ARTICLES

STUDIES ON THE COMPARATIVE SUSCEPTIBILITY OF *ANOPHELES FREEBORNI* AITKEN, *A. QUADRIMACULATUS* SAY, *A. LABRANCHIAE ATROPARVUS* THIEL AND *A. SUNDAICUS* RODENWALDT TO *PLASMODIUM GONDERI* WITH A REPORT OF TRANSMISSION BY *A. FREEBORNI*

WILLIAM E. COLLINS, FRANCES E. JONES, CHARLES G. DOBROVOLNY [1] AND HARVEY AKINS [1]

National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Parasite Chemotherapy, Section on Cytology, P.O. Box 190, Chamblee, Georgia

The malaria parasite *Plasmodium gonderi* naturally infects monkeys of Africa and was first seen by Gonder and Barenberg-Gossler (1909) in the blood of a mangabey (*Cerocebus fuliginosus*) and described under the name of *P. kochi*. Rodhain and van den Berghe (1936) renamed the parasite *P. gonderi* and showed that it had tertian periodicity.

The infection was first transmitted by mosquito bite to a rhesus monkey by Rodhain and van Hoff (1940) using *A. maculipennis*. Garnham (1957) reported the infection of *A. maculipennis* and *A. stephensi* but had 13 unsuccessful transmission attempts. Garnham *et al.* (1958) were able to infect *A. aztecus* and *A. maculipennis* var. *atroparvus* on a strain of *P. gonderi* recently isolated from a drill (*Mandrillus leucophaeus*). Viable sporozoites were produced in both species of mosquitoes and were found in the salivary glands 9 days after the blood meal. *Anopheles aztecus* appeared to be the more susceptible species. The infection was readily transmitted to rhesus monkeys by the intravenous inoculation of infected salivary glands.

Reported here are results of attempts to infect four species of *Anopheles* with *P. gonderi* and to transmit the parasite through *A. freeborni* from monkey to monkey.

MATERIALS AND METHODS. Fresh parasitized blood received in 1959 from Professor P. C. C. Garnham, (London School of Hygiene and Tropical Medicine) was used to establish the parasite in the *Macaca mulatta* monkey in our laboratory. It was maintained in this species and was the source of *P. gonderi* for transmission studies during the subsequent four years.

The *A. quadrimaculatus* Say (Q-1 strain), originally from Southeastern United States, has been maintained in the laboratory since 1941, and the *A. freeborni* Aitken (F-1 strain) from Marysville, California, since 1944. The *A. sundaicus* Thiel, originally from Java, and the *A. labranchiae atroparvus* Rodenwaldt from England, have been in our insectary since 1962 and 1963 respectively, when they were obtained from the London School of Hygiene and Tropical Medicine, London, England, through the courtesy of Mr. G. Davidson.

Mosquitoes were allowed to feed through a screened cage directly on the monkey and were then held in the insectary (78 to 80° F.) receiving daily feedings of 5 percent Karo syrup in a cellulose pledget. Dissections for oocyst counts were made beginning on the 6th day after infection, and examinations of salivary glands for the presence of sporozoites were initiated the 11th day after infection.

RESULTS. *Infectivity Studies.* Mosquito infectivity studies were made during the

[1] Present address: Communicable Disease Center, 1600 Clifton Road, N.E., Atlanta, Georgia 30333.

last four years in connection with efforts to transmit *Plasmodium gonderi* to humans. During this period a total of 27 lots of *A. quadrimaculatus* were fed on five monkeys infected with *P. gonderi;* the results of these feedings are shown in Table 1. Of these lots, 17 (63 percent) demonstrated that sporozoites were present in the salivary glands of 31 of 72 mosquitoes between 11 and 16 days after the infective blood meal.

Preliminary comparative infectivity feedings between *A. quadrimaculatus* and *A. freeborni* were done on seven

TABLE 1.—Results of mosquito infectivity studies with *Plasmodium gonderi* using *Anopheles quadrimaculatus* mosquitoes.

Monkey no.	Day of parasitemia	Oocysts on gut			Glands pos./no. dissected
		Pos. guts/no. dis.	Percent inf.	Ave. no. oocysts per gut	
C-86	10	6/8	75	125	5/10
	11	5/5	100	20.2	..
	12	7/15	47	3.6	..
	17	4/10	40	1.5	..
	19	4/15	27	5.0	..
	27	7/10	70	4.5	..
	28	6/10	60	2.9	..
	34	7/7	100	35.7	5/7
	49	3/5	60	21.6	0/18
	50	3/5	60	3.8	0/5
S-230	8	13/25	52	4.0	..
	9	2/10	20	0.5	..
	10	1/10	10	0.6	..
B-767	9	0/8	..	..	..
	14	1/15	7	0.1	..
C-828	6	5/5	100	200	10/14
	7	2/5	40	8.4	0/5
C-868	19	7/10	70	14.0	2/13

subsequently proved to be infected as determined by the presence of oocysts on the gut. Average densities of as high as 200 oocysts per gut were obtained. Salivary glands examined from 7 lots of *A. quadrimaculatus* showed that 22 of 72 mosquitoes contained sporozoites between 11 and 16 days after the infective blood meal.

A total of 12 of 15 lots of *A. freeborni* were infected with *P. gonderi.* The results of these feedings are shown in Table 2. Mosquitoes became infected in this case when allowed to feed from 6 to 34 days after parasites were found in the monkey's blood. Densities as high as 125 oocysts per gut were obtained. The examination of 7 lots of *A. freeborni* occasions. The *A. freeborni* had an infection rate of 44.8 percent (26 of 58 mosquitoes) and a gut infection index (average number of oocysts per 100 guts) of 2836. The *A. quadrimaculatus* had 40 percent infection (26 of 65 mosquitoes) with a gut infection index of 2095. It appeared that if any difference in susceptibility to *P. gonderi* existed between these two mosquitoes the *A. freeborni* was the more susceptible.

To study further the comparative susceptibility of mosquitoes to *P. gonderi* infection, an additional series of feedings was made on an infected monkey (C-139). The *Macaca mulatta* monkey (C-139) used for these comparative infectivity studies, was inoculated intravenously with

TABLE 2.—Results of mosquito infectivity studies with *Plasmodium gonderi* using *A. freeborni* mosquitoes.

Monkey no.	Day of parasitemia	Oocysts on gut: Pos. guts/no. dis.	Oocysts on gut: Percent inf.	Oocysts on gut: Ave. no. oocysts per gut	Glands pos./no. dissected
C-86	12	2/5	40	1.6	..
	17	9/10	90	13.6	3/9
	34	4/5	80	40.6	3/7
A-690	14	8/11	73	62.8	..
	29	17/24	73	12.7	4/13
A-689	12	7/13	54	16.8	..
W-756	19	5/9	56	11.6	6/15
	20	7/10	70	7.9	3/9
B-767	9	1/14	7	0.3	..
	14	1/15	7	0.2	..
C-828	6	5/5	100	125.4	10/14
	7	4/4	100	166	2/5

fresh parasitized blood. