MALARIA TRANSMISSION BY *ANOPHELES LITORALIS* KING, A SALT-WATER BREEDER, IN PANGUTARAN, SULU, REPUBLIC OF THE PHILIPPINES

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INTRODUCTION

During our filaria survey in Sulu archipelago some two years ago, we came across a number of people suffering from high fever and chills which we tentatively diagnosed as malaria cases. These were later on verified by the presence of malaria parasites in their blood smears. We were in the small island of Pangutaran which we surveyed from end to end. Not a single fresh-water river or stream was found that could serve as breeding place for *Anopheles minimus flavirostris*, the established principal vector of malaria in the Philippines, yet we found several cases of malaria. Preliminary inquiries indicated that malaria was confined to the northern portion of the island with the exception of one or two barrios in the southern portion. Out of 116 night blood smears taken during the filaria survey, 12 or 10.3 per cent were found positive for malaria parasites. Approximately half of these cases were *Plasmodium falciparum* and the rest were *Plasmodium vivax*. The adult parasite rate was 4.8 per cent, children (7-9 years) had a rate of 16 per cent while infants had a rate of 25 per cent (Cabrera, 1968). These figures, no doubt, point to an active transmission of the disease in an area where no possible breeding sites for *Anopheles minimus flavirostris* exist. These interesting facts prompted the principal investigator of the filaria project to pursue the problem.

Pangutaran is one of several islands in Sulu archipelago, located northwest of Jolo island (Fig. 1). It is actually a huge coral reef with only a thin layer of top soil. The island is shaped like a boot with the toe pointing west northwest and the leg in an approximated north northeast direction. The length is about 17 kilometers and around 7 kilometers wide at its broadest portion. The location is 120° 34' East longitude and 6° 18' North latitude and with an area of 98.4 square kilometers.

The island has twelve organized barrios with Simbahan as the premier barrio and the main center of activity in the area. This is located on the eastern shore midway between the heel and the toe of the boot (Fig. 2). No accurate and up-to-date figures are available for the population of the island; however, the Malaria Eradication Unit at Jolo estimates the population to be around 13,000 as of the year 1967, (M.E.U. Report). Majority of the inhabitants are Samals and Tausogs. The principal industry is fishing and the main product is copra. Fruits and vegetables are scarce and one can observe only a handful of cattle, goats and poultry. There are hardly any trees other than coconuts.

Dug wells as deep as 20 feet pass through the layer of coral reef with very scanty water that is slightly salty. Drinking water is

