WELL-BREEDING *ANOPHELES DIRUS* AND THEIR ROLE IN MALARIA TRANSMISSION IN MYANMAR

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Abstract. Mosquitos were collected with human and animal baits from March 1996 to January 1998 in four villages located along the Yadana gas pipe line in Yepyu township, Dawae district, Tanintharyi Division, southern Myanmar. A total of 23 anopheline species were collected. *Anopheles dirus* were abundant in pre-monsoon (May/June) an post-monsoon (October) months. All *An. dirus* caught both humans and cattle were assayed with specific, sporozoite enzyme-linked immunosorbent assays (ELISAs). A total of 5/250 (2%) caught with human bait was found positive with *Plasmodium vivax* from Eindayaza, Ohnbinkwin and Thaechaung during rainy and cool-dry months. Larval surveys also showed *An. dirus* larvae/pupae were caught from domestic wells (6 to 46% found positive). Clinical surveys indicated that transmission is hyperendemic and occur all year round in all four villages.

INTRODUCTION

Since 1978 malaria has been declared the first health priority in Myanmar. Of the 37 anopheline species recorded in Myanmar *An. dirus* (Peyton and Harrison, 1979) is one of the primary vectors in Myanmar and its area of prevalence can be taken to coincide with the distribution of forest (Scanlon and Sanhinand, 1965; Tin and Tun, 1991; Gingrinch *et al,* 1990). In Myanmar, *An. dirus* is a widespread species, particularly in the upper central, Mandalay Division, Bago Yoma, Mon State, Kayin State, Tanintharyi Division and is found in forest and forested foothill areas (Myo-Paing *et al,* 1989, 1990a).

A preliminary survey made in June 1995 revealed the two primary vectors *An. dirus*, and *An. minimus* with the secondary vectors *An. sundaicus*, *An. annularis*, *An culicifacies* and other potential vectors for focal transmission like *An. aconitus, An. maculatus gp, An. philippinensis* were also caught with human and cattle baits from this study area. Unlike other parts of Myanmar *An. dirus* were found breeding in domestic wells in southern Myanmar. In this paper, we report the bionomics of *An. dirus* and its role in malaria transmission in Yepyu township, Dawae district, Tanintharyi Division, Southern Myanmar.

MATERIALS AND METHODS

Study area

Yepyu township is one of the townships in Dawae district, Tanintharyi Division. It is in the south-eastern part of Myanmar. Four villages from Yepyu township, *viz* (l) Eindayaza, (2) Michaung-laung, (3) Ohnbinkwin and (4) Thaechaung each with 97 houses and 652 inhabitants; 59 houses and 331 inhabitants; 280 houses and 1,595 inhabitants and 242 houses and 1,436 inhabitants respectively, were selected as study areas for this investigation (Fig 1). All these villages are not far from the Andaman sea and the Tanintharyi mountain ranges runs from north to south. The area is mountainous and covered with forest, and creek runs around or through the villages. Most villagers remain permanently inside the village. The main occupation of villagers is cashewnut plantation, lead digging, wood-cutting and seeking forest products. However, villages like Ohnbinkwin and Thaechaung have many rice fields. These areas are highly endemic for malaria. Previous surveys showed the spleen rate in the 2 to 9 year old age group to be 52.6% and the crude parasite rate was >30%. This is an unsprayed area.

Mosquito collection

Fixed mosquito catching stations were chosen in four selected villages. Indoors and outdoors biting and landing catches were conducted. All catching was done in fixed stations throughout the study as follows:

- Human bait hand catches with glass tubes and WHO sucking tubes from 18.00 hours to 06.00 hours of the next day, both indoors and outdoors catching were conducted.

- Human baited and animal baited big bed net catches were also conducted with WHO sucking tubes starting from 18.00 hours to 06.00 hours of the next day.
- Day-time indoor resting collections were also conducted in houses and cow-sheds.

Larval surveys

For identification of breeding sites larval surveys were conducted in and around the study villages. Domestic wells and stream/creeks were the major breeding habitats emphasized. However, during rainy season all the water pockets, coconut shells, discarded tins and utensils bamboo stumps including footprint of buffalos, elephants, etc, were examined. The captured larvae and pupae were put in labeled plastic bags and brought back to the laboratory for species identification.

Anopheles mosquitos were identified by species according to Peyton and Scanlon (1966), Reid (1967), Harrison (1980) and Myo-Paing *et al*, (1990b).

