

## *Anopheles dirus* and its role in malaria transmission in Myanmar

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**ABSTRACT:** *Anopheles dirus* is one of the primary vectors of highly drug-resistant *Plasmodium falciparum*, which causes cerebral malaria resulting in high mortality. Mosquito collections were conducted in a forest wood-extraction area (Bago Division), an irrigated plain area near foothills (Mandalay Division), a coastal plain (from domestic wells in the Mudon area, Mon State) near the foothill area, as well as a hilly area (deep forest timber extraction camp, Tanintharyi Division) from May 1998 to March 2000. This study examined adult bionomics of *An. dirus* and its relationship to malaria transmission as an aid in the control of malaria in different ecological settings in these particular regions. Within these areas, Mudon, Mon State, has a high incidence of malaria. To investigate this malaria, blood smear examinations were conducted among the local people in Mudon, Mon State. During the study period, malaria blood smear slide-positive rates ranged between 9.9% and 34.28% throughout the year. The ultimate goal of these studies was to help in formulating an improved malaria control program involving microbial control agents in this area. *Journal of Vector Ecology* 28 (2): 175-183. 2003.

**Keyword Index:** *Anopheles dirus*, ecology, malaria.

### INTRODUCTION

*Anopheles dirus* is a member of the *An. balabacensis* species complex and an important vector of malaria in Southeast Asia (Rosenberg 1982). In Myanmar, malaria has been identified as one of the most important public health problems in every national health plan since 1978. Since 1984, the number of confirmed malaria cases has consistently been above 60,000, with >80% due to *Plasmodium falciparum*. Since 1986, the annual mortality has been >8/100,000 population (>3,000 deaths), with most being non-immune adult males who became infected while working in the forest (Tin and Tun 1991). About 60% of all malaria cases in Myanmar are reported from the forest and forest fringe areas (Lin et al. 1995). Dense forests cover an area of about 48% of the total area of Myanmar where 20% of the population lives. Studies on forest malaria transmission have been carried out in Myanmar by Lin et al. (1995), as well as the neighboring countries of Bangladesh (Rosenberg and Masheswary 1982) and Thailand (Gingrich et al. 1990, Rosenberg et al. 1990b).

Thirty-seven species of anopheline mosquitoes have been recorded in Myanmar. Of these, *An. dirus* is

one of the primary vectors. Its areas of prevalence can be assumed to coincide with the distribution of forest (Scanlon and Santhinand 1965, Tin and Tun 1991, Gingrich et al. 1990). In Myanmar, *An. dirus* is a widespread species, particularly in the Mandalay, Tanintharyi and Bago (Bago Yoma forest) Divisions, as well as in Kayah and Mon States and is especially found in forest and forested foothill areas (Paing et al. 1989, 1990). The primary objective of this study was to examine the ecology and bionomics of mosquitoes involved in malaria transmission to form a basis for further studies aimed at reducing the incidence of malaria. The secondary objective was to consider the dangers of mosquitoes breeding in domestic wells. In Mudon, a coastal town in Mon State in South Myanmar, *An. dirus* was found breeding in domestic wells, a situation not encountered in other parts of Myanmar (Oo et al. 2002).

Mudon is only 9.45 meters above sea level and is located close to the slope of the Tanintharyi mountain range. Among the townships of Mon State, Mudon shows one of the highest incidences of malaria; more than 75% of the population is at risk and the foci of chloroquine resistant strains of *Plasmodium falciparum* were detected from this township (VBDC unpublished

reports 1982, 1992, 2000). Malaria is clearly identified as one of the most important public health problems in Mudon, with a very serious effect on the economy of the state. Thus, in light of this situation, Mudon Township was selected as another study area to investigate the ecology of *An. dirus*, its relevance for malaria transmission, and blood smear examination. The ultimate goal of these studies was to help formulate an improved malaria control program in this area.

## MATERIALS AND METHODS

### Collection areas

Collection areas were chosen in three different ecological conditions. (Figure 1). The locations of collection sites were as follows:

(A) *Forest wood-extraction area*: Kyauktagar Township, Bago Division; collection sites were situated in the Bago Yoma mountain range.

Site 1: Htiphado village- deep forest wood-cutting camp, foothill village.

Site 2: Phado village- temporary settlements on edge of forested foothill.