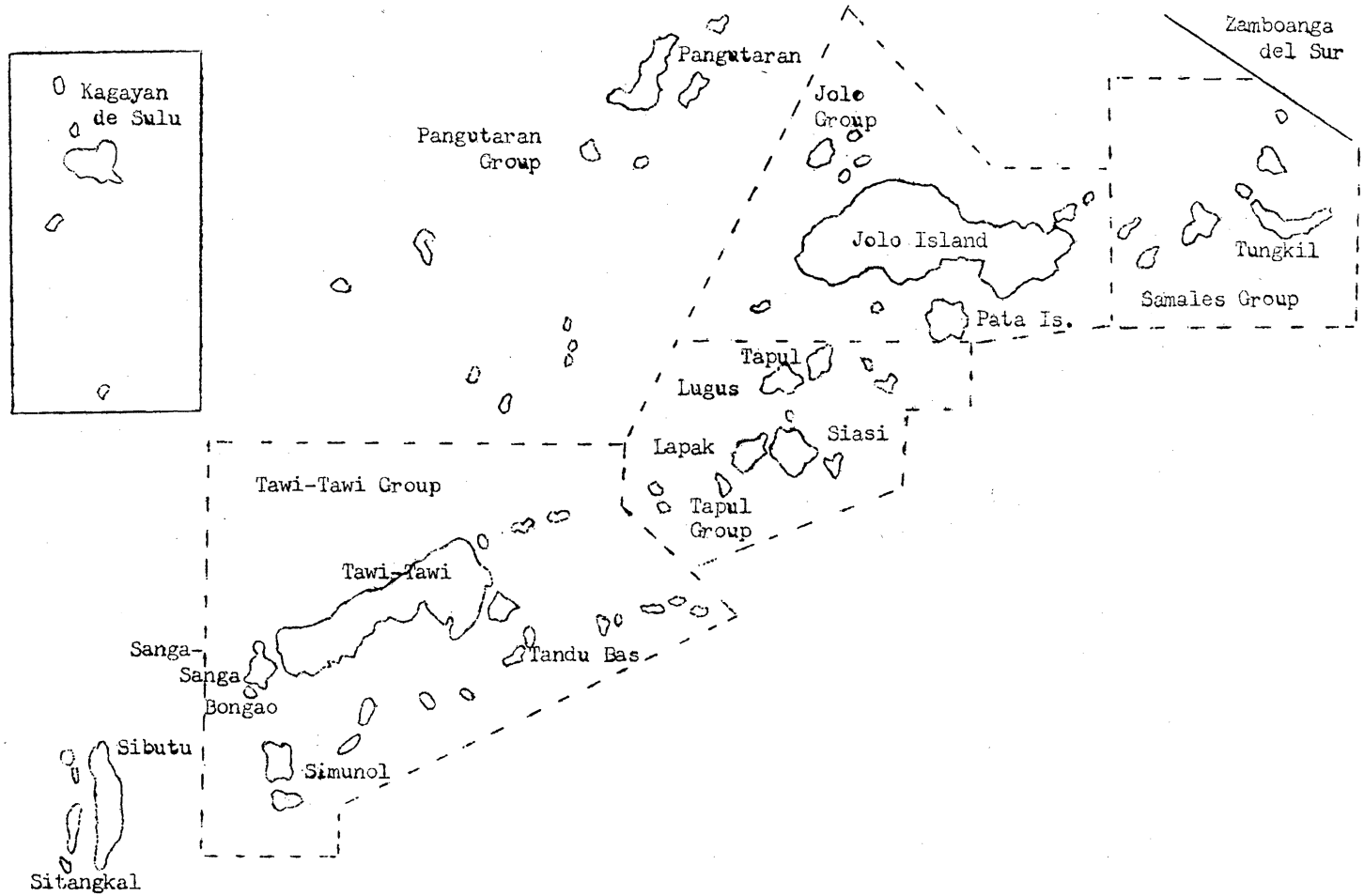

Fig. 1—Map of Sulu Archipelago.

transported daily by motor boats (kumpits) from Jolo, the largest and most populated island in the entire Sulu archipelago. No mountains or hills of significant elevations are present which explains the absence of fresh-water rivers or streams. Viewed from afar as the kumpit approaches the island, we see a flat terrain full of coconut trees. The mean elevation from sea level is around 3-4 meters while the highest elevation is 8 meters. Along the eastern coast are mangrove swamps and coral depressions behind coastal embankments which could serve as ideal breeding sites for salt-water breeding mosquitoes. It is not uncommon to find holes and/or depressions on rocks or coral reefs filled with brackish water yielding several mosquito larvae in a single dipping.

The objectives of this project were: a) to make a preliminary malaria survey in the entire island to determine the prevalence of the disease and to find out whether or not transmission of malaria exists; b) to study the anopheles mosquito fauna and attempt to incriminate the vector of malaria in a place where *Anopheles minimus flavirostris* is entirely absent. The data presented here will undoubtedly be of value both to the national and international program of malaria eradication.

MATERIALS AND METHODS

Blood survey procedure:

This constitutes the first phase of the study covering all age groups of the population. A thick and thin blood smear per person was taken in school buildings, in houses and in health centers. The smears were dried and after staining with Giemsa, were sent by air to our home base in Manila for microscopic examination. Among infants and children, in addition to blood smears, spleen palpation was done. This phase was meant to determine the extent and distribution of malaria and to provide information as to whether or not active transmission of the disease was going on.