Incrimination of vector

Guts and salivary glands of *An. dirus* were dissected for oocysts and *Plasmodium* sporozoites. Enzyme-linked immunosorbent assays (ELISA) test for circumsporozoites antigen were supplemented according to Wirtz *et al*, (1987) for vector incrimination studies.

Parasitology and spleen rate

100-200 samples of thin and thick films of peripheral blood were taken from every study village. All children between 2 and 9 years age group were examined for spleen enlargement using Hackett's index (Bruce-Chwatt, 1985). The data were collected seasonally. Surveys were conducted during cool-dry (January), hot-dry (March), pre-monsoon (May/June), mid-monsoon (July/August) and post monsoon (September/October) months.

Meteorological data

Data were obtained from TMEP weather station Kanbauk, Dawae (Fig 2). The study area experiences its maximum rainfall during the southwest monsoon (May to October) with coastal areas receiving higher levels than the interior uplands. High intensity storms are fairly common in the area. Each year, approximately eight storms exceed 100 mm of rainfall during 24 hour period. Rain gauge data indicate that high rainfall and longer-duration, can be particularly frequent in August. Rainfall levels during the rainy season are generally such as to effectively prohibit the mosquitos to come out from their hiding places. Maximum daily temperatures

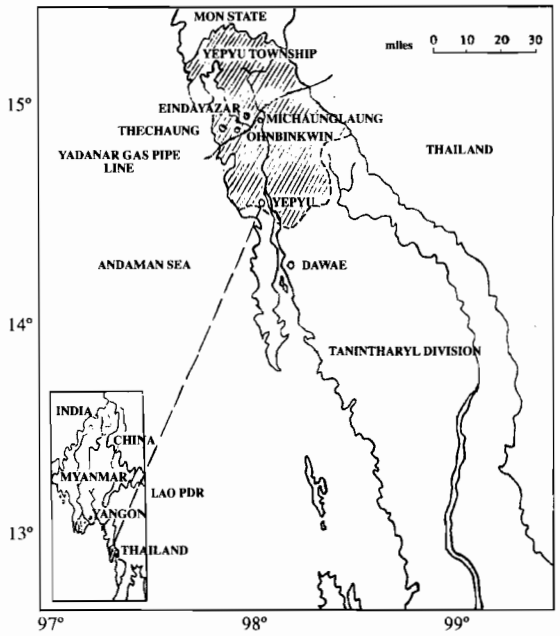

Fig 1–Map of Yepyu township showing four study sites.

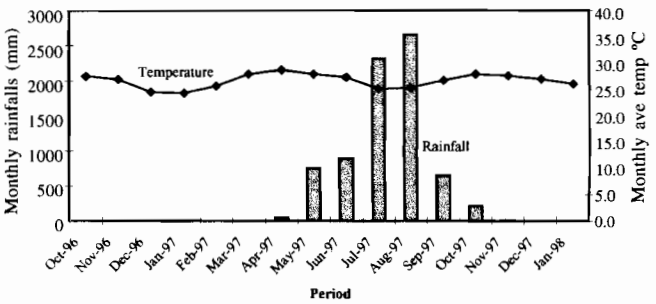

Fig 2–Meteorological data of study area: Yepyu township.

are 28-30°C during the southwest monsoon and 32-34°C during the northeast monsoon.

RESULTS

Table 1 shows the diversity of anopheline mosquitos in the study area. 23 anopheline species were collected from this area including *An. dirus* and *An. minimus*, the two primary vectors together with *An. sundaicus*, *An. annularis* and *An. culicifacies*, the secondary vectors and other potential and suspected vectors like *An. aconitus*, *An. maculatus*, *An. philippinnsis* were also caught. Monthly variation in rainfall influenced the prevalence of mosquito

Table 1
Species and number of mosquitos collected with human bait (HB) and cattle bait (CB) from four study villages of Yepyu township along "Yadana" gas pipe corridor area.

