

## Surveys on Simian Malaria Parasites and Their Vector in Palawan Island, the Philippines

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**Abstract:** Preliminary surveys on simian malaria were tried in January 1971 and December 1971–January 1972 during the period of investigations on human malaria in Palawan Island, the Philippines. Out of 20 long-tailed macaques examined, 11 were infected with malaria parasites each of which closely resembled *Plasmodium cynomolgi*, *P. inui*, *P. knowlesi* and / or *P. coatneyi*. This infection rate (55%) is much higher than those previously known (8.6%–18.3%) in the Philippine monkey. The evidence that the most important vector of simian malaria in Palawan is *Anopheles balabacensis* has been confirmed by results of mosquito collection by monkey-bait net trap and experimental infections of *A. balabacensis* females which were fed on a malaria positive monkey. Importance of further studies on simian malaria in the Philippines was also discussed.

So far four species of simian malaria parasites have been known to be distributed in the Philippines. Although there were no proper scientific papers published, strains of *Plasmodium inui* had been isolated from monkeys which were sent to the United States and Europe from the Philippines, and had been used in laboratories for studying mosquito infections since two decades ago. Thus the first record appeared in papers by Mohiuddin (1957) and Sezen (1958) (cited from Howard and Cabrera, 1961, and Garnham, 1966). Soon the first authentic record on the Philippine simian malaria parasite has been published by Howard and Cabrera (1961) who reported the detection of *P. inui* from monkey blood smears taken from Palawan and Mindanao. Lambrecht *et al.* (1961) in the United States also reported the presence of three species of simian malaria parasites (*P. knowlesi*, *P. cynomolgi* and *P. inui*) in monkeys sent from the Philippines. The fourth species, *P. coatneyi*, was detected by Eyles *et al.* (1963) in

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Malaya from a rhesus monkey inoculated with a blood sample which was shipped from the United States where the blood was taken from a Philippine monkey originally from Cebu Island. Working on the exoerythrocytic stages of simian malaria parasites, Held *et al.* (1968) used four different strains of *P. inui* including both CDC and Cebu strains originated from the Philippine monkeys. Sekikawa *et al.* (1974) examined blood smears of various monkeys which were imported from tropical countries into Japan and found that 22 out of 120 Philippine monkeys were infected with *P. inui*. With only one exception of the report by Howard and Cabrera (1961), as mentioned above, all the isolation or detection of the Philippine simian malaria parasites were done at laboratories outside the Philippines. In relation to the simian malaria parasites of the Philippines, as far as we know, nothing has been recorded about natural nor experimental anopheline vector, too.

From 1969 to 1973 field studies on the human malaria were performed in the Philippines, mainly in Palawan Island, by the members of the Nagasaki University Medical Team together with some members of the Malaria Eradication Service, Department of Health, Republic of the Philippines (Nakabayashi *et al.*, 1973; Nakabayashi *et al.*, 1974; Tsukamoto *et al.*, 1975). During the period of these investigations, preliminary surveys on blood parasites of small wild animals of Palawan Island were also attempted by us. Except for monkeys, results of detection of some blood parasites of wild animals, including a new species of *Trypanosoma* and malaria parasites of the genus *Hepatocystis*, were published by Miyata (1975) and Miyata and Tsukamoto (1975). In the present paper, therefore, results of surveys on simian malaria parasites and, in this connection, those of survey on anopheline mosquitoes as possible vectors in Palawan Island have been reported.

## MATERIALS AND METHODS

**Survey Area and Period:** the surveys were carried out twice mainly at the Montible Subcolony, Iwahig Penal Colony, Palawan, during the dry season: January in 1971 and from December 1971 to January 1972. Geographical and natural situations of the survey area were briefly described in a previous paper (Miyata and Tsukamoto, 1975).