Entomological studies:

The second phase, meant to accomplish the main objective of this study, is entomological in nature. Mosquito traps, using cows as baits, were set late in the afternoon in the vicinity of houses with malaria cases. Early the next morning, the baits were removed, and the trap doors were closed. All mosquitoes inside the traps were collected by means of glass-tube aspirators and placed in cylindrical cardboard containers with the open ends covered with bobbinette. Since the mosquitoes were mostly blooded, we allowed two days for the insects to digest the blood meal before dissections were done. Inasmuch as human malaria is transmitted only by mosquitoes of the genus anopheles, only anophelines were sorted according to species prior to dissection. However, the rest of the mosquitoes were also indentified but were not dissected. The mosquito guts were dissected out and examined for oocysts while the salivary glands were exposed and examined for sporozoites according to standard dissection techniques (Barber and Rice, 1936; Russell, 1963). Trap settings were made daily with an average dissection rate of 200-300 mosquitoes per day. Mosquito larvae were collected from rock holes and/or depressions on coral reefs by means of dippers and/or glass pipettes with a round rubber bulb attached to one end. In this phase of the work we expect to determine the anopheline fauna in the island as well as the density of each species. Furthermore, we hoped to pinpoint the most probable natural vector of malaria in the island as per result of our dissections.

Experimental infection:

It is always preferable as standard procedure that the field findings be duplicated in the laboratory. Hence, we also did experimental feeding of wild-caught anopheline mosquitoes on a gametocyte carrier malaria case. Gametocyte counts in the blood smear and exflagellation procedures were carried out prior to experimental feeding so as to know the density and capability of these gametocytes to develop inside the mosquito. The engorged mosquitoes were kept in cylindrical cardboard cages for a period ranging from 9-14 days to allow development of the parasite inside these insects. Mosquitoes that were weak prior to the end of the incubation period were dissected both for oocysts in the guts and sporozoites in the salivary glands.

RESULTS

Distribution and prevalence:

A total of 1,957 persons of all ages were examined for malaria parasites during the first phase of this work. Since one of the more immediate objectives was the establishment, in a more definite way than could be obtained from skimpy records, of the malaria prevalence for the island of Pangutaran, emphasis was placed on parasite rates. There were 1,004 females examined as against 953 males as shown in *Table* 1. Proportion-wise, the slight over-representation amounts to about 105 females per 100 males.

Table 1
Prevalence of Malaria by Barrios, Pangutaran Island, Sulu, 1968.

Barrios	Male			Female			Both Sexes		
	No. Exam'D'	No. Pos.	% Pos.	No. Exam'D'	No. Pos.	% Pos.	No. Exam'D'	No Pos.	% Pos.
1. Bangkilay	26	2	7.7	25	3	12.0	51	5	9.8
2. Kawitan	64	2	3.1	74	2	2.7	138	4	2.9
3. Kihiniog	108	12*	11.1	123	15	12.2	231	27	11.7
4. Lantong-Babag	84	17	20.2	101	15**	14.9	185	32	17.3
5. Lumadapdap	107	4	3.7	96	2	2.1	203	6	3.0
6. Pandaniog	68	1	1.5	42	1	2.4	110	2	1.8
7. Panitikan	26	-	0.0	22	-	0.0	48	-	0.0
8. Patutol	39	4	10.3	52	-	0.0	91	4	4.4
9. Seipang	102	7	6.9	112	5	4.5	214	12	5.6
10. Simbahan	147	33	22.4	177	21	11.8	324	54	16.7
11. Tubig-Nonok	50	-	0.0	25	-	0.0	75	-	0.0
12. Tubig-Sallang	132	10	7.6	155	12	7.7	287	22	7.7
Total	953	92	9.65	1004	76	7.57	1957	168	8.58

* Includes one *P. malariae* infection in a 3-year-old male from Kihiniog.
**Includes one *P. malariae* infection in a 12-year-old female from Lantong-Babag.

The over-all positivity rates revealed a difference of around 2 per cent (9.65% as against 7.5%) in favor of males. This trend is generally reflected in all barrios except in four, where the female prevalence appeared to be slightly above that of the males. These are in barrios Bankilay, Kihiniog, Pandaniog and Tubig-Sallang. These exceptions could, however, be explained by sampling variation.