Site 3: Kyarkyaungthaik village- temporary settlements on edge of forested foothill.

Site 4: Ngokto village- 0.5 kilometers from the forest edge (forested foothill with paddy fields on one side).

Site 5: Gwegone village- located 2.4 kilometers from the forest edge (forested foothills with paddy fields on one side).

(B) *Irrigated plain area*: Madaya Township, Mandalay Division.

Site 6: Botegone village- near foothills and extensive irrigation area.

(C) *Plain area*: Mon State, rural area.

Site 7: Innwaing village, Mawlamyine Township- coastal plain near foothills.

Site 8: Mudon Town, Mudon Township- edge of town near rubber plantation and forested foothill area.

Site 9: Wetlay village, Thanbyuzayat Township- coastal plain near foothills.

(D) *Hilly area*: Kawthaung Township, Tanintharyi Division.

Site 10: Namtun camp- deep forest timber extraction camp.

### Mosquito collections

Human-baited, night-biting catches (both indoors

and outdoors) of mosquitoes were conducted. Catches were scheduled for seven consecutive days every month. Catches were made by two men, armed with flashlights and aspirators, seated facing each other on stools inside a house and a second pair similarly equipped seated outdoors 15 feet away from each other. The feet, legs, arms, and heads of the collectors were bare. Two groups of four men were used each night. One group worked from 6 p.m. to midnight, the second from midnight to 6 a.m. One night's biting catch consisted of 12 episodes of 45 min uninterrupted catching. Each episode began on the hour, and all mosquitoes resting on or biting either member of a pair were collected and stored in a paper cup cage. Each of the 24 cages collected nightly was labelled according to the hour and station and stored in humidified buckets until the next morning.

In animal-baited bed-net catches, a domestic animal, usually a cow or water buffalo, was selected from the village. An animal-baited trap net (330 x 330 x 180 cm) was placed close to where the animal was customarily kept overnight. Before sunset, the animal was tied securely using a short tether attached to wooden or metal pegs driven firmly into the ground. At sunset, mosquitoes were collected by means of a flashlight and aspirator at intervals of three hours (18:00, 21:00, 24:00, 03:00, and at sunrise).

Daytime indoor resting collections involved the examination of about 10 houses in each village to provide a representative sample. The collection of mosquitoes in each house took place early in the morning (from 07:00 to 09:00). The entire house was examined, or if it was too large, up to 15 minutes was spent searching each room. Special attention was paid to those parts of the house most likely to harbor mosquitoes, including rooms in which people had slept the previous night or the washing room. Those rooms selected had few external openings. The search for mosquitoes on walls, ceilings or the roof was aided by a flashlight. Rooms were searched systematically, starting from the door and moving clockwise around the inside of the house, checking for specimens on the wall hangings and curtains, behind and under furniture, and inside large pots and jars.

### Vector incrimination

Early in the morning following a catch, mosquitoes were identified and dissected. Guts and salivary glands of *An. dirus* (from human bait) were dissected to assess the rate of infections with *Plasmodium* sporozoites according to the WHO method (1975). The choice of organ to be dissected depended on the type of data required. The stomach was dissected to investigate the presence of oocysts, their number, stage of development, and positioning on the midgut. If oocysts were observed,

Table 1. Relative abundance of anopheline mosquitoes in the Mudon area during 1999.

Mosquito species	Total number of female mosquitoes collected											
	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
<i>An. dirus</i>	37	29	26	38	53	42	39	56	58	68	30	22
<i>An. aconitus</i>	14	2	0	0	9	7	2	2	12	36	22	11
<i>An. annularis</i>	50	53	47	50	62	58	60	61	62	72	58	40
<i>An. barbirostris</i>	32	12	10	28	9	5	0	0	18	23	18	20
<i>An. jamesii</i>	19	9	20	10	12	7	0	0	10	13	8	3
<i>An. karwari</i>	4	2	1	0	0	0	0	2	11	19	9	8
<i>An. maculatus</i>	4	1	1	1	3	0	0	0	0	0	0	1
<i>An. minimus</i>	1	0	1	0	1	0	0	0	0	0	0	0
<i>An. philippinensis</i>	5	14	20	18	23	8	11	18	23	45	21	11
<i>An. ramsayi</i>	2	0	5	1	0	0	0	0	0	0	0	0
<i>An. splendidus</i>	3	0	0	0	2	0	0	0	0	0	0	1
<i>An. tessellatus</i>	9	3	0	3	2	1	0	0	2	1	2	1
<i>An. vagus</i>	29	19	2	8	14	3	0	3	3	5	3	12

Table 2. Average human biting rates per hour of *An. dirus* at Phado, Bago Division (1998 to 1999).