**Monkeys:** The long-tailed monkey or crab-eating monkey, *Macaca irus*, was a subject for the survey. The scientific name for this Philippine monkey adopted in "Checklist of Philippine Mammals" by Alcasid (1970) is *Macaca philippinensis philippinensis* for that from Luzon, Palawan and Calamianian Islands, and *M. philippinensis mindanensis* for that from Mindanao and Negros. Since some local people caught and kept monkeys for their own pets or to sell to local dealers, we have often had chances to prepare blood smears from those monkeys. Some of them were prepared even on the road with the owner's permission when we saw monkeys. A total of 20 monkeys (7 in the first year of the surveys and 13 in the second season) were examined for blood parasites. One of those monkeys, A-55, was under our

continuous observations for nearly two weeks until the end of the surveys.

**Blood Smears:** Both thick and thin blood smears were prepared by pricking the finger of each monkey in a similar way as usual blood examinations for human. The blood smears were stained in a 3% Giemsa solution which was prepared with apparently clean running water brought from a local stream or a spring. Relative parasitaemia was expressed on the basis of thick blood smear, in accordance with human cases, as follows:

- : No malaria parasite was detected at least in 100 microscopic fields of thick blood smear with the magnification of  $1,000\times$ ,
- + : 1–10 parasites in 100 microscopic fields,
- ‡ : 1–10 parasites in 10 microscopic fields, and
- ‡‡ : 1–10 parasites in one microscopic field.

**Mosquito Survey:** Anopheline mosquitoes were collected at night during the 1971–1972 winter season mostly by using a large mosquito net of the monkey-bait trap, human-bait trap, or carabao-bait trap which were set up in forest areas, mainly Taguliat of the Montible Subcolony, where at least at that time numbers of wild Philippine monkeys could be observed frequently. After collecting mosquitoes by closing the net, identification of mosquito species and examination of natural infection of malaria parasites, *i.e.*, detection of either oocysts or sporozoites, were carried out. Monkey cages were set about 1.2–2 m high in the mosquito net.

**Experimental Infections of Mosquitoes:** Small numbers of newly emerged (and hence uninfected) adults of *Anopheles balabacensis* (s. str.) were obtained by rearing field-collected larvae, then they were allowed to feed on a monkey (A-55) which had already confirmed to be “malaria positive”. With certain intervals after blood sucking, a small portion of the mosquitoes were examined for any development of the sexual stages of the malaria parasites. During the observation and examination, larvae, pupae and adult mosquitoes were reared in a small house protected by nylon mesh either from invasion of outdoor mosquitoes or escape of them to the outside.

## RESULTS

### 1. Detection of Malaria Parasites from Monkeys

In total 20 Philippine monkeys were examined for blood parasites. Among them 11 were infected with malaria parasites of the genus *Plasmodium*: in more detail, malaria “positive” monkeys were 3 out of 7 examined in the first year’s survey and 8 out of 13 in the second year. In general, even in positive cases, parasitaemia in some monkeys was quite low, and in some cases we have experienced that a monkey which was once “positive” but in the next examination became “negative” and *vice versa*. The results of such blood examinations of monkeys were pooled in Table 1. Practically it was impossible to identify the accurate species of malaria parasites when only few ring forms were detected from a blood smear because some of young trophozoites of malaria parasites resemble each other. Therefore diagnosis of these simian malaria parasites was tried only in some cases such as monkey Nos. A-7, A-9, A-11,

Table 1. Results of blood examinations for malaria parasites  
in Philippine monkeys in Palawan Island