Anopheline species	Nos. of mosquitos collected from							
	Eindayaza		Michaunglaung		Ohnbinkwin		Thaechaung	
	HB	CB	HB	CB	HB	CB	HB	CB
Cellia								
aconitus	26	263	15	24	32	153	33	161
annularis	734	1,699	20	167	29	125	37	112
cuhcifacies	0	58	0	9	0	10	0	0
dirus	15	24	12	10	200	157	50	47
filipinae	10	48	0	0	12	9	0	4
jamesii	13	1,325	3	45	24	1,198	92	398
karwari	23	246	9	59	117	394	20	168
kochi	31	146	25	91	25	91	6	28
maculatus	205	1,041	79	335	63	149	4	38
minimus	42	337	23	87	5	25	0	38
nivipes	0	49	2	9	2	60	26	49
pampanai	0	11	0	0	2	2	0	4
philippinensis	11	108	8	41	34	238	145	649
ramsayi	0	11	0	5	1	3 8	0	0
splendidus	6	66	0	1	0	0	0	0
stephensi	0	3	0	0	1	19	0	6
subpictus	3	48	0	4	10	86	0	1
sundaicus	55	215	25	92	278	586	77	166
tesselatus	0	11	0	17	13	65	27	36
vagus	21	1,118	12	95	24	374	108	1,101
varuna	3	121	2	13	1	54	0	9
willmori	2	21	25	131	2	3	0	2
Anopheles								
barbirostris gp	9	196	3	198	32	126	14	75

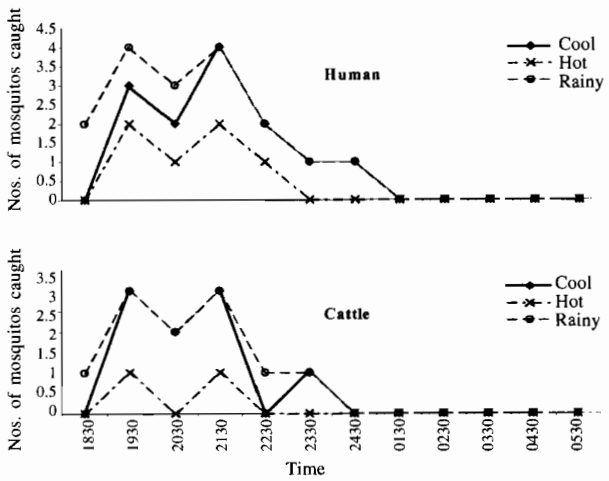

Fig 3–Biting activities of *An.dirus* on human and cattle through cool, hot and rainy seasons.

caught. A total of 18,660 *Anopheles* mosquitos representing 23 species or species group were collected during the two years study period (*ie* March 1996 to January 1998).

Table 2 shows man-vector contact and host-preference of *An. dirus* in Eindayaza, Michaunglaung, Ohnbinkwin and Thaechaung. The densities of *An. dirus* varied from month to month, with major peaks between May and October. In cool-dry (January) and hot-dry (March) months the densities were low with an average less than 1 mosquito per man/night. However, in wet season (May to October) the densities increases rapidly, starting from late May and reached its peaks in August 3.2 mosquitos per man/night with human bait and 3.7 mosquitos per cattle per night respectively. During monsoon the greater numbers of *An. dirus* were collected in May and October (Table 2).

Table 2
Man-vector contact and host preference of *An. dirus* complex at Yepyu township study areas from March 1996 to January 1998.

Period	Eindayaza			Michaunglaung			Ohnbinkwin			Thaechaung		
	MBR		ABR	MBR		ABR	MBR		ABR	MBR		ABR
	BI	BO	B/N	BI	BO	B/N	BI	BO	B/N	BI	BO	B/N
1996												
March	0	0	0	0	0	0	0.2	1.0	0.8	*	*	*
May	0.2	0.8	1.8	0	0.5	0.3	0.6	3.2	3.7	*	*	*
Aug	0.1	0.1	0.4	0	0	0.2	0	1.9	2.6	*	*	*
1997												
Jan	0	0	0	0	0	0	0.0	0.1	0.3	0	0	0.5
March	0	0	0	*	*	*	0	0.7	0.9	0.2	1.1	1.5
May	0	0.4	0.3	0	0.5	0.4	0	3.1	1.8	0	3.1	1.8
Aug	0	0.2	0.1	0	0.2	0.2	0.3	2.1	0.3	0	0.5	0.3
Oct	0	0.5	0.4	0	0.6	0.3	0	3.8	1.2	0	0.6	0.6
1998												
Jan	0	0.1	0	0	0	0	0	0.2	0.1	0	0.8	1.0

* indicated no survey was conducted that period. For Thaechaung village it was only in January 1997 the surveys were initiated.
MBR = Man biting rate; BO = Biting outdoors catches; ABR = Animal biting rate; BI = Biting indoors catches

Table 3
Larval surveys and results of *An. dirus* positive wells from four study villages during March 1996 to January 1998.