Only three out of the twelve barrios showed prevalence rates exceeding 10 per cent. These were Kihiniog, Lantong-Babag and Simbahan having rates of 11.7, 17.3 and 16.7 per cent respectively. Geographically speaking, these observations indicate that malaria is most prevalent in the central and upper barrios along the eastern coast of the island (Fig. 2).

Age and sex distribution:

Table 2 shows the breakdown of persons examined by age and sex. The most significant detail portrayed here is the trend of parasite rates in relation to age of subjects examined. The higher rates were observed among infants, pre-school children and the juveniles. All these indices exceeded 10 per cent. For the young infants (less than one year) the parasite index appeared to be the highest (28.6 per cent) indicating that about 3 out of every 10 infants examined were positive for malaria. Majority of infants and children found positive for malaria also had enlarged spleen of varying sizes.

Distribution by species of malaria parasites:

In order to determine whether there was a predominance of any one species of plasmodia among the 168 cases detected during the survey, the appropriate classifications were made and is presented in Table 3. Of the 168 cases of malaria, 83 were found to be *Plasmodium vivax*, 79 were *Plasmodium falciparum*, 4 were mixed infection of *P. vivax* and *P. falciparum* and 2 were *Plasmodium malariae*. The 2 cases of *Plasmodium malariae* came from barrios Kihiniog and Lantong-Babag.

Mosquito identification and dissection:

The results of anopheles mosquito dissections during the first field trip in 1968, which unfortunately coincided with the dry season, is shown in Table 4. A total of 842 female anopheles mosquitoes belonging to 7 species were dissected for oocysts and sporozoites. Out of this number, 831 or 98.7 per cent were *A. litoralis*, two *A. franciscoi*, two *A. tessellatus*, three *A. baezai*, two *A. subpictus indefinitus*, one *A. annularis* and one *A. kochi*. So far, with these few mosquitoes dissected, we have not encountered any mosquito that harbored oocysts and/or sporozoites. Culicine mosquitoes also entered the traps and were identified but not dissected as follows: 402 *Armigeres malayi*, 15 *Armigeres joloensis*, 5 *Armigeres manalangi*, one *Armigeres baisasi*, 19 *Aedes dux*, 15 *Aedes poecilus*, 18 *Aedes nocturnus*, 4 *Culex tritaeniorhyncus*, 3 *Culex fatigans*, one *Culex nigropunctatus* and one *Culex annulus*.

Larval dippings were done during the first and third trips and the results are shown in Table 5. Out of 651 larvae collected from 3 barrios, 616 or 93 per cent were *Anopheles litoralis*, 22 or 3.3 per cent *Culex sitiens* and 13 or 2 per cent *Aedes sp.*

During the months of November and December 1969, another field trip was undertaken. This time the island was having moderate precipitation almost daily. Whereas during the dry season an average of 15-20 anopheles mosquitoes entered our traps per setting, during this wet season at least a thousand anopheles mosquitoes (mostly *A. litoralis*) entered the traps. Since *Anopheles litoralis* is the predominating species comprising 98.7 per cent of the anopheles catches,