| Monkey No. | Sex       | Size (Age) | Date of examination | Malaria detection | Remark   |
|------------|-----------|------------|---------------------|-------------------|--|
| M-1        | Female    | Baby       | Jan. 9, 1971        | +                 |  |
|            |           |            | 13                  | -                 |  |
| M-2        | Male      | Young      | Jan. 8, 1971        | +                 |  |
| M-3        | Male      | Baby       | Jan. 14, 1971       | ++                |  |
|            |           |            | 15                  | ++                |  |
| M-4        | Male      | Young      | Jan. 20, 1971       | -                 |  |
| M-5        | Male      | Young      | Jan. 24, 1971       | -                 |  |
| M-6        | Female    | Baby       | Jan. 24, 1971       | -                 |  |
| M-7        | No record | Young      | Jan. 25, 1971       | -                 | On the road  |
| A-3        | Female    | Adult      | Dec. 14, 1971       | +                 |  |
|            |           |            | 15                  | +                 |  |
| A-5        | Female    | Baby       | Dec. 5, 1971        | ++                | Young rings only   |
| A-6        | Female    | Baby       | Dec. 19, 1971       | -                 |  |
|            |           |            | Jan. 10, 1972       | -                 |  |
|            |           |            | 19                  | -                 |  |
|            |           |            | 27                  | -                 |  |
| A-7        | Female    | Adult      | Dec. 21, 1971       | ++                | ( <i>P. cynomolgi</i> )  |
|            |           |            | Jan. 4, 1972        | -                 |  |
|            |           |            | 19                  | +++               |  |
|            |           |            | 27                  | +++               |  |
| A-8        | Male      | Baby       | Dec. 22, 1971       | -                 |  |
|            |           |            | Jan. 4, 1972        | -                 |  |
|            |           |            | 19                  | -                 |  |
|            |           |            | 27                  | -                 |  |
| A-9        | Male      | Baby       | Dec. 23, 1971       | ++                | ( <i>P. inui</i> )   |
|            |           |            | Jan. 4, 1972        | -                 |  |
|            |           |            | 10                  | +++               |  |
|            |           |            | 19                  | +                 |  |
|            |           |            | 27                  | +                 |  |
| A-10       | Female    | Baby       | Dec. 27, 1971       | -                 |  |
|            |           |            | Jan. 4, 1972        | -                 |  |
|            |           |            | 10                  | -                 |  |
|            |           |            | 19                  | +                 | Young rings only   |
| A-11       | Male      | Baby       | Dec. 27, 1971       | +                 | ( <i>P. inui</i> and <i>P. knowlesi</i> )  |
| A-12       | Female    | Baby       | Jan. 4, 1972        | -                 |  |
|            |           |            | 19                  | -                 |  |
|            |           |            | 27                  | -                 |  |
| A-XY       | Male      | No record  | Jan. 5, 1972        | -                 | On the road  |
| A-13       | Female    | Baby       | Jan. 7, 1972        | -                 |  |
|            |           |            | 19                  | -                 |  |
|            |           |            | 27                  | -                 |  |
| A-14       | Female    | Young      | Jan. 7, 1972        | ++                | ( <i>P. inui</i> , <i>P. cynomolgi</i> and<br><i>P. knowlesi</i> or <i>P. coatneyi</i> ) |
|            |           |            | 10                  | ++                |  |
|            |           |            | 19                  | -                 |  |
|            |           |            | 27                  | +                 |  |
| A-55       | Male      | Young      | Jan. 12, 1972       | +++               | ( <i>P. inui</i> , <i>P. knowlesi</i> or<br><i>P. coatneyi</i> )                         |
|            |           |            | 14                  | +++               |  |
|            |           |            | 15                  | ++                |  |
|            |           |            | 16                  | ++                |  |
|            |           |            | 17                  | ++                |  |
|            |           |            | 18                  | ++                |  |
|            |           |            | 19                  | +++               |  |
|            |           |            | 20                  | +++               |  |
|            |           |            | 21                  | +                 |  |
|            |           |            | 22                  | +                 |  |
|            |           |            | 23                  | ++                |  |
|            |           |            | 24                  | ++                |  |

Total: 11 "positive" out of 20 monkeys examined (55%)

A-14 and A-55 where relatively higher parasite numbers were counted on thin blood smears. From monkey A-7, malaria parasites morphologically most resembling *P. cynomolgi* were detected, namely infected erythrocytes were quite enlarged, stippled, and large *vivax*-typed trophozoites were found within the host erythrocytes. Monkey A-9 was infected with malaria parasites of various stages. On December 23, for example, 1971, there were ring forms, band forms, large old trophozoites, early schizonts and nearly mature schizonts where the number of divided chromatin bodies ranged from 10 to 13. The nucleus or nuclei showed to be pleomorphic in developed large trophozoites or early schizonts, and malaria pigments are in small granules, dark yellowish brown, and abundantly scattered in the cytoplasm. The infected erythrocytes sometimes showed a slight enlargement, if any. From these characters of the parasite, the most suggestive species was *P. inui*. The erythrocytes of monkey A-11 seemed to be parasitized mainly by *P. inui* (Figs. 26-27) with a mixed infection of *P. knowlesi*. In the case of monkey A-14, at least three species of malaria parasites seemed to be involved: the major one had small granules of malaria pigment in the cytoplasm of large trophozoites and early schizonts within unenlarged erythrocytes, suggestive of *P. inui*; and the second one had large rice grain-shaped pigments in the cytoplasm of old trophozoites and schizonts similar to those in *P. coatneyi* or *P. knowlesi*. Fig. 30 shows a microgamete of the parasite closely resembles *P. coatneyi*. The third species was minor in number among them but the erythrocytes infected with the trophozoite were more or less enlarged and stippled sometimes heavily or slightly (Figs. 28-29): thus suggested parasite species is *P. cynomolgi*.

For monkey A-55 continuous blood examinations were carried out in more details from January 12 to 24, 1972, because of its rather high parasitaemia. In this case too, mixed infections due to at least two, possibly three, species were suggestive. Early trophozoite showed the thick cytoplasm and a clear vacuole (Figs. 1-3). Young trophozoites were usually in typical ring forms with a round nucleus on or within a slightly ragged circle of cytoplasm (Figs. 4-5, 8-10). The chromatin dot was sometimes curvilinear or circle around a small clear spot (Figs. 11-12), or divided into two dots of different sizes (Fig. 13). These characters suggest the parasite to be *P. knowlesi*. Deformed ring forms could be observed too (Figs. 14-15). The ring form with an exceptionally large spherical nucleus (Figs. 6-7) was suggestive of *P. inui*. Developed trophozoites and early schizonts were classified into three groups with regard to the size and shape of malaria pigments: the first one had fine granules (*inui*-type), the second had prominent rather round granules or grains (*knowlesi*-type), and the last one had large rice-grains (*coatneyi*-type). All the infected host erythrocytes remained in about normal size.

## 2. Anopheline Mosquito Survey

Within the forest area at Taguliat of Montible, totally 7 species of anopheline mosquitoes were collected by traps. Among them 6 species were attracted to monkey-bait trap. For the bait, several monkeys were kept in 2 cages placed inside of a big mosquito net which had an opening curtain for the entrance. A total of 57 anopheline mosquitoes were collected for 7 nights using the monkey bait. Among them 30 (53%) were identified to be *Anopheles balabacensis*, and 10 *A. kochi*, 6 *A. vanus*, 5 *A. minimus flavirostris*, 5 *A. maculatus*, and 1 *A. peditae-*

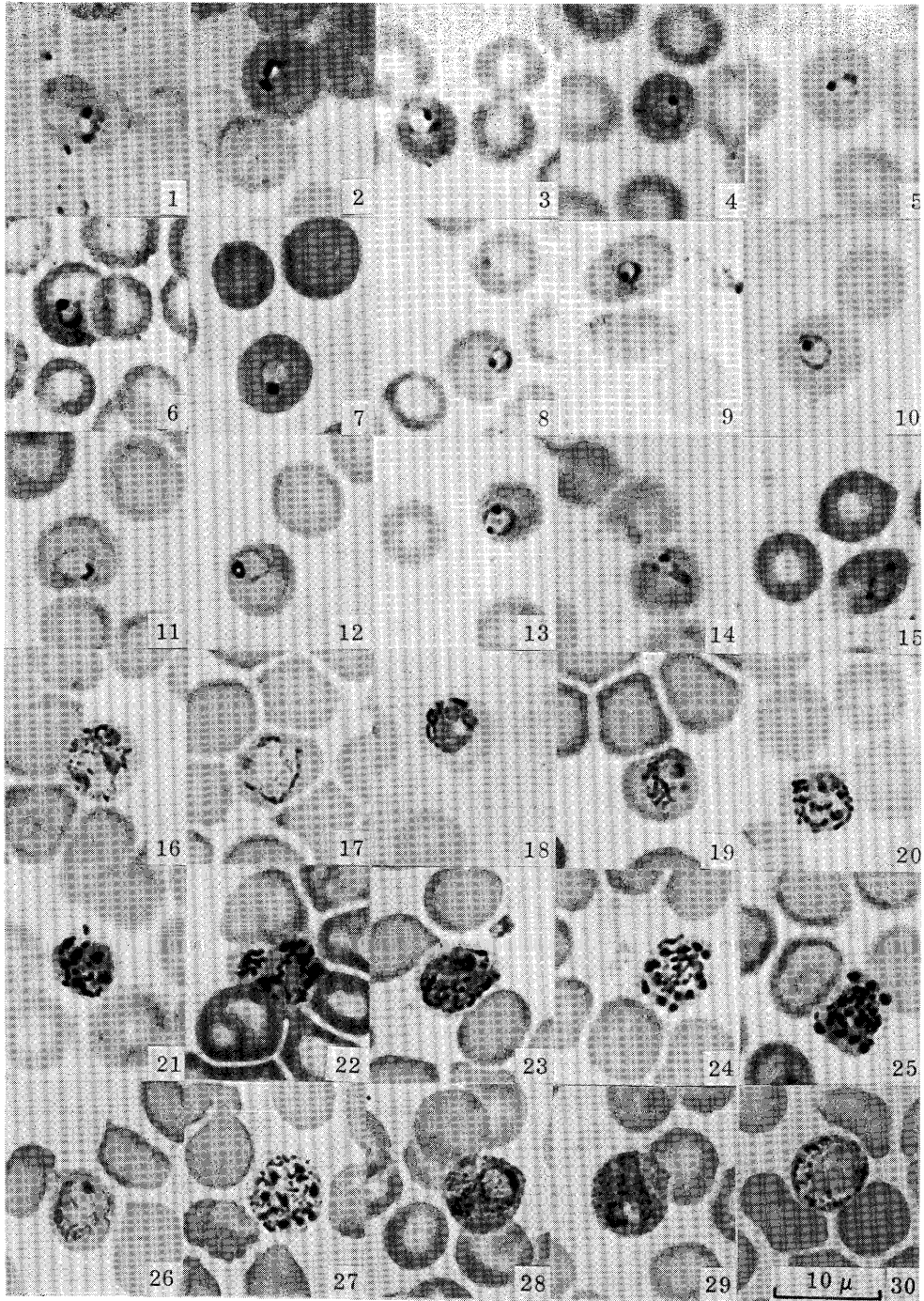


Plate I. Examples of various malaria parasites of different blood stages detected from Philippine long-tailed macaques. Figs. 1-25: from monkey A-55. Figs. 26-27: from monkey A-11. Figs. 28-30: from monkey A-14. See text for details.

*niatus* were also attracted to monkey baits. By the carabao-bait trap, on the other hand, among 1,026 mosquitoes of the genus *Anopheles*, only 29 individuals of *A. balabacensis* were caught in pooled 8 nights (the rate was thus 2.8%). *A. kochi*, *A. minimus flavirostris*, and *A. maculatus* were major ones in descending order. Although the number of trials and that of collected mosquitoes were too insufficient, it should be noticed that one individual of *A. balabacensis* was caught by the human-bait trap. Table 2 presents the summarized results of anopheline mosquito collections which were carried out on different nights by using 3 different kinds of the bait.

### 3. Natural and Experimental Malaria Infections of Mosquitoes

A few numbers of mosquitoes collected by the monkey-bait trap were dissected and examined for the presence of oocysts or sporozoites. Results on natural infections of sexual stages of malaria parasites were shown in Table 3. Out of 24 *A. balabacensis* dissected, 7 (29%) and 3 (13%) females were found to be infected with oocysts and sporozoites, respectively. Although those mosquitoes were attracted to monkeys in the natural forest, it is still unknown whether these oocysts and/or sporozoites were due to truly simian malaria parasites or due to other sources such as human or avian malaria parasites. Then preliminary experimental infections of mosquitoes were attempted under the field condition.

Newly emerged adults of *A. balabacensis* were obtained by rearing larvae which were

Table 2. Number (and rate in parentheses) of anopheline mosquitoes attracted to carabao-, monkey-, and human-bait traps in forest areas of Montible, Palawan, in January 1972

| Mosquito species               | Carabao<br>(8 nights) | Monkey<br>(7 nights) | Human<br>(4 nights) |
|--------------------------------|-----------------------|----------------------|---------------------|
| <i>A. kochi</i>                | 528 (51.5)            | 10 (17.5)            | 0 (0.0)             |
| <i>A. minimus flavirostris</i> | 251 (24.5)            | 5 (8.8)              | 4 (57.1)            |
| <i>A. maculatus</i>            | 121 (11.8)            | 5 (8.8)              | 1 (14.3)            |
| <i>A. peditaeniatus</i>        | 57 (5.6)              | 1 (1.8)              | 0 (0.0)             |
| <i>A. vanus</i>                | 31 (3.0)              | 6 (10.5)             | 0 (0.0)             |
| <i>A. balabacensis</i>         | 29 (2.8)              | 30 (52.6)            | 1 (14.3)            |
| <i>A. parangensis</i>          | 9 (0.9)               | 0 (0.0)              | 1 (14.3)            |
| Total                          | 1,026 (100%)          | 57 (100%)            | 7 (100%)            |

Table 3. Natural infections with malaria parasites in anopheline mosquitoes collected by monkey-bait trap in January 1972

| Mosquito species               | Number of mosquitoes |        |                  |        |            |
|--------------------------------|----------------------|--------|------------------|--------|------------|
|                                | Dissected            | Parous | Nulli-<br>parous | Oocyst | Sporozoite |
| <i>A. balabacensis</i>         | 24                   | 12     | 12               | 7      | 3          |
| <i>A. kochi</i>                | 4                    | 1      | 3                | 0      | 0          |
| <i>A. vanus</i>                | 3                    | 1      | 2                | 0      | 0          |
| <i>A. minimus flavirostris</i> | 2                    | 2      | 0                | 0      | 0          |
| <i>A. maculatus</i>            | 2                    | 0      | 2                | 0      | 0          |

collected from various water sources in jungle such as muddy puddles, carabao hoofprints, wheel ruts, and so on.

On January 12, 1972, a group of the uninfected mosquitoes was fed on monkey A-55 which was temporarily anesthetized by injecting small amounts of Nembutal. More than 100 of mosquitoes were used but only few portions of females engorged. Five days later, 2 mosquitoes were dissected and the formation of oocysts was observed in one female. Seven days later, 3 mosquitoes were examined for oocysts and 2 mosquitoes showed the presence of oocysts. In a similar way, 5 mosquitoes were dissected 11 days later, and this time sporozoites could be detected from salivary glands of 3 mosquitos. Thus, a total of 6 out of 10 mosquitoes dissected were infected with sexual stages of simian malaria parasites. From these evidences summarized in Tables 2, 3 and 4, it is clearly indicated that *A. balabacensis* is the most important vector of simian malaria in Palawan Island.

Table 4. Preliminary experimental infection in *Anopheles balabacensis* after feeding on a "malaria positive" monkey, A-55

| Date             | Days after feeding | Number of mosquitoes |        |            |
|------------------|--------------------|----------------------|--------|------------|
|                  |                    | Dissected            | Oocyst | Sporozoite |
| January 12, 1972 | 0                  | —                    | —      | —          |
| January 17       | 5                  | 2                    | 1      | 0          |
| January 19       | 7                  | 3                    | 2      | 0          |
| January 23       | 11                 | 5                    | 0      | 3          |

## DISCUSSION

As mentioned before, almost all the reports on the simian malaria parasites of the Philippines were based on the isolation or detection of parasites from monkeys sent to other countries from the Philippines. A short report by Howard and Cabrera (1961) was the only exception. In their paper, however, no actual data on the detection of malaria parasites or on details of the captured places of monkeys were given. Therefore, the present paper must be the first published data which give detailed informations on the Philippine simian malaria although the total number of monkeys examined was quite insufficient. The results reported here are also still preliminary because the blood surveys of monkeys were carried out as one of the side works during the period of studies on the human malaria in Palawan Island, the Philippines.

Identification of the parasite species based on the blood smear examination alone under field conditions is generally very difficult and our opinion is that without further studies, including experimental subinoculations of malaria parasites to uninfected monkeys, especially to rhesus monkeys, conclusive diagnosis should be avoided unless the situation is undoubtedly clear. In nature frequency of mixed infections must be much higher than that thought, and this may mislead one's diagnosis. According to Garnham (1966), natural infections of *P. inui* were often confused in the early records with those of *P. cynomolgi* and probably with other



parasites. Before the description as a new species, *P. coatneyi* was also at first mistaken to be *P. knowlesi* (Eyles *et al.*, 1962). In the present studies especially with monkey A-55, cycle of erythrocytic parasites was first attempted to know but no clear cut explanation was obtained probably because of a mixed infection of malaria parasites.

In the case of monkey A-14, parasites morphologically resembling *P. coatneyi* or *P. knowlesi* was involved in addition to *P. inui*. Early schizont of this parasite is characterized by its heavy large grain-shaped malaria pigments. According to description of *P. coatneyi*, however, the parasite is characterized by the presence of predominant ring forms and the absence of schizonts from the peripheral blood. From these situations it is more plausible to assume that the present malaria parasite in question is *P. knowlesi*.

Although some informations about simian malaria parasites of the Philippines have been available in literature as noted above, nothing has been known about the vector of the Philippine simian malaria. Up to date, about 40 species and subspecies of *Anopheles* mosquitoes were recorded in the Philippines (Basio, 1971), of which 7 species have been collected during the period of our surveys in Montible, Palawan, by using baits of various sources, such as monkey, carabao, and human. Among them the following anopheline mosquitoes were attracted to monkeys: *Anopheles balabacensis*, *A. kochi*, *A. vanus*, *A. minimus flavirostris*, *A. maculatus*, and *A. peditaeniatus*.

In other countries, mainly in the United States, some species of anopheline mosquitoes which are known to be distributed in the Philippines were examined experimentally for ability as vectors of simian malaria. The following anopheline species are known to transmit simian malaria or at least to produce sporozoites in salivary glands (for more details, see Coatney *et al.*, 1971):

- Plasmodium cynomolgi*: *Anopheles balabacensis*, *A. kochi*, *A. maculatus*,  
*A. peditaeniatus*, *A. philippinesis*, *A. tessellatus*,  
and *A. vagus*
- Plasmodium inui*: *A. balabacensis*, *A. maculatus*, and  
*A. philippinesis*
- Plasmodium coatneyi*: *A. balabacensis*, *A. maculatus*, *A. kochi*,  
*A. philippinesis*, and *A. vagus*
- Plasmodium knowlesi*: *A. balabacensis* and *A. maculatus*

It should be noticed that all these species are also distributed in Palawan Island, whereas in our preliminary field surveys in Montible the most attracted mosquito species to monkeys was *A. balabacensis* followed by *A. kochi*.

Results of a preliminary experimental infection of *A. balabacensis* after feeding on a "malaria positive" monkey, A-55, also gave us a strong evidence to confirm our finding on natural infections in Palawan. According to Coatney *et al.* (1971), sporozoites of *P. cynomolgi*, *P. coatneyi* and *P. knowlesi* can be seen in salivary glands of *A. balabacensis* 10-11 days at 25°C after blood feeding, whereas in the case of *P. inui* 15 days are necessary to produce sporozoites under a similar condition. Therefore, our own results of the experimental feeding

