Epidemiological Study of Malaria in Palawan

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INTRODUCTION

Malaria continues to be the major parasitic disease in the tropical and subtropical areas of the world, with more - than 200 million people infected and more than one million deaths annually.[1] Prior to World War II it was estimated that 2 million eases of malaria were seen annually in the Philippines.[2] During World War II there was an increase in malaria with an estimate of greater than 4 million cases occurring annually after the war.[3] The Philippines reported 97,500 cases in 1982 and 90,300 in 1983.[4] There are undoubtedly many more who are chronically infected but unreported because they are suffering no acute manifestations.

Previous studies[5,6] on the island of Palawan consisted primarily of point prevalence surveys for malaria. The selection of a site for longitudinal studies allows work necessary to understand more fully the epidemiology of malaria in the Philippines. This information can then be used to make decisions regarding modifications in control and treatment programs.

Longitudinal. studies allow one to define seasonal variation in the vector and disease as well as to determine incidence of the disease.

The site selected for our studies is located on the west coast of the island of Palawan, with coordinates 9^{O}43', north latitude and. 118^{O}28', east longitude (Figure 1). The village or barangay of Napsan consists of 3 smaller divisions or settlements called sitios: Napsan proper, Labtay, and Santo Niño which are spread over a distance of approximately 12 kilometers along the South China Sea. Santo Niño is located at the northern end of the barangay with Labtay at the southern end and Napsan proper located midway between these two sitios. The barangay is located in the foothills of the very mountainous central portion of the island. The terrain is quite heavily forested with many small streams/rivers during the rainy season. During the dry season, most flowing streams with the exception of a few main rivers tend to dry up. There are approximately 3,000 residents living in this area including members of the Tagbanua minority. Although a number of immigrants from the Visayas have settled here, most of the people are long-term residents. The villagers utilize slashing and burning techniques prior to planting rice in the foothills. This practice, as well as the gathering of rattan, necessitates living and working farther in the foothills away from the beach, during particular periods of the year such as at rice planting or harvest time.

MATERIALS AND METHODS

Surveys to detect prevalence and distribution of infections with plasmodium and the abundance and nature vector were conducted in March, June, and September of 1986, and

January, April and July of 1987. The initial survey was conducted only in Napsan proper, although 99 people residing in the outlying sitios of Labtay and Santo Niño were seen at that time. The three subsequent surveys included blood collection and mosquito studies in all three sitios within the barangay. The first survey conducted in March occurred well into the dry season, with the rainy season beginning in May and lasting until December (Table 1).

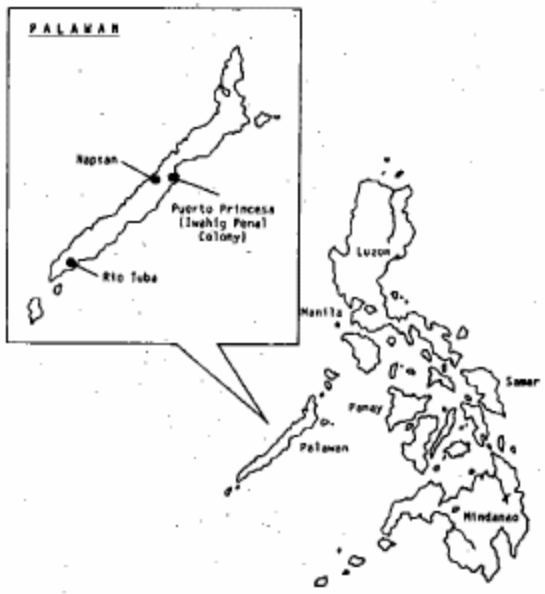

Figure 1. Map of the Philippine Islands showing the location of Napsan on the island of Palawan

Table 1. Rainfall data Puerto Princesa, 1950-1980 (National Institute of Climatology, Manila, R.P.)