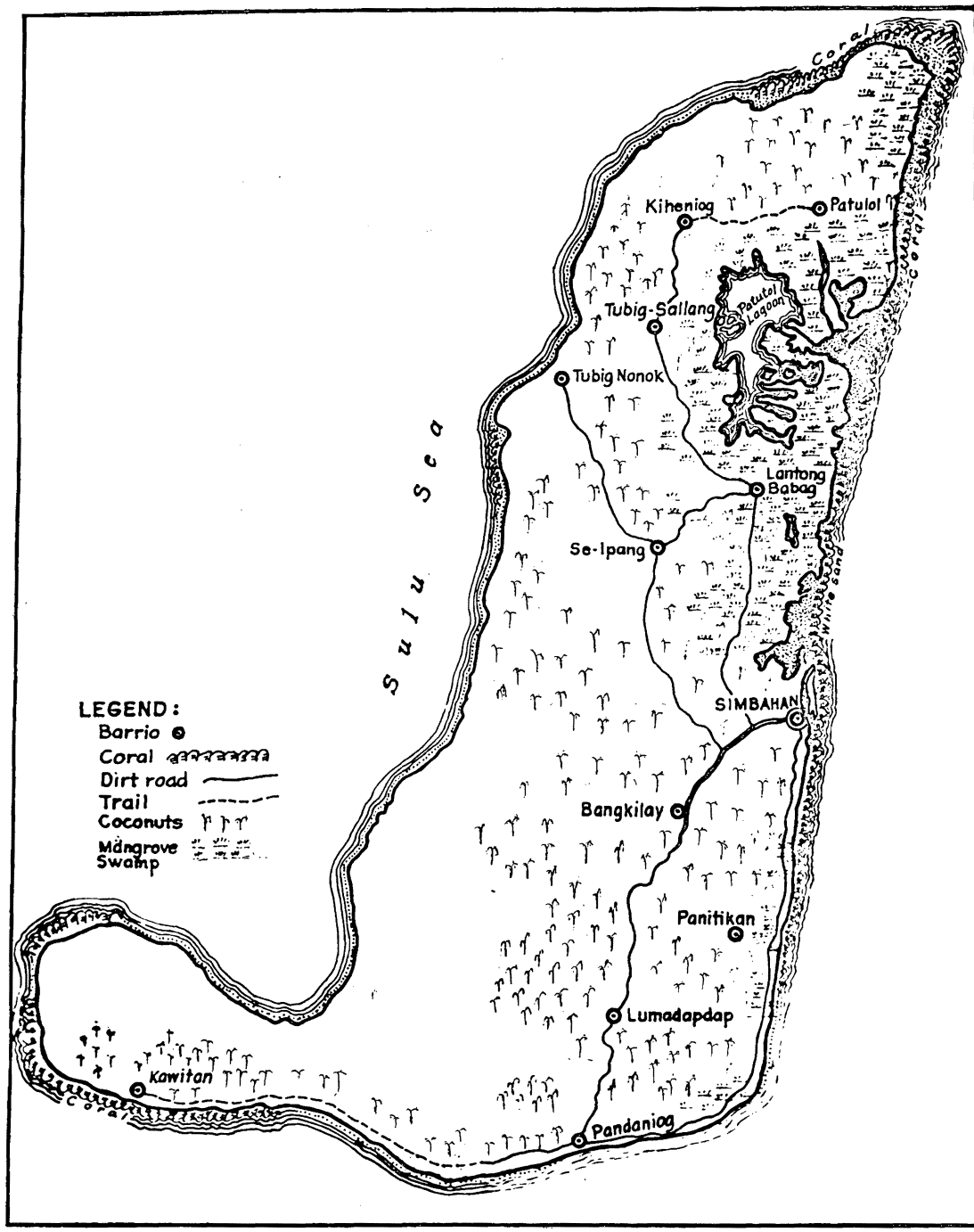

Fig. 2 — Map of Pangutaran Island.

Table 2

Prevalence of Malaria by Age and Sex, Pangutaran Island, Sulu, 1968.

Age Group	Male			Female			Both sexes		
	No. Exam'D'	No. Pos.	% Pos.	No. Exam'D'	No. Pos.	% Pos.	No. Exam'D'	No. Pos.	% Pos.
0–1	22	7	31.8	13	3	23.1	35	10	28.6
1–2	37	3	8.1	33	5	15.2	70	8	11.4
2–4	122	21*	17.2	103	18	17.5	225	39	17.3
5–9	199	32	16.1	219	24	11.0	418	56	13.4
10–14	216	18	6.5	202	14**	6.9	478	32	6.7
15–24	128	10	7.8	183	8	4.4	311	18	5.8
25–34	89	-	0.0	161	3	1.9	250	3	1.2
35–54	73	1	1.4	82	1	1.2	155	2	1.3
55	5	-	0.0	7	-	0.0	12	-	0.0
Not stated	2	-	0.0	1	-	0.0	3	-	0.0
Total	953	92	9.65	1004	76	7.57	1957	168	8.58

* Includes one *P. malariae* infection in a 3-year-old male from Kihiniog.
**Includes one *P. malariae* infection in a 12-year-old female from Lantong-Babag.

