	19	100	8	13	100

Cx.quinq =Cx.quinquefasciatus, An.mini=An.minimus, Taxo= Toxorhynchities

Table 2. Shows that detail breeding habit of *Anopheles*, *Culex* and *Aedes* larvae in water wells and creeks in study areas in Yee Township. A total of 72 and 25 wells were examined and found that 49 (68.05%) and 19 (76.00%) wells were positive for mosquitoes larvae in Koe Mine and Man Aung village. Highest number of *Cx. quinquefasciatus* habitats (Water wells) were found in Koe Mine village 40(81.63%) followed by 11(57.90%) in Man Aung village. *Anopheles minimus* larvae were breed 6(12.25%) and 3(15.79%) wells in Koe Mine and Man Aung villages. In both villages *Cx.quinquefasciatus* and *An. minimus* larvae were bred together in 3(6.12%) wells in Koe Mine and 2(10.53%) wells in Man Aung. Only 2(10.53%) wells from Man Aung village were found *Aedes*

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larvae positivity. Of this one well was found *Aedes aegypti* and *Ae. albopictus* larvae and another well was positive with *Ae. cogilli*. In 14 Mine (Ma Hlew Taung =Ma Hlew hill) area larval survey was done in 31 places in creek of the Ma Hlew Taung, and found that 3(23.08%) of the *An. minimus* habitats and 10(76.93%) of the *An.maculatus* breeding habitats were found beside the creek where the water is slowly running.

Table 4. Susceptibility and sporozoite positivity status of collected *Anopheles* mosquitoes from Koe Mine and Man Aung villages in Ye Township

Sr.No.	Species	samples	Susceptibility test		Sporozoite detection by ELISA	
			impregnated papers (WHO)	Results	Test samples	Sporozoite positivity
Koe Mine village	*An. minimus* *An. maculatus* *An. jamesii* *An. willmori* *An. annularis* *An. kochi* *An. aconitus* *An. philippinensis* *An. barbirostris*	40 80 20 20 40 20 19 20 40	DDT4%, Permethrin0.75%, Cyfluthrin 0.15% Deltamethrin 0.05%	Susceptible	45 358 27 33 47 37 19 31 81	Negative
Man Aung village	*An. minimus* *An. maculatus* *An. jamesii* *An. philippinensis* *An. barbirostris*	3 33 27 17 9	DDT4%, Permethrin 0.75%, Cyfluthrin 0.15% Deltamethrin 0.05%	Susceptible	3 33 27 17 9	Negative

Table 4. shows that all collected *Anopheles* mosquitoes from Koe Mine and Man Aung villages were highly susceptible to WHO recommended Insecticides as DDT4%, Permethrin 0.75%, Cyfluthrin 0.15% and Deltamethrin 0.05% as well as sporozoite positivity status of collected *Anopheles* mosquitoes were found sporozoite negative by ELISA test.

Discussion

Sporadic studies on different species of mosquitoes suggest ecological and biological differences in types of larval habitat, seasonality and behaviour according to species [15-17] but such differences also occur for specific species within their distributions and could relate to key environmental factors. Variation in behaviour, such as early biting or ovipositing in wells, might determine vector capacity and adaptation to changing environment. Even so, *Anopheles* species exploit a variety of breeding habitats that vary considerably in size, altitude, vegetation cover and topography [18-19].

Of the 37 Anopheline species recorded in Myanmar *An.dirus* and *An.minimus* are primary vector of malaria in Myanmar other local vector as *An.annularis* is a vector of malaria in Rakine State and *An. sundigus* and *An. subpictus* are the vector of costal areas as Taninthayi and Mon state [2]. In the present study occurrence of *Anopheles* mosquitoes in Ye township found that a total of 9 species of *Anopheles* mosquitoes were collected, the highest density of *An. maculatus* 358 (52.39%) from Koe Mine and 33 (30.34%) from Man Aung villages were collected followed by *An. barbirostris* 81(11.84%) in Koe Mine and *An. jamesii* 27(30.34%) in Man Aung by cattle bate k-net collection within 5 days. Although the main vector *An. minimus* was collected 45(6.58%) in Koe mine and 3(3.37%) in Man Aung village were lower density when compared with the secondary vector *An maculatus*. Similarly high density of *An.minimus* and *An.maculatus* were observed from Katinehtit and Kinedaw villages Kamamaung Township Kayin State and *An.*