Period	Human biting rate (br/h)	Rainfall (mm)
<u>1998</u>		
September	1.14	346
October	1.8	112
November	0.44	0
December	0.95	0
<u>1999</u>		
January	0.25	0
February	0	0
March	0	0
April	0	14
May	0.45	92
June	0.73	371
July	0.91	537
August	1.36	1039
September	1.04	276

Table 3. Results of the resting behavior of *An. dirus* in wells from Mudon, Mon State (07:00 to 09:00) during 1999.

Period	Mosquitoes collected		
	Male	Female	Total
January	0	5	5
February	1	1	2
March	0	2	2
April	1	4	5
May	1	8	9
June	1	5	6
July	1	3	5
August	2	7	9
September	2	9	11
October	3	11	14
November	2	8	10
December	1	6	7

Table 4. Numbers of *An. dirus* caught by different techniques in various catching stations during 1998 and 1999.

Catching Stations	Period	Total no. of <i>An. dirus</i> collected (daytime)	Total no. of <i>An. dirus</i> collected (night)	Collected by different techniques*							
				Human Bait				Cattle bait			
				I	II	III	IV	I	II	III	IV
<b>Mandalay Div.</b>											
Botegone village	1998	0	264	1	79	108	34	1	6	33	0
<b>Mon State</b>											
Innwaing village	1998	5**	296	12	84	123	33	4	9	29	2
Thanbyuzayut	1999	2**	196	14	51	77	12	9	12	18	3

\*Roman numerals indicate first, second, third, and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00.

\*\*Daytime outdoor collection.

the mosquito was considered "infected." However, in order to determine whether a mosquito was "infective," the salivary glands were dissected and examined for the presence of sporozoites. This was carried out routinely for establishing the parity, sporozoite and oocyst rate.

Live mosquitoes were killed either with chloroform, ether, or carbon dioxide. After immobilization, the insect was held by one wing to remove the legs and afterwards the body was pulled off the wings. The insect was then placed on a dry slide and arranged in a more suitable position for dissection of the gland or stomach.

#### Blood smear examination

Blood smear examinations were conducted on all people residing in Mudon Town, Mon State, during the dry (May), rainy (October), cold dry (January), and hot dry (March) seasons by the Township Medical Officer. During each survey about 200 samples (from four age groups: 0-4 years, 5-10 years, 11-15 years, 16 years and above) of blood were drawn for preparation of both thin and thick blood smears. All blood films were stained with Giemsa and examined in the field and again checked in the laboratory by viewing 50 microscopic fields under 1,000 X oil immersion.

## RESULTS

### Seasonal prevalence

*An. dirus* was abundant in post-monsoon months (August to October). Table 1 shows the relative abundance of anopheline mosquitoes in the Mudon area during the study period. Table 2 shows human biting

rates (br/h) of *An. dirus* at Phado, a foothill village of Bago Yoma Range. The highest biting rate was also recorded for the period August to October.

### Resting habits

Male and female *An. dirus* were found resting in crevices and on vegetation around the inner walls of the domestic wells during daytime at Mudon Town, Mon State (Table 3). Daytime indoor resting catches carried out in various areas did not yield *An. dirus*. This species is highly exophilic (Table 4).