Table 3

Distribution of Malaria Cases by Barrio and Type of Infection, Pangutaran Island, Sulu, 1968.

Barrios	No. Exam'D'	Number positive				
		P.V.	P.F.	P.M.	Mix Infec.	All types
1. Bangkilay	51	2	3	-	-	5
2. Kawitan	138	2	1	-	1	4
3. Kihiniog	231	12	14	1	-	27
4. Lantong-Babag	185	23	8	1	-	32
5. Lumadapdap	203	5	1	-	-	6
6. Pandaniog	110	-	2	-	-	2
7. Panitikan	48	-	-	-	-	-
8. Patutol	91	2	2	-	-	4
9. Seipang	214	8	3	-	1	12
10. Simbahan	324	18	35	-	1	54
11. Tubig-Nonok	75	-	-	-	-	-
12. Tubig-Sallang	287	11	10	-	1	22
Total	1957	83	79	2	4	168

dissection for oocysts and sporozoites were confined to this species. Results of the dissections are shown in Table 6. Out of 3,539 anopheles mosquitoes dissected for oocysts in their guts, 3,511 or 99 per cent were *Anopheles litoralis* and only 28 or 1 per cent were *Anopheles franciscoi*. Of the 3,511 guts of *Anopheles litoralis*, 6 or 0.17 per cent were found positive for oocysts. Likewise, of 3,531 *Anopheles litoralis* dissected for sporozoites in the salivary glands, 4 or 0.11 per cent were found positive for sporozoites.

For a more detailed examination of the sporozoites, the specimens were fixed with methyl alcohol and stained with Giemsa and examined in Manila under a compound microscope using oil immersion. Measurements of 25 sporozoites each from the four positive mosquitoes were made and the averages are shown in Table 7. A study of the morphology of the stained sporozoites in specimens 1 and 2 showed that they have narrow elongated bodies, majority of them being straight or slightly curved. They have tapering ends or with one extremity more blunted than the other. The nuclei are located centrally, oval-shaped, single but at times fragmented (plate 1). On the other hand, sporozoites from specimens 3 and 4 are sickle-shaped with both ends equally pointed. The nuclei are also centrally located, either single or split into several discrete dots (plate 2).

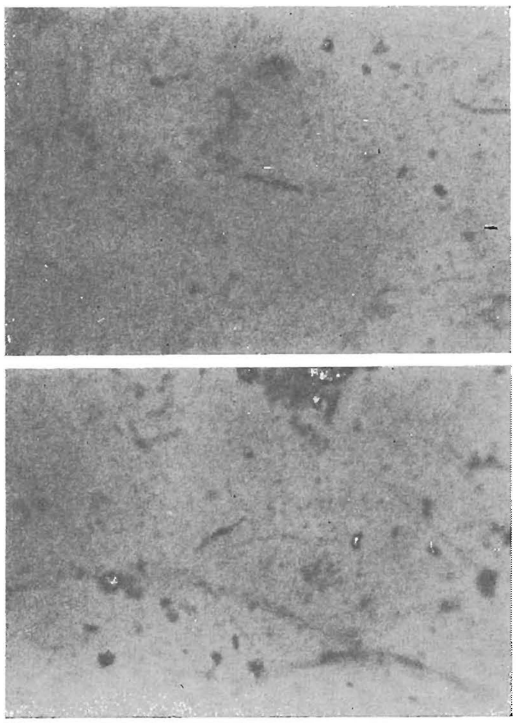

Plate 1—Sporozoites of *P. vivax* from specimens 1 & 2.