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minimus and *An.dirus* were found potential vector of malaria in this area [20]. In the present study main vector *An. dirus* have not collected in both areas although monsoon is starting. Other researchers observed that high density of *An. dirus* were collected in monsoon period in Yephyu Township Taninthayi, Oktwin Township Bago Regions and low density was observed in Kamamaung Township Kayin State[21-23]. Study carried out in different areas reported that *An.dirus* and *An.minimus* are the major vectors of malaria although *An. culicifacies, An.vagus, Anmaculatus, An.annularis* and *An. philipinansis* are the secondary or suspected vectors in Myanmar[2]. *Anopheles dirus , An.minimus, An.philippinensis, An.culicifacies, An. stiphensi, An. fluviatelis* are vector of malaria in India[24], and *An. culicifacies* B and E are present in India and Sri Lanka[25,26]. In the present study main vector *An.minimus* was found highly active from 21.00 hr to 24.00 hr in outdoor in both Koe Mine and Man Aung village and pick biting time was found between 22:00 and 23.00 hr. Although Thailand researchers revealed that *An. minimus* showed biting activity at 19:00-21:00 hr indoors and again at 19:00-20:00 hr and 23:00-00:00 hr in outdoors[27]. In a previous study in western Thailand, the main biting activity of *An. minimus* was indoors between 01:00 and 04:00 with a peak at 02:00, whereas outdoor feeding started around 22:00, reaching a peak near midnight[27]. Recently, Kwansomboon et al.,[29] reported that *An. minimus* fed preferentially outdoors along the Thailand-Myanmar border (0.57 and 0.58). Maculatus group consisted essentially of *An. rampae* which is highly zoophilic[30] and has no confirmed role in malaria transmission[30,31]. Same finding was observed in present studied *An. maculatus* was abundantly collected from cattle bate collection and they are suspected vector of malaria in Myanmar. In the present study all collected mosquitoes were found soprozoite negative by ELISA method. Although other researcher found that vector incrimination study of *An. dirus, An. minimus, An. kochi* and *An. maculatus* were sporozoite positive in Bubpin Township in Thaninthayi Region and *An. minimus* and *An. culicifacies* were found sporozoite positive in Ann Township, Rakhaine State Myanmar[32]. Maung Maung Mya and his associate reported that *An. minimus* was *Pf* circumsporozoite positive which was collected indoor at 11 pm in Katine Htit village and one *An. dirus* was Pv_{210} circumsporozoite positive which was collected from outdoor human bait collection at 10:00pm in Kine Taw village Kamamaung Township, Kayin State Myanmar[20].

Highest numbers of mosquitoes were collected from cowshed in both areas than indoor and outdoor mosquito collection in both Koe Mine and Man Aung villages. Of this six species of *Anopheles* mosquitoes were collected. The highest number of *An. maculatus* was collected in Koe mine and Man Aung villages in cowshed morning resting collection. Main vector *An. minimus* was collected higher in Koe Mine than Man Aung from cowshed resting morning collection. *Anopheles* mosquitoes were mostly collected under the hanging cloths and bomboo baskets as resting position. *Culex* mosquitoes were found higher in all indoor, outdoor and cowsheds in both areas and these mosquitoes were mostly found under the bed, cloths, baskets, open bags and moisture areas. Other researcher revealed that highest number of *An.dirus* mosquitoes were collected biting outdoor in May 1996 and 1997 in Ohnbinkwin village, Yephyu Township Taninthayi Region[21].

Breeding habitats of different mosquito species in Koe Mine, Man Aung and 14 Mine (Ma Hlew Taung) villages in Ye Township Mon State were found *An. minimus*, *Culex* and *Aedes* larvae were bred in domestic water wells, no water wells were observed in 14 Mine (Ma Hlew Taung) village. A total of 72 and 25 wells were examined and found that 49 (68.05%) and 19 (76.00%) wells were positive for mosquitoes larvae in Koe Mine and Man Aung village. Almost all of the larval positive wells were found full of the vegetative on the surface of the wells and debris in water. *Anopheles minimus* larvae were breed 6(12.25%) and 3(15.79%) wells in Koe Mine and Man Aung villages. In both villages *Cx.quinquefasciatus* and *An. minimus* larvae were bred together in 3(6.12%) wells in Koe Mine and 2(10.53%) wells in Man Aung. Only 2(10.53%) wells from Man Aung village were found *Aedes* larvae, one well was positive with *Aedes aegypti* and *Ae. albopictus* larvae and one well was found *Ae. cogilli* positivity. Highest number of *Culex*

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quinquefasciatus were breeding in wells 40(81.63%) in Koe Mine village and 12(63.16%) in Man Aung village. It is the first time report of the *An.minimus, Ae.aegypti, Ae. albopictus, Ae.cogilli* and *Cx.quinquefasciatus* larvae in water well in Myanmar. And also *Ae. cogilli* (new species) was first time found in Myanmar in Man Aung village from Ye Township Mon State. Although other researchers revealed that *An. dirus* larvae were highly breeding in water wells in Mon state and Taninthayi Region[8,21] but they are deep forested breeding in rock pools[22]. Although Maung Maung Mya and his associate reported that *An. dirus* larvae were found in water wells and *An.minimus* larvae were found in sand pools and slowly running water in Kamamaung Township Kayin State in rainy season[20]. In a coastal village, Chantaburi Province of Thailand near the Cambodian border, the breeding of *An.dirus* is in small water-collections (gam pits)[33]. Although in Thabewa village Oktwin Township Bago Region found *An.dirus* larvae were highly breed in rock pools in forested hilly area and *An.minimus* was observed in slowly running water in Thabewa creek and water pools near the creek[22]. The result are agreed with the present study it was found that 3(23.08%) of the *An. minimus* habitats and 10(76.93%) of the *An.maculatus* breeding habitats were found beside the creek of Ma Hlew Taung where the water is slowly running. Pe Than Htun et al., found that *An.minimus* were abundantly present in rice fields[34]. Other researcher revealed that *An.minimus* were co breeder with *An. culicifacies* in sand pools in Pakkaung and Phyu Townships Bago Region and abundantly collected in slowly running water in Yunsalin creek of Kamamaung Township Kayin State[35,23,20]. Other researcher from India revealed that *An. stephensi* was bred in high temperature water (35 d C) in overhead tank and in wells water minimum 26.58 °C in December and maximam 28.59 °C in May[36].
In both Koe mine and Man Aung villages 81.63% and 63.16% of the domestic wells were found *Culex quinquefasciatus* larvae in large numbers. The wells were found different in shape, size and depth. They were approximately 10 to 50 feet deep and some were circular and some were square in shape as well as some are muddy well covered with bamboo or woods and some are covered with break some were made large concrete pipes. Most of the wells were under shade of trees and Shelters. A researcher from India revealed that 66.67 % of wells and 63.64 % of other breeding habitats with *An. stephensi* breeding were observed to co-inhabit with either *Culex* or *Aedes* species. However, co-inhabitation of anophelines with other mosquito species in Over Head Tanks was rare (5 %) [36]. Farjana et al., [37] revealed that drains were the most common aquatic habitat of *Anopheles* and *Culex* larvae in Boyra, Kewatkhali and Balashpur and *Culex quinquefasciatus* is widely distributed over all the localities, similar findings have been reported in Dhaka city[38]. The results were agreed with the abundant of *Cx. quinquefasciatus* larvae and co breeder of *An. minimus* larvae in both study areas. It may be due to the fact that in dry season mosquitoes breeding habitats are dried in both areas therefore polluted water bred *Cx. quinquefasciatus* and slowly running water bred *An. minimus* may change their breeding habit and habitats to permanently remained wells water.

In the present study 2(10.53%) of the domestic water wells were found *Aedes* larvae positivity. Of this 5.26% well was found *Ae. aegypti* and *Ae. albopictus* larvae. Other researchers revealed that certain *Aedes* species, as *Ae. albopictus* and *Ae.aegypti* are highly breed in artificial containers Car tyres, metal drums, plastic drums, Bago jars, earthen pots concrete jars in Myanmar[22,39,40], tree holes and other variety of container in Northen Nigeria[41]. Certain *Aedes* species, including *Ae. albopictus*, are able to breed in artificial containers[42]. *Aedes aegypti* is an urban species, whereas *An. albopictus* prefers to breed in the rural conditions in breed both in artificial and natural containers[43]. In the present study, one of the water well in Man Aung village was observed *Ae. cogilli* larvae. This is the first time report of the *Ae.cogilli* in Myanmar in Man Aung village Ye Township Mon State. Same findings also found the first time has been recorded *Aedes* (Phagomyia) *cogilli* in Sri Lanka and morphologically confirmation and molecular characterization was supported the presence of *Ae. cogilli* as well as *Ae. cogilli* had developed resistance to common insecticides and developed adaptations to urban environment, raising health and environmental concerns and it was collected in the canals of the Jaffna Municipality area in Sri Lanka[44]. Other researchers revealed that *Ae. cogilli* is regarded as a sylvatic species mainly breeding in tree holes and hollow bamboos[45]. Although in the present study found *Ae. cogilli* was bred in water well in Man Aung village. *Ae. cogilli*

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is a dominant species in India[46]. *Aedes aegypti* and *Cx. nebulosus* showed highest preferences for breeding in automobile Tyres, *Cx. horridus* and *Cx, quinquefqsciatus* larvae occurred most in wells[41]. Other researcher reported that *Aedes vittatus* is currently found in Africa, Asia and Europe, where it acts as a vector of pathogens causing animal and human diseases and *Ae. vittatus* and *Ae. cogilli*, while similarities with other, public databases provided a ≥ 99% similarity with sequences for two *Aedes* mosquitoes[42] (Fernández et al., 2018). Larvae of *Ae. vittatus* have been recorded in a variety of habitats including rock pools, tree holes, domestic containers and hoofprints[47,48]

World Health Organization estimated that 40% of the 506 insect species of medical importance had evidence of resistance to various insecticides[2]. Most documented cases of resistance have involved organochlorine, organophosphate, and carbamate class compounds compared with the relatively more recent introduction of broad-spectrum pyrethroids[49]. In present study found that all collected *Anopheles* mosquitoes from Koe Mine and Man Aung villages were highly susceptible to WHO recommended Insecticides as DDT4%, Permethrin0.75%, Cyfluthrin 0.15% and Deltamethrin 0.05%. Same insecticides susceptibility status of Anopheles mosquitoes collected from Pin Oo Lwin township Mandalay Region and Kamamaung Township Kayin state were found 100% sensitivity to WHO recommended insecticides[23]. Although other researcher observed that *An. dirus* s.l. is susceptible to DDT[50] but due to exophilic behaviour, females avoid treated walls[51] or even avoid the sprayed huts by biting more outdoors after residual spraying[52]. An Indian researcher working on unwashed PermaNet in India found that 100% mortality in both *An. minimus* and *An. dirus*[53]. *Anopheles dirus* was found to have 100% knockdown and mortality against brand-new, one and three year duration of unwashed PermaNet 2.0 and very sensitive to deltamethrin treated different LLINs nets on *An.minimus* and secondary vector of malaria in different parts of Myanmar .[54,55]. A Thailand researcher revealed that contact irritancy is a major behavioral response of both *An. minimus* A and C when exposed directly to any of the three compounds commonly used agricultural insecticides, carbaryl (carbamate), malathion (organophosphate) and cypermethrin (pyrethroid),, whereas only cypermethrin produced a significant repellency response in *An. minimus* species A[56]. Although WHO report from 2010 to 2016 revealed that there was no clear indication of an overall global increase in resistance frequency to the other three insecticide classes commonly used in malaria vector control. However, increased resistance since 2010 was evident for the WHO South-East Asia Region for organochlorines (predominantly DDT) (12% rise, from 17% to 29%), carbamates (9% rise, from 0% to 9%) and organophosphates (11% rise, from 1% to 12%). There was also some evidence of an increase in organochlorine resistance frequency in the WHO Eastern Mediterranean Region since 2010 (9% rise, from 24% to 33%). The only increase in resistance frequency for nonpyrethroid insecticides identified for any African subregion was an increase in carbamate resistance in central Africa (6% rise, from 6% to 12%) [57].

In conclusion

Main vector *An. minimus* adult and larvae were collected in both Koe Mine and Man Aung villages and larvae were found in domestic water well in both areas and larvae of An. minimus and An.maculatus were collected in the Ma Hlew Taung creek of 14 Mine . Secondary vector *An. maculatus* was collected in high density, *An. philippinensis* and *An.jamesii* adult was observed low density in both areas. Other species of *An. willmori An. annularis An. kochi* and *An. aconitus* were collected in Koe Mine village. All these mosquitoes were also collected in indoor, outdoor and cow shed morning collected. Susceptibility status of all collected *Anopheles* were found susceptible to DDT4%, Permethrin0.75%, Cyfluthrin 0.15% and Deltamethrin 0.05%. Although main vector of *An.minius* and secondary vectors of *An.minimus, An.philippinensis, An. jamesii, An.kochi, An. willmorri* and *An.annularis* were available in villages moreover there is also filarial main vector of *Culex quinquefasciatus* mosquitoes larvae were observed high density in water wells and they are co breeder with *Anopheles* larvae and also DHF vector Aedes aegypti and *Ae.albopictus* and a new species of *Ae.cogilli* were breeding in water wells. Therefor there is a high risk for the transmission of malaria, filarial and DF/DHF in both

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study areas need to proper control measure as use of insecticide treated bed nets and application of focal residual spray for adults as well as treatment of abate or larvivorus fishes in water wells for larvae control to protect vector borne diseases transmission in these areas. In prevention and control, at selected areas are important part of the programme in Ye township Mon State.

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