Period	*An. dirus* larvae/ pupae positive well from			
	Eindayaza	Michaunglaung	Ohnbinkwin	Thaechaung
1996				
Mar	1/18 (5.6%)	0/1	3/30 (10%)	-
May	1/18 (5.6%)	0/1	7/30 (23.3%)	-
Aug	3/18 (16.7%)	0/1	9/30 (30%)	-
1997				
Jan	1/18 (5.6%)	0/1	4/30 (13.3%)	3/30 (10%)
Mar	1/18 (5.6%)	0/1	3/30 (10%)	4/30 (13.3%)
May	2/19 (10.5%)	0/1	5/30 (16.7%)	8/30 (26.7%)
Aug	2/19 (10.5%)	0/1	13/30 (43.3%)	14/30 (46.7%)
Oct	0/19 (flooded)	0/1	12/30 (40%)	13/30 (43.3%)
1998				
Jan	2/19 (10.5%)	0/1	6/30 (20%)	10/30 (33.3%)

The biting cycle of *An. dirus* on human and cattle is shown in Fig 3. The biting rhythm of *An. dirus* on human and animal showed no significant different. The peak biting activities was in the first and second quarters of the night. However, during the monsoon *An. dirus* was found to bite earlier compared to the other two seasons. As expected in August (1997) the rainfall was over 2,654 mm and many larval breeding habitats were flooded (7 wells from Eindayaza were flooded during August 1997) and washed away by rain. Also the adults could hardly come out from their hiding places because of continuous rain and strong wind.

In the present study the outdoors biting activities of *An. dirus* rose sharply from 18.30 hours and attaining its first peak at 19.30 hours followed by a

Table 4
Nos. of *An. dirus* mosquitos captured on human and positive with *Plasmodium* sporozoites by ELISA technique.

Locality	% positive with ELISA assays during					Reamarks
	Hot-dry	Monsoon months			Cool-dry	
		Pre	Mid	Post		
Eindayaza	0	0	0	7.7%(1/13)	0	All mosq
Michaunglaung	0	0	0	0	0	CS positive
Ohnbinkwin	0	0	3%(1/33)	5%(2/40)	0	are only
Thaechaung	0	0	0	0	8.3%(1/12)	with *Pv*

Table 5
Clinical (malaria) data of the four studied villages.

Period	Prevalance of parasite and spleen rates (< 2-9 yrs) from local peoplc of							
	Eindayaza		Michaunglaung		Ohnbinkwin		Thaechaung	
	SPR	SR	SPR	SR	SPR	SP	SPR	SR
1996								
March	19.9	24.2	8.8	12.3	10.7	14.0	-	-
May	28.5	37.6	53.6	17.5	21.9	35.2	-	-
August	24.5	41.7	38.4	52.6	28.5	47.7	-	-
1997								
January	10.7	21.5	12.1	17.4	8.2	4.8	-	-
March	12.9	17.2	Nil	Nil	8.7	27.1	15.3	5.2
May	38.0	13.0	54.0	18.0	18.2	26.8	34.0	4.0
August	34.0	12.0	12.0	17.0	16.6	23.8	24.0	0.0
October	39.0	18.0	30.0	22.0	19.8	30.1	35.0	17.02
1998								
January	23.0	13.0	15.0	8.0	21.6	30.4	12.0	2.4

SPR= Slide positive rate; SR= Spleen rate

second peak at 21.30 hours.

Table 3, showed the results of larval survey from March 1996 to January 1998. Since there are more than 100 wells in both Ohnbinkwin and Thaechaung, adequate samples of 30 wells were selected using allocation of proportion to site methods. However all wells from Eindayaza and the only well from Michaunglaung were all chosen for study. Wells that are found positive with *An. dirus* larvae was learnt to be under shade and less frequently used. In Michaunglaung there is only one well and was negative throughout the study period. The reason is there are lots of fishes (*eg* catfish and ophicephalus) in this well. Edges of streams/creaks and all water pockets were also surveyed, but no *An. dirus* except some *An. annularis*, *An. culicifacies*, *An. maculatus*, *An. vagus* and *An. jamesii* larvae were caught.

Other than mosquito gut and salivary gland dissections, enzyme linked immunosorbent assays (ELISAs) for *Plasmodium* circumsporozoite (CS) proteins detection (Wirtz *et al*, 1987) were conducted and the results are shown in Table 4. The malaria situation in the study area is shown in Table 5. The parasite positive rate ranged between 8.8% and 54% during the study period. Slide positive rates are higher during rainy season.

DISCUSSION

This vector (*An. dirus*) elusive and it is difficult to control its breeding sites. Very few workers had been able to study the breeding habitats of *An. dirus*. In Chantaburi Province of Thailand near the Cambodian border, the breeding of *An. dirus* is in small water-collections (gem pits). The place is Tha

Mai, a coastal village near Chantaburi (Scanlon and Sandhinand, 1965). The area receives a particularly heavy rainfall during most parts of the year. However, in Yepyu township, Tanintharyi Division, Myanmar, the breeding of *An. dirus* is in domestic wells.

In Yepyu township, *An. dirus* larvae were found in large numbers in domestic wells which had been dug for daily usage. These wells were of various sizes, depth and shape, approximately 15 feet to 40 feet deep, some circular and some square in shape. Most of the wells were dug under the shade of coconut, plums, mango, banana, durian, cashewnut, jack-fruit trees etc. These wells were located within the compound under shade and near the houses. The soil is not extremely friable but with porous soft laterite rocks.

The larval densities per type of habitat were also critically observed. It was found that wells (deep) under full-time shade with plenty of debris (organic food) and vegetation (with herbs, shrubs and grass around the inner walls of the wells) harbored the greatest numbers which followed by wells (deep) partial shade with debris and the least numbers were in wells (shallow) under partial shade, with very little debris. All wells studied are lined by laterite rocks. These rock may give a sustained cooling effect which together with shade, debris (organic food) on the water surface and other factors, creates an ideal micro-environment for the breeding of *An. dirus*. The spilling over of *An. dirus* from the forest fringes and adaptation to the well-breeding condition in the Yadana gas pipe line corridor areas is a possibility.

The results of the biting cycle on human showed that *An. dirus* were early night biters, especially in the first and second quarters of the nights. Naturally, this situation increases the chances of man-vector contacts since those time periods are coincident with various activities of the people around their houses.

The best measure of malaria transmission is a combination of the study of anopheline mosquito prevalence and the determination of infection rates. Unfortunately, adult *An. dirus* are difficult to catch in large numbers. Enzyme linked immunosorbent essays (ELISA) for plasmodium circumsporozoites protein detection were conducted. The result were intriguing showing 1/13 positive with *Plasmodium vivax* (*Pv*) from Eindayaza during post-monsoon, 1/33 and 2/40 positive with *Pv* from Ohnbinkwin during mid-monsoon and post-monsoon months, and 1/12 with *Pv* from Thaechaung village during cool-dry (January) months' caught mosquitos. This confirm that the *An. dirus* mosquitos are responsible vectors in the study area.

In the present study malaria slides positive rates were recorded through all three seasons (Table 5). The seasonal pattern of malaria transmission was evident that the parasite positive rate was low prior to monsoon months, and gradually building coming up in monsoon months reaching its peak in May and October.

The result of the present study showed that *An. dirus* bite both humans and cattle with the animal biting densities higher in 1996. However, in 1997 onward *An. dirus* females were caught more from humans. The prevalence of *An. dirus* mosquito was influenced by monthly rainfall relative humidity and air temperature. Malaria transmission in Yepyu township is intense and perennial because of highly efficient vector *An. dirus* involvement throughout the year. Similar findings were observed in Thailand (Rosenberg *et al,* 1990).

The most obvious aspect of the data obtained from Yepyu township is the present of this deep forest breeder *An. dirus* found in the community of pipe line corridor villages. Ecological diversity determines the persistence of forest related malaria system in Yephu township. Stability of these systems is particularly remarkable in the central sections of the ecosystem (deep-forest, forest-fringes) and to a lesser extent in the peripheral areas. Another recent impact of humans upon species distribution is probably the extensive destruction of forest. In these villages people use firewood for cooking. For this purpose alone nearby forest are being effected every year, gross ecological changes have taken place. The change in ecology has influenced the mosquito fauna, vector prevalence, their behavior, etc. In addition to the vector and human behavior, environmental changes are also causing this efficient vector *An. dirus* to thrive in ever increasing numbers in the community, all aggravated the malaria situation and caused an increase of malaria in the area.

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