of *A. balabacensis* on "malaria positive" monkey (as shown in Table 4) were due to other simian malaria parasites than *P. inui* which is most predominant in the survey area. From these evidence, it is obvious that *A. balabacensis* is the most important vector of the Philippine simian malaria. In Palawan Island, *A. balabacensis* rarely comes to bite human to open places where human lives; we have, however, collected two specimens of this mosquito species within a house. Further it should be noticed that one female of *A. balabacensis* attacked one of us (I.M.) in jungle at night. Indeed, this species is an important vector of human malaria in North Borneo, Burma, Assam and Indo-china (Sandosham, 1965) as well as that of simian malaria. In Iwahig, of which territory is vastly extended to jungles of mountain areas, usually colonists working at central areas do not suffer from malaria but colonists working at mountain areas frequently suffer from malaria. Therefore, in deep jungles they might have many chances to be attacked by *A. balabacensis* which had been infected with any simian malaria parasite. Importance of simian malaria to human was noticed by some malariologists after natural and accidental infections of simian malaria parasites to man occurred as well as experimental infections through mosquito bites (Bray, 1963; Coatney *et al.*, 1971). *P. knowlesi* is the easiest simian malaria parasite to infect human (Knowles and Das Gupta, 1932) and more recently natural infection of human was found in Malaya (Yap *et al.*, 1971) and the parasite was at first diagnosed as *P. malariae* by routine microscopic examinations. In the case of *P. cynomolgi*, including its various strains or subspecies, succeeding accidental infections at various laboratories in the United States and England in 1960 are famous and followed by other similar cases in Taiwan and in the United States few years ago (Cross *et al.*, 1973; Most, 1973). One of us also experienced an imported case of human malaria which was suspected to be due to natural infection with a simian malaria parasite (Tsukamoto, 1977). Therefore, the importance of simian malaria to human should not over-looked in the Philippines too, and it should be kept in mind that actual human infection with simian malaria parasites might have occurred much more than those thought.

From the taxonomic viewpoint, several types, either strains or subspecies, of *P. cynomolgi* are known in Asian countries, and hence it is interesting to determine what type of the parasites is involved in the Philippines. In neighbouring areas of the Philippines, for example, the following simian malaria parasites are reported: *P. inui* var. *cyclopis* (= *P. cynomolgi cyclopis*) by Inoki *et al.*, (1942), *Plasmodium knowlesi* var. *arimai* and *Plasmodium taiwanensis* (= *Hepatocystis taiwanensis*) by Yokogawa (1941) from Taiwan; and *Hepatocystis semnopithecii* by Eyles and Warren (1963) from Java. In the present study no typical evidence was available for the presence of the simian malaria parasite of the genus *Hepatocystis*, though from squirrels and fruit bats from the same areas in Palawan *H. vassali* and *H. pteropi* were detected respectively as reported in a previous paper (Miyata and Tsukamoto, 1975). However, this does not mean to deny the presence of *Hepatocystis* malaria parasites of monkeys in the Philippines.

In conclusion, much more detailed studies on simian malaria, as well as human malaria, are still necessary in the Philippines to establish appropriate taxonomic situation and to understand their ecology in relation to malaria eradication of human malaria.

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#### フィリピン・パラワン島の猿マラリアと媒介蚊

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本研究は文部省科学研究費補助金海外学術調査費 (代表者中林敏夫)によるフィリピンでのマラリアの調査研究の一環として行われたものであって、1971年1月および1971年12月から翌年1月までの2回の野外調査の結果をまとめたものである。今迄フィリピンの猿マラリアについては *Plasmodium inui*, *P. cynomolgi*, *P. knowlesi* および *P. coatneyi* の4種が分布するものと云われているが、そのほとんど全部が外国においてフィリピンから送られたカニクイザルからのマラリア原虫の検出と分離を報告したもので、フィリピン国内における調査は簡単な報告が1つあるにすぎない。従って、フィリピン国内で行われた調査研究に関する詳細な報告としてはこれが最初のものである。パラワン島イワヒグ地区のモンテブレ支所付近で厚層および薄層血液標本を作成することのできたカニクイザルは総計20頭で、そのうちの11頭がマラリア原虫を血液中に持っていた (陽性率55%)。これは今迄知られていた記録よりもかなり大きな値である。マラリア原虫としては各種の原虫種の単独感染や混合感染が認められた。またフィリピンの猿マラリアの媒介蚊については今迄全く報告がなかったが、カニクイザル、ヒト、水牛などを餌に用いた誘引採集や、マラリア感染陽性のカニクイザルにおける蚊の実験感染などの結果から、パラワン島では *Anopheles balabacensis* が最も重要な猿マラリアの媒介者であることを示した。この蚊は他の東南アジアの諸国では人間のマラリアの媒介者でもあるので、最近でも世界的に猿マラリアの人体感染例が知られていることに関連して、フィリピンの猿マラリアについての問題点についても論議した。