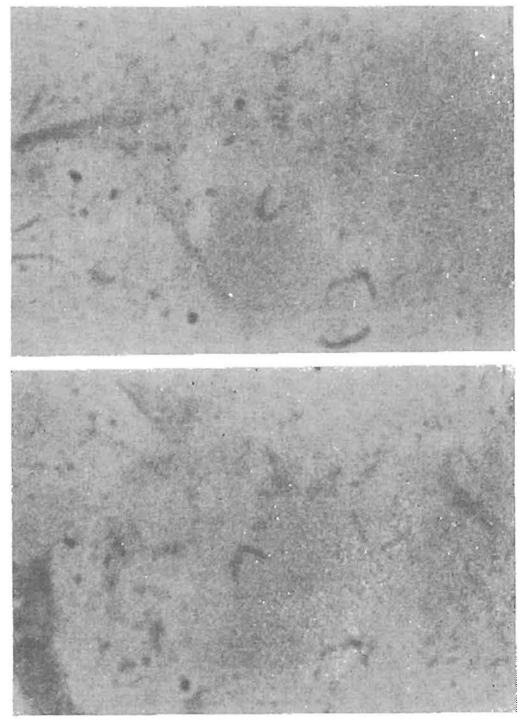

Plate 2—Sporozoites of *P. falciparum* from specimens 3 & 4.

Experimental infections:

Wild-caught *Anopheles litoralis* were allowed to bite a gametocyte carrier during the second trip but due to the temporary closure of Jolo airport, all the mosquito

specimens died on the return trip to Manila. Another attempt was made during the third trip whereby a total of 475 wild-caught *Anopheles litoralis* were fed on gametocyte carriers. Dissections were made from the first day to the fourteenth day. Of this number only one mosquito showed oocyst in the gut and not a single mosquito harbored sporozoites in the salivary gland.

DISCUSSION

It is interesting to note that infants had the highest parasite index. If we combine the group of younger infants (less than a year) with the older infants (more than a year but less than 2 years) the infant parasite index becomes 17.1 per cent. These figures convey very definitive information regarding the amount of transmission during the period immediately preceding the survey. Three infants suffering from malaria out of every ten examined is a good indication of the intensity with which active transmission has been going on. In the meager efforts toward control and/or eradication of the disease in the island by the authorities concerned, it is reasonable to assume that the same degree of transmission, if not higher, is currently taking place.

A spot check into the places of residence of the 18 infants found positive for malaria revealed that all of them came from barrios previously found to have the highest prevalence rates. For the present, however, we can only take this as being suggestive rather than corroborative evidence since relatively fewer infants were examined in the other barrios.

An analysis of the frequencies obtained shows that the two major species (*P. vivax* and *P. falciparum*) are about equally represented in the totality of positive cases. This is generally true for the different barrios of the island, with the exception of barrios Lantong-Babag and Simbahan. In the former, there are three times as many cases of *P. vivax* infection than *P. falciparum* infection (24 against 8) while in the latter, there is a reversal of the proportionate frequencies ... twice as many *P. falciparum* than *P. vivax* (35 against 18). Whether these conflicting pictures are real can only be verified by further observations. The distance between these barrios is only about two kilometers and it is quite difficult at this time to speculate on the reasons why the predominant species will differ in the two places, if such a predominance really exists.

From the mosquito dissection data (Tables 4 and 6) there is no doubt that the most prevalent anopheles mosquito is *Anopheles litoralis*. It is interesting to note that during the dry season, 98.7 per cent of the anopheles mosquitoes caught in the traps were *Anopheles litoralis*. This figure is likewise duplicated during the wet season. Although the infection rates in *A. litoralis* are low compared to those in *Anopheles minimus flavirostris* as found by other local workers (Dy and Gapuz, 1948; Manalang; Russell, 1934.) we should not forget that the latter is our principal malaria vector and therefore is a more efficient transmitter. Then we should also take into consideration the endemicity of the disease in the area at the time of the survey. That *Anopheles litoralis* adult is the most predominant in the catches is borne out by the larval collections (Table 5) in which the larvae of this same species are the most abundant whether it be during the dry or wet season of the year.

While it is true that sporozoites recovered from wild-caught anopheles mosquitoes may not necessarily be of human origin, we have strong evidences to believe that the sporozoites we found from *Anopheles litoralis* are human in origin. Information gathered from

Table 4
Mosquito dissections for oocysts and sporozoites,
Pangutaran, Sulu, 1968.