Month	Average Rainfall (mm)	Average # Days with rain
**January	31.5	4
Feburary	18.7	2
*March	39.5	4
**April	40.8	5
May	150.3	13
*June	188.4	15
**July	180.2	16
August	181.9	17
*September	193.0	16
October	209.2	16
November	211.8	14
December	146.3	9

*Months in.1986 in which surveys were conducted by NAMRU-2

**Months in 1987 in which surveys were conducted by NAMRU-2

Parasitology

In March, blood samples were obtained by finger puncture using a sterile disposable lancet. Blood was collected by venipuncture on all subjects during subsequent visits except in infants, where sterile disposable lancets were employed. Thick and thin blood smears were prepared on a single slide for each patient, stained with 2% Giemsa, and examined by light microscopy at a magnification of 1,000x (oil immersion). Two hundred fields were examined on the thick smear before considering the smear negative. Persons having blood smears positive for malaria parasites were treated on each occasion. Venous blood was allowed to clot at ambient

temperature. Serum was removed after centrifugation and stored on dry ice for transport to laboratory facilities in Manila.

The indirect flourescent antibody (IFA) test, as originally described with modification,[7,8] was performed on all serum specimens collected in June using a Gambian strain of *P. falciparum* from culture. Serum specimens collected at later dates were tested by IFA only if initially negative and patients subsequently demonstrated a positive peripheral blood smear. The sera were tested at dilutions from 1:16 to 1:2048. All remaining sera were frozen and stored for further immunological testing.

Entomology

The malaria vectors in this area were determined using the man-biting technique. Collecting times were from 1800-2400 and 2400-0600; each collection was conducted by a 4-person team. The collectors used oral aspirators and red-filtered flashlights to capture mosquitoes as they landed on their exposed legs. Following identification, a sample of *Anopheles species* was dissected to determine sporozoite rates; others were frozen to be tested later for sporozoites using the ELISA technique. During the preliminary survey in March 1986 the mosquitoes were sampled for only 2 nights at Napsan proper. During the following 5 trips, mosquitoes were sampled for 9 nights (3 nights each at Napsan, Labtay, and Santo Niño).

RESULTS

The slide positive rate of malaria for the entire barangay, which includes the three sitios of Napsan proper, Labtay, and Santo Niño, was lowest in the month of March during the dry season (Table 2). The ratio of vivax to *P. falciparum* was approximately 1.1:1 at that time. An increase in the point prevalence of malaria occurred after the onset of the rainy season. The ratio of *P. falciparum* to *P. vivax* increased during the rainy season with a corresponding increase in mixed infections of these two species. June of 1986 yielded a 1.7:1 ratio of *P. falciparum* to *P. vivax* with 2.2% of the infections being mixed. The overall percentage of slide positive cases remained high at around 30% from June of 1986 through January of 1987 falling with the next dry season as reflected by sampling in April 1987 and rising again by July of 1987, The ratio of *P. falciparum* to P. vivax in July of 1987 was 2.9:1, the highest seen during the study.

Table 2. Malaria slide-positive rate by Plasmodium species and date, Barangay Napsan, Palawan, 1986-1987

	March 86		June 86		September 86		January 87		April 87		July 87	
	Cases	(%)	Cases	(%)	Cases	(%)	Cases	(%)	Cases	(%)	Cases	(%)
P. falciparum	40	(8.0)	174	(18.6)	140	(15.7)	122	(15.1)	108	(12.6)	192	(21.9)
P. vivax	44	(9.0)	101	(10.9)	102	(11.4)	92	(11.4)	73	(8.5)	67	(7.7)
P. malariae	-		-		1	(0.1)	4	(0.5)	3	(0.4)	1	(0.1)
Mixed (P. falciparum + P. vivax)	4	(0.8)	21	(2.2)	13	(1.5)	12	(1.5)	7	(0.8)	14	(1.6)
Overall	88/ 490	(18.0)	295/ 934	(31.6)	253/ 890	(28.4)	230/ 807	(28.5)	191/ 857	(22.3)	274/ 875	(31.3)

Nine cases of *P. malariae* were detected during this study. This represented a rate that varied from 0% to 0.5% of the population and from 0% to 1.7% of the malaria positive slides.