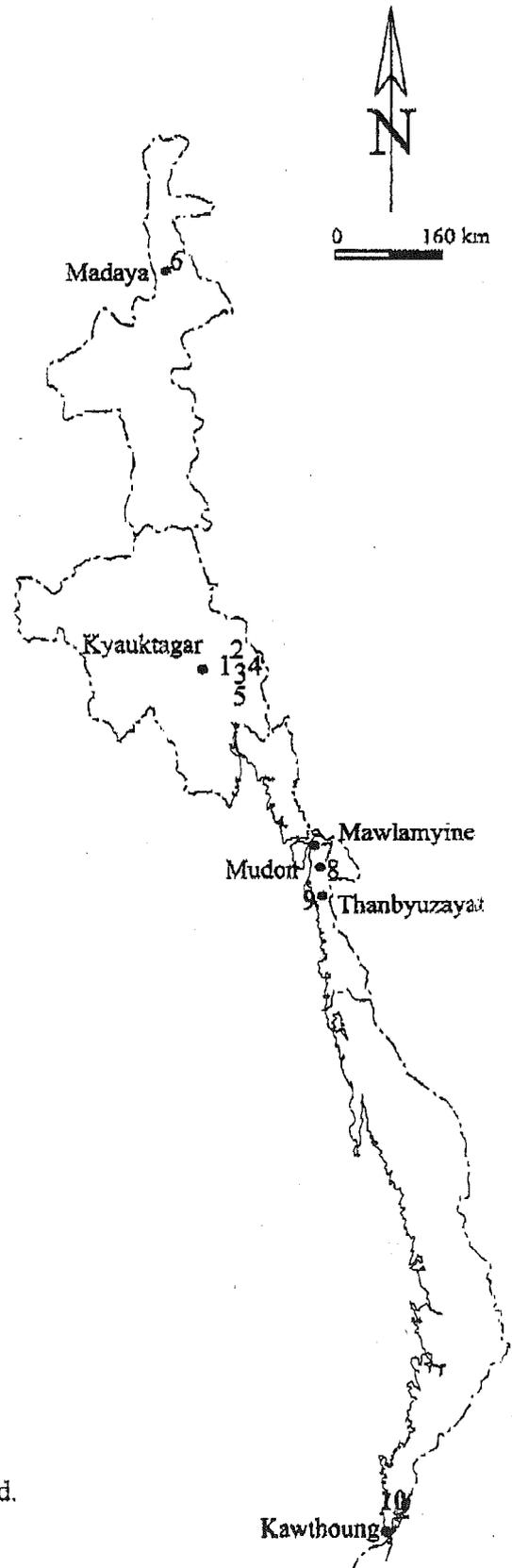
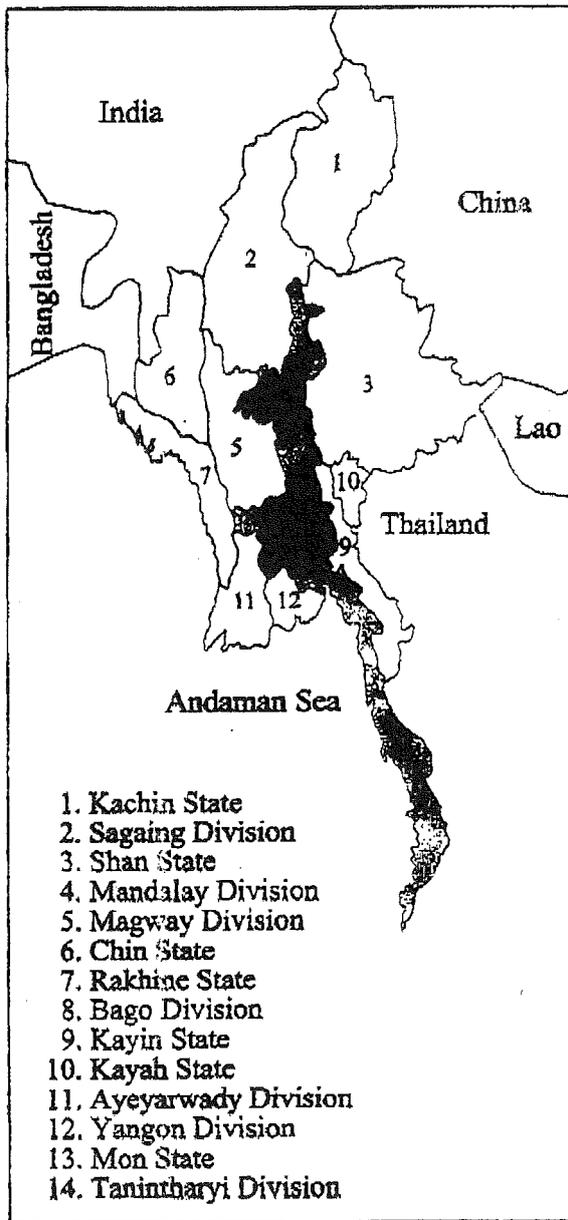
### Biting activity and host preference

In the present study, the outdoor biting peak occurred in the 2nd and 3rd quarter of the night, 21:00 to 03:00 (Table 4). The preference of *An. dirus* for human blood is well documented by the results collected during different periods in various areas. However, a similar study conducted in Mon State revealed that *An. dirus* can be more zoophilic, although the breeding sites are situated very close to human dwellings within a domestic area (Table 5).

### Sporozoite and slide positive rates

Table 6 shows the dissection records on *An. dirus* infection (oocyte and sporozoite) rates at different localities. In Mudon Town, Mon State, the malaria slide positive rate (obtained from the Township Medical Officer) ranged from 9.9% (July) to 30.0% (October) (Table 7) during 1999. *Plasmodium falciparum* was the dominant parasite species in this community.

Figure 1. Location map of the study areas in Myanmar.



1 to 10 = Locations where *An. dirus* were collected.

Table 5. Occurrence of *An. dirus* in Mudon area during 1998.

Period	Average number of mosquitoes collected /hour	
	Human bait	Cattle bait
January	0.6	1.0
February	0.4	0.7
March	0.5	1.1
April	0.4	0.9
May	1.5	1.7
June	0.9	0.9
July	0.7	0.7
August	0.7	0.9
September	1.4	3.1
October	1.9	4.2
November	0.8	1.2
December	0.2	0.7

Table 6. Dissection records of *An. dirus* infection rates (gut and gland) in different localities during the study period.

Localities	No. of <i>An. dirus</i> dissected	No. of <i>An. dirus</i> with gut infection	No. of <i>An. dirus</i> with gland infection	Infection rate (%)
<b>Mandalay Division</b>				
Botegone village	250	1	2	1.2 (3/250)
<b>Bago Division</b>				
Htiphado village	250	1	6	2.8 (7/250)
Phado village	250	1	4	2.0 (5/250)
Kyarkyaungthaik village	250	1	3	1.6 (4/250)
Ngokto village	250	1	2	1.2 (3/250)
Gwogon village	250	0	3	1.2 (3/250)
<b>Mon State</b>				
Mudon	200	3	5	4 (8/200)
Innwaing village	200	0	0	0
Thanbyuzayut	250	0	1	0.4 (1/250)
<b>Tanintharvi Division</b>				
Namtun camp	250	1	3	1.6 (4/250)

Table 7. Malaria blood smear positive rates from local people in Mudon.

Month in 1999	No. of slides examined	Total no. of slides positive	Slide positive rate (%)
January	200	31	15.50
February	248	34	13.70
March	262	37	14.12
April	260	42	16.15
May	268	57	21.26
June	209	23	11.00
July	212	21	9.90
August	208	21	10.09
September	246	49	19.91
October	210	63	30.00
November	240	51	21.25
December	276	34	12.31

Table 8. Comparison studies of *An. dirus* human biting rate per hour and infection rates with malaria blood smear positive rates from Mudon, Mon State in 1999.

Period	Human biting rate (br/h)	Infection rate (%)	Slide positive rate (%)
January	0.6	2.50	15.50
March	0.5	2.43	14.12
May	1.5	3.33	21.26
October	1.9	4.42	30.00

## DISCUSSION

It has long been recognized that *An. dirus* is one of the most important vectors of human malaria in the Oriental Region (Baimai et al. 1988, Rosenberg et al. 1990), including Myanmar. The presence of this mosquito is always associated with dense forest and forest fringes, where it finds its favorable biotopes (Scanlon and Sandhinand 1965). It is often found in high densities in hilly areas covered with thick forest, whereas its density decreases in relation to distance away from the jungle edge. Thus, for example, Scanlon and Sandhinand (1965) and Kyi<sup>5</sup> used vegetation maps of Thailand and Myanmar respectively as a rough guide to the distribution of *An. balabacensis* (*An. dirus*), the species found wherever the maps indicated tropical evergreen or monsoon forests.

The seasonal abundance of *An. dirus* is influenced by several factors. Among the biological factors, adult and larva densities were found to be positively correlated with rainfall (Tables 1 and 2). For instance, in the Mudon area a total of 13 anopheline species was collected, with *An. annularis* as the dominant species followed by *An. dirus*, the vector most responsible for malaria in the area, and *An. philippinensis*. Two other important malaria vectors, *An. minimus* and *An. maculates*, were also found, but their numbers were low compared to *An. dirus*, which was collected throughout the year. Moreover, more *An. dirus* mosquitoes were caught during the rainy season than other seasons. Baimai et al. (1988), in their study at Ban Kang Rieng, Thailand, also reported similar results. *An. dirus* are usually found in forest vegetation during the day (Scanlon and Sandhinand 1965). However, in Mudon Town, *An. dirus* was found in crevices of deep wells, and on vegetation around the inner walls of the

wells (Table 3). This finding is in agreement with the observations by Paing et al. (1989) and Aung<sup>6</sup>. Scanlon and Sandhinand (1965), Baimai et al. (1988), and Rosenberg (1982) also found a significant increase in biting activity during the wet season in Thailand and Bangladesh and that there was a close relationship between rainfall and biting rate. Our observations are in agreement with their findings (Table 2). *An. dirus* appeared to be transmitting malaria throughout the year in the Mudon community especially in the post-monsoon months (i.e. September and October). The present finding is similar with the findings of Rosenberg et al. (1990a) and Ismail et al. (1974).

The present study indicated that the human biting rate per hour of *An. dirus* is correlated with the infection rates and malaria blood smear positive rates in Mudon area (Table 7 and Figure 2). The highest blood smear positive rate was observed in October and a moderate level of transmission was recorded all year. However, the seasonal pattern of malaria transmission was evident in that the parasite positive rate was low prior to the monsoon months (i.e., the hot/dry season), then gradually increased during the monsoon months (rainy season) reaching its peak in October (post-monsoon). The incidence of malaria could be categorized as stable in this area. A preponderance of *P. falciparum* resistant to 4-aminoquinoline in Mudon Town could result in high morbidity among this population. Finally, Figure 3 shows the possible factors influencing or associated with the man-mosquito contact in Mudon area. This close contact of malaria vector with humans could be one of the reasons for the increasing numbers of malaria cases in the region.

There is a great need to use biological control agents in integrated vector control programs (WHO, 1975).

<sup>5</sup>Kyi, K.M. 1974. Further observations on *Anopheles balabacensis* and malaria in Burma. (Unpublished documents WHO/MAL/74.838).

<sup>6</sup>Aung, H. 1975. Ecological and genetic studies of the *Anopheles dirus* complex in relation to malaria transmission in Mudon township, Mon State, Myanmar. Ph.D. Thesis, Mahidol University, Thailand.

Figure 2. Comparative studies on *An. dirus* infection rate and blood smear positive rate with human biting rate/hour.