Locality	Species	No. dissected		No. Infected		Total No. dissected
		Gut	Sal. Gld.	Gut	Sal. Gld.	
Simbahan & Lantong-Babag	*A. litoralis*	-	831	0	0	831
	A. franciscoi	-	2	0	0	2
	A. tesselatus	-	2	0	0	2
	A. baezai	-	3	0	0	3
	A. subpictus indefinitus	-	2	0	0	2
	A. kochi	-	1	0	0	1
	A. annularis	-	1	0	0	1
	Total	-	842	0	0	842

Table 5
Larval collections, Pangutaran, Sulu, 1968 and 1969.

Locality	Species Collected		
	Anopheles litoralis	*Culex sitiens*	*Aedes* sp.
Simbahan	486	16	12
Lantong-Babag	20	-	-
Kawitan	110	6	1
Total	616	22	13

Table 6
Mosquito Dissection for Oocyst and Sporozoites
Pangutaran, Sulu, 1969.

Locality	Species	No. Dissected		No. Infected		% Infected	
		Gut	Sal.Gld.	Gut	Sal.Gld.	Gut	Sal.Gld.
Simbahan	*A. litoralis*	3511	3531	6	4	0.170	0.113
	A. franciscoi	28	28	0	0	0	0
	Total	3539	3559	6	4	0.169	0.112

Table 7
Measurement of Sporozoites from *A. litoralis*
Pangutaran, Sulu, 1969.

Mosquito Specimen	No. of Sporozoites	Ave. length (in microns)	Ave. width (in microns)
1	25	11.08	1.21
2	25	11.36	1.21
3	25	12.42	1.60
4	25	12.02	1.58

the local inhabitants tends to support our own observation that there are no monkeys and wild birds in the barrios where we constructed our mosquito traps, more so in barrio Simbahan which is the center of activities in the island, located along the eastern coast. There are no trees or group of trees where monkeys could dwell probably because of the lack of top soil, mountains and rivers. Moreover, the morphology of the sporozoites found in *Anopheles litoralis* (specimens 1 and 2) fit into the description of *Plasmodium vivax* sporozoites, while those found in specimens 3 and 4 fit into those of *Plasmodium falciparum* according to Garnham (1966). This finding is quite understandable since the prevalence of the two species of human plasmodia in the island is about equal. Although ideally, it is best to undertake experimental infection of the suspected vector to conclusively prove its vectorial capacity, a review of literature showed that many malaria vectors have been incriminated on the basis alone of recovery of sporozoites from naturally infected wild-caught anopheline mosquitoes. This holds true also for *Anopheles minimus flavirostris*, the principal vector of malaria in the Philippines (Dy and Gapuz, 1948; Manalang; Russell, 1934). In this report we demonstrated oocysts in only one of 475 experimentally fed mosquitoes.

SUMMARY AND CONCLUSION

Pangutaran is one of several islands of Sulu archipelago which is unique in that it is composed of a huge coral reef. It has a very flat terrain without fresh-water rivers or springs for possible breeding places of *Anopheles minimus flavirostris*. However, despite all these, our blood survey gave a prevalence rate of malaria of about 9 per cent with infants showing the highest rates. About half of all malaria cases had *Plasmodium vivax* infection while the other half had *P. falciparum* infection. Four cases had mixed infections and two had *P. malariae* infection.

Practically all mosquitoes caught in our traps were *Anopheles litoralis* with identical findings in our larval dippings. If only anophelines caught in the traps were considered then almost 100 per cent were *Anopheles litoralis*. About 0.17 per cent of this species of mosquito had oocyst in the guts while 0.11 per cent had sporozoites in their salivary glands. Not a single *Anopheles minimus flavirostris* was found in the island.

From the above findings we may conclude that there is active transmission of malaria in the island of Pangutaran with *Plasmodium vivax* and *Plasmodium falciparum* equally predominant. We are convinced that the

sporozoites and oocysts found in the *Anopheles litoralis* are of human origin and therefore believe that we have incriminated this mosquito as the principal vector of malaria in this island.

With the above findings we anticipate that a similar and/or identical situation exists in several small islands in the Philippines particularly in the Sulu archipelago. We would suggest, therefore, that other malaria workers in the field should continue this type of work so that the National Government can pinpoint all islands where malaria is present but *Anopheles minimus flavirostris* is absent. Malaria control and/or eradication programs in such areas may be modified accordingly.

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