A crude measure of incidence for each sampling period was obtained by considering as new cases, those persons who initially had negative smears but developed positive smears upon follow-up examination at 3-month intervals. In March, 490 blood smears were obtained and 220 of these persons returned in June, 189 of whom had negative smears in March. The smears collected in June revealed that 38 (20.1%) of these patients now had positive smears. Between

June and September of 1986, 43 of 213 (20.2%) of the patients with negative smears converted to positive and between September of 1986 and January of 1987, 50 of 281 (17.8%), converted to smear positive. These figures are without regard to age, sex, or parasite species.

The slide positive rates were examined by age and date (Table 3) and by sitio and date (Table 4). Patients below the age of 10 consistently had higher rates of slide-positive blood smears than those 10 years of age or older. A seasonal variation with increasing malaria during the rainy season occurred in all age categories. Overall, the sitios of Labtay and Santo Niño had higher rates of malaria than Napsan proper. The distribution of slide-positives by all three parameters of age, sitio, and date is shown in Table 5. The data from all study periods in the three sitios, show a higher rate of malaria in Labtay and Santo Niño. The most striking increases in the prevalence rates are seen in June and September in these two sitios among the 0-4 and 5-9 age groups. Napsan proper had a much higher overall point prevalence than expected in July of 1987 primarily reflected in the prevalence among persons below the age of 10 years. This age group had a slide-positive rate of 64/107 (59.8%) at this time.

Table3. Malaria slide-positive rate by age and date, Barangay Napsan, Palawan, 1986-1987

	March 86		June 86		September 86		January 87		April 87		July 87	
Age	Cases	(%)	Cases	(%)	Cases	(%)	Cases	(%)	Cases	(%)	Cases	(%)
0-4	16/96	(16.7)*	66/144	(47.2)	45/138	(32.6)	47/129	(36.4)	44/145	(302)	45/121	(37.1)
5-9	29/103	(28.2)	77/183	(42.0)	78/190	(41.1)	71/174	(40.8)	57/173	(32.9)	113/212	(53.3)
≥10	43/291	(14.8)	150/607	(24.7)	130/562	(23.1)	112/504	(22.2)	90/539	(16.7)	116/542	(21.4)
Total	88/490	(18.0)	295/934	(31.6)	253/890	(28.4)	230/807	(28.5)	191/857	(22.3)	274/875	(31.3)

*Slide positive rates are recorded as the number positive/total number tested in the age group;
(%) rounded to the nearest tenth, regardless of Plasmodium species

Table 4. Malaria slide-positive rate by sitio and date, Barangay Napsan, Palawan, 1986-1987

	Sitio					
Date	Napsan Proper		Labtay		Santo Niño	
March (1986)	71/391	(18.2.)*	14/70	(20.0)	3/29	(10.3)
June (1986)	94/367	(21.6)	104/278	(37.4)	97/289	(33.6)
September (1986)	65/380	(17.1)	109/275	(39.6)	79/235	(33.6)
January (1987)	70/286	(24.5)	76/261	(29.1)	84/272	(30.1)
April (1987)	50/250	(20.0)	78/390	(20.0)	63/217	(29.0)
July (1987)	105/285	(36.8)	74/357	(20.7)	95/233	(40.8)
Average Overall Rate	445/1959	(22.7)	455/1631	(27.9)	421/1275	(33.0)

*Slide positive rates are recorded as the number of positive/total number sampled from the sitio;
(%) rounded to the nearest tenth.

The IFA test results performed on 786 patients' serum specimens are shown in Table 6. Seven hundred thirty three (93.3%) had titers of 1:16 or greater. The age distribution demonstrates an early exposure and an increased percentage of persons having a positive IFA with increasing age. The antibody titer measured by IFA also shows a direct relationship to age. Fifty percent of the patients below the age of 5 had titers that were either considered to be borderline positive (1:16), or were negative. The geometric mean titers for the age groups were as follows: 0-4 (1:49), 5-9 (1:103), 10-14 (1:138), and 15 and older (1:186).

The various species of anopheles mosquitoes collected using the man-biting technique during 6 separate surveys is shown in Table 7. Of the 12 species collected, *An. flavirostris* was by far the most common, comprising 90.4% of the total. Vector abundance is lowest during the dry season, increases in June as the rain begins, peaks during the rainy season, and then declines with the onset of the dry season. Of 363 *An. flavirostris* dissected, 1, which was collected during June from Labtay, was found positive resulting in a sporozoite rate of 0.3%. Limited dissections of other species were all negative. ELISA testing for sporozoites is being conducted, as well as more extensive salivary gland dissections.

Table 5. Malaria slide-positive rate by sitio, age, and date within the barangay of Napsan, Palawan, 1986-1987

Age	Date	Napsan Proper		Labtay		Santo Niño	
0-4	March	14/79	(17.7%)*	2/10	(20.0%)	0/7	(0%)
	June	17/48	(35.4)	30/46	(65. 2)	21/50	(42.0)
	September	6/24	(23.0)	27/55	(49.1)	12/30	(40.0)
	January	12/42	(2 8.5)	16/38	(42.1)	19/49	(38.7)
	April	10/40	(25.0)	20/71	(28.2)	14/34	(41.2)
	July	26/40	(65.0)	12/56	(21.4)	7/252	(8.0)
5-9	March	23/81	(28.4)	6/17	(35.3)	0/5	(0)
	June	16/56	(28.5)	28/58	(48.2)	33/69	(47.8)
	September	19/67	(28.4)	28/65	(43.1)	31/58	(53.4)
	January	18/57	(31.5)	21/52	(40.3)	32/65	(49.2)
	April	11/46	(23.9)	20/72	(27.8)	26/55	(47.1)
	July	38/67	(56.7)	29/75	(38.6)	46/70	(65. 7)
≥10	March	34/231	(14.7)	6/43	(14.0)	3/17	(17.6)
	June	61/263	(23.1)	46/174	(26.4)	43/170	(25.2)
	September	40/259	(15.4)	54/1563	(4.6)	36/147	(24.5)
	January	40/184	(217)	36/174	(20.6)	36/156	(23.0)
	April	29/1641	(7.7)	38/247	(15.4)	23/128	(08.0)
	July	41/178	(23. 0)	33/226	(14.6)	42/138	(30.4)

*Slide-positive rates are recorded as the number of positive/total sampled from the sitio in the particular month and age group; (%) rounded to the nearest tenth.

Table 6. Indirect fluorescent antibody results on sera from Napsan, Palawan collected in June 1986

	AGE							
	0 - 4		5 - 9		10 - 14		≥ 15	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Negative	14	(25.9)	13	(8.7)	9	(6.3)	17	(3.9)
1:16	13	(24.1)	26	(17.3)	19	(13.3)	28	(6.4)
1:32	7	(13.0)	8	(5.3)	6	(4.2)	29	(6.6)
1:64	10	(18.5)	24	(16.0)	21	(14.7)	60	(13.7)
1:128	3	(5.6)	32	(21.3)	31	(21.7)	72	(16.4)
1:256	6	(11.5)	29	(19.3)	24	(16.8)	91	(20.7)
1:512	1	(1.9)	11	(7.3)	24	(16.8)	94	(21.4)
1:1024	0	(0)	7	(4.7)	5	(3.5)	38	(8.7)
1:2048	0	(0)	0	(0)	4	(2.8)	10	(2.3)
Total	54		150		143		439	
Positive (1:16)	40/54	(74.1)	137/150	(91.3)	134/143	(93.7)	422/439	(96.1)

IFA – Gambian strain of *P. falciparum* used

Table 7. Various species of Anopheles mosquitoes collected using man-biting technique at Napsan, Labtay, and Santo Niño from March 1986 to July 1987.

	Average number of mosquitoes caught/man/night					
Species	March	June	September	January	April	July
Anopheles flavirostris	7.4	16.1	59.4	35.2	29.1	119.8
An. annularis	1.1	1.9	1.7	3.6	0.3	11.2
An. peditaeniatus	0.0	0.4	0.1	1.2	0.0	0.1
An. balabacensis	0.0	0.2	1.2	0.0	0.0	1.0
An. tesselatus	0.1	0.1	0.9	0.1	0.0	0.2
An. subpictus	0.1	0.5	0.0	0.1	0.1	0.1
An. maculatus	0.0	0.0	0.6	0.1	0.1	0.2
An. karwari	0.0	0.0	0.1	0.4	0.0	0.1
An. litoralis	0.2	0.0	0.0	0.0	0.0	0.0
An. kochi	0.0	0.0	0.1	0.1	0.0	0.0
An. mangyanus	0.0	0.0	0.0	0.1	0.0	0.0
An. ludlowae	0.1	0.0	0.0	0.0	0.0	0.0
Totals	9.0	19.2	64.1	40.9	29.6	132.7

DISCUSSION

The description of the history and nature of malaria in the Philippines is generally not based upon longitudinal studies from single sites. More often, information comes from point prevalence studies, which have been conducted at different sites at various times of the year, or from cases seen at health care facilities. We have been able to study malaria at a single site on the island of Palawan during periods, which include the beginning, middle, and end of the rainy season as well as the dry season. In a discussion of the epidemiology of malaria, others have alluded to variation in the occurrence of the vector, which may be influenced both by the dry season and heavy rains, which might flush mosquito larvae from small streams.[5,9] As expected, we found the lowest prevalence of malaria in the dry season. The prevalence rose with the rainfall and remained high throughout the rainy season. The rains, despite being quite heavy, did not reduce malaria during the middle of the rainy season as experienced in other locations (personal communication - G. Schultz). In fact, the anopheles population continued to increase throughout this period. Numerous factors including the nature of the vector, amount of rainfall, and geographical characteristics help to establish this pattern of malaria prevalence. A thorough understanding of the seasonal variation is important when planning both control measures and other investigations such as vaccine or drug prophylaxis trials.

The particular site chosen for extensive longitudinal studies of malaria should be representative of the greater area of interest such as a region or country where data can be extrapolated and similarly applied. In the Philippines this is quite difficult since there are numerous islands spread over a great distance. Palawan is known to be one of the more malarious areas in the Philippines (Malaria Eradication Service - Personnel Communication). The site selected is probably representative of the entire island since the population characteristics, geography, and rainfall vary little throughout the island.

We found seasonal variation in the overall slide-positive rate within the barangay from 17.8% in March of 1986 to 31.6% in June of 1986 with a similar seasonal pattern in 1987. One of the sitios (Labtay) within the barangay reached a prevalence of 39.6% in September of 1986 and another (Santo Niño) reached 40.8% in July of 1987. A study conducted in 1968 within the Iwahig Penal Colony, on the east coast of Palawan, demonstrated a parasite rate of 55.9% previous to, and 38.8% subsequent to, a mass drug administration.[10] This very high rate primarily in adults could be a result of non-immune population brought here from areas of the Philippines not as endemic for malaria. These prevalence rates are quite high when compared to reports of other studies on Palawan.[5,6] The two sitios of Labtay and Santo Niño generally had higher slide-positive rates than Napsan proper, probably because they are located more directly in the foothills with many of the inhabitants planting rice in heavily forested areas. The very high prevalence rate found in Napsan proper in July of 1987 could represent an epidemic introduced from the surrounding area. *Anopheles flavirostris*, the primary vector, breeds in flowing fresh water, which is shaded by overhanging vegetation, and therefore is more abundant in the more heavily forested foothills, found in Santo Niño and Labtay.

Our calculated crude incidence of malaria slide-positivity due to all species of plasmodium for each three-month period ranged between 17.8% and 20.2%. This is probably inaccurate since it is derived from two point prevalence studies and not based on monitoring actual attacks of malaria over time in the population. The initial results of measurement of *P. falciparum* anti-sporozoite antibody conversion from negative to positive tend to support this conclusion. Preliminary data from measurement of anti-sporozoite antibody development indicates an exposure rate to *P. falciparum* alone, over the three-month period of March to June 1986, to be greater than 37%. Attack rate studies carried out during a recently completed (unpublished) drug prophylaxis study support this conclusion. Between January 1988 and April 1988, an overall attack rate of greater than 50% was seen in a group of persons receiving the placebo.