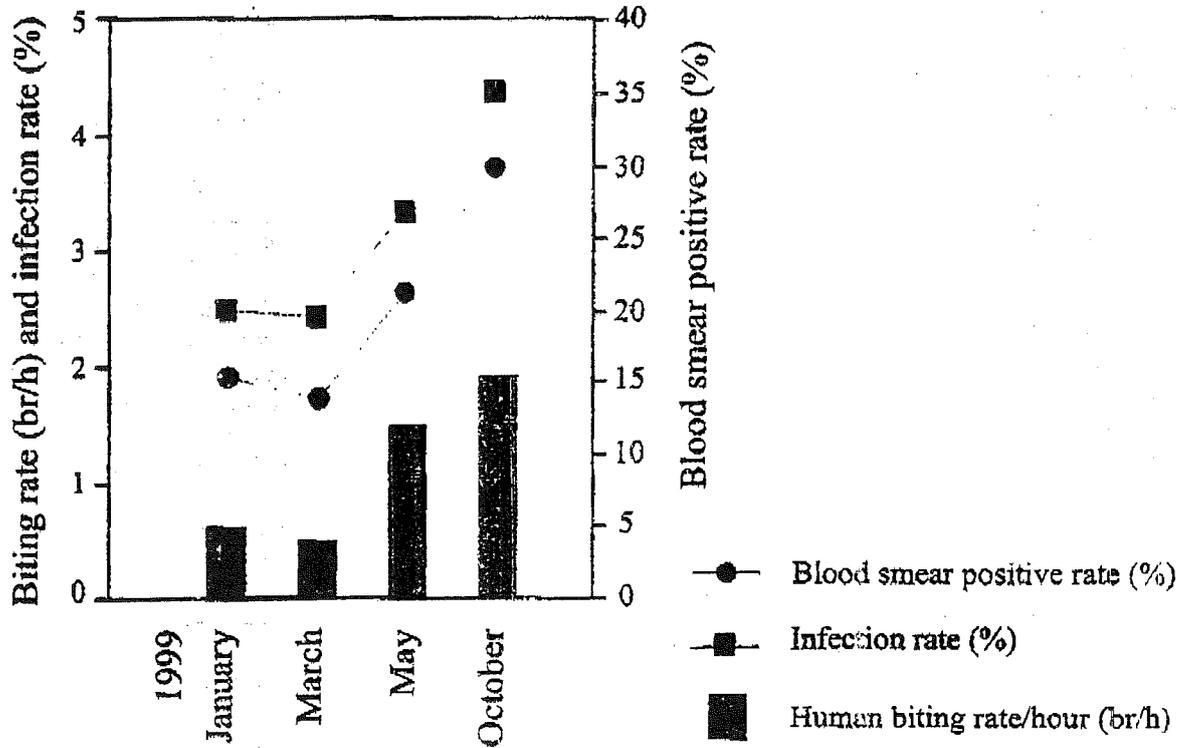
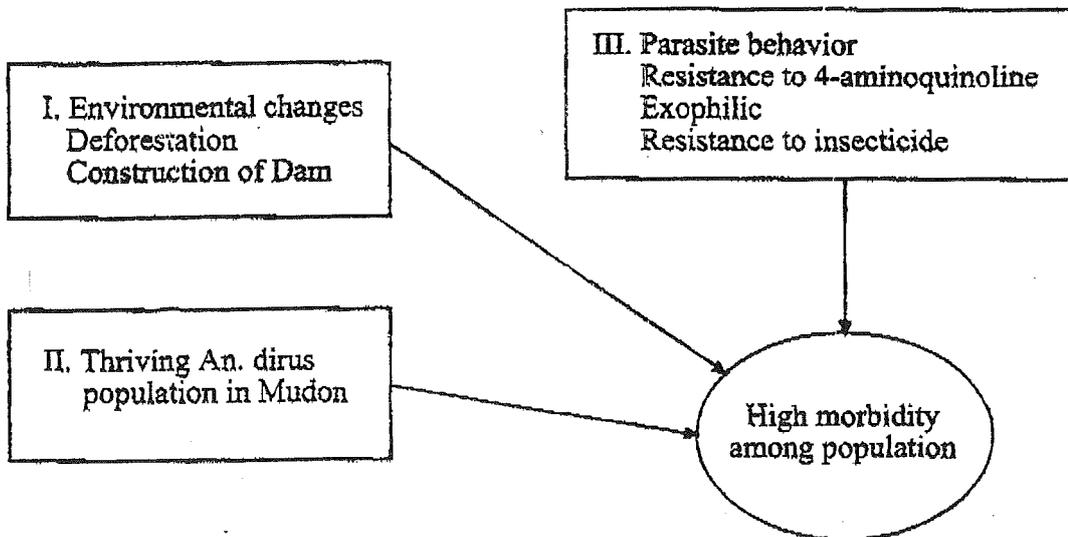


Figure 3. Factors influencing or associated with malaria morbidity in Mudon area.



Results obtained from laboratory trials show that microbial control agents (*Bacillus sphaericus* and *Bacillus thuringiensis* var. *israelensis* H-14) work favorably against *Anopheles* (Paing et al. 1987). The larval breeding habitats of the target *Anopheles* species must be known for practical use in control programs. In forest areas, *An. dirus* breeds mainly in puddles on footpaths, pits, in small temporary pools of streams, in small rock pools, and in bamboo stumps (Paing et al. 1987). Because of these widely distributed small breeding sources, larval controls seem to be unsuitable, and practical use of microbial agents or chemicals, no matter how effective, is almost impossible. However, in the domestic area of Mudon, microbial control agents could be used in wells to control *An. dirus* much more easily and effectively.

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