Prior to World War II the predominant species of plasmodium was *P. vivax*. *Plasmodium falciparum* was most prevalent during World War II, however, *P. vivax* again predominated after the war and prior to the initiation of malaria control measures.[11] Between 1967 and 1971, 63% of the positive smears were *P. falciparum.* By 1972 this number had risen to 65%. In 1976 the relative prevalence of *P. falciparum* and *P. vivax* were reported to be 67.0% and 30.6% respectively.[12] In contrast to these figures, *P. falciparum* represented 58.1% and *P. vivax* represented 35.9% of our 1,334 positive slides. Mixed infections of *P. falciparum* and *P. vivax* accounted for 5.3% of the positive slides. *P. malariae* accounted for 0.7% of the malaria positive slides. The study conducted by Alves et al[10] revealed a *P. falciparum* to *P. vivax* ratio of 1.5:1 in February and 5.3:1 in October of 1967. Our study showed a similar seasonal trend, probably associated with rainfall, with an increase in *P. falciparum* during the rainy season. During the course of our study, *P. falciparum* prevalence ranged between 8.0% and 21.9% and for *P. vivax* from 7.7% to 11.4%. Cases of ovale and *P. malariae* have been described from Palawan.[6,10] We identified nine cases of *P. malariae* during this study.

Despite the fact that the IFA may not be an accurate measure of immunity but rather past exposure, it is interesting to note that the number of negative tests is higher in the younger age groups, and that there is a direct relationship between IFA titer and age. The prevalence of malaria based on examination of peripheral blood smears was higher in persons below the age of 10. Further tests of immunological status are being conducted. There are 5 recognized malaria vectors in the Philippines, those being *An. flavirostris, An. balabacensis*, *An. maculatus*, An. *litoralis*, and *An. mangyanus.*[13] Although all of these vectors were found in the Napsan area of Palawan, *An. flavirostris* is clearly the primary vector. The other 4 species, because of their rarity, play at most an incidental role in malaria transmission.

The careful evaluation of parasitological, entomological, epidemiological and immunological aspects of malaria are required in order to produce baseline data for planning and evaluation of future studies including drug prophylaxis and vaccine trials. Our report describes the initial phase of such a study being conducted in the Philippines.

SUMMARY

During a longitudinal study on the island of Palawan from March 1986 to July 1987, the prevalence of malaria was found to vary between 18.0% and 31.6. The increase in malaria positive slides during the season of greatest rainfall was due primarily to an increase in *P. falciparum.* The increased prevalence of positive blood smears during this season was well correlated with increased numbers of the primary vector *An. flavirostris*, collected using man-biting techniques. The average number of mosquitoes ranged from 7.4/man/night during the dry season to 59.4/ man/night in the wet season of 1986, down to 29.1 in April of 1987, and up to 119.8 in July of 1987. These rates are much higher than we have seen in other locations in the Philippines. The prevalence of Giemsa smears positive for plasmodium in persons below age 10 ranged from 22.6% o t 47.4% and was consistently higher than that for those above age 10 which ranged from 14.8% to 24.7%. Residents of the more heavily forested areas within the village consistently had higher prevalences of malaria positive smears. Indirect fluorescent antibody studies indicate early and continued exposure; 91.3% of those 5-9 years of age were positive, and titers increased with age. This study is the first long term longitudinal study on Palawan to carefully examine the seasonality of malaria and vector prevalence over time. The dry season appears o t be the major influence in reducing parasite prevalence at this time. Prolonged rainy seasons will have a significant effect on the amount of malaria seen in this area. Other parameters such as attack rates, sporozoite rates in mosquitoes, insecticide effectiveness and drug effectiveness are being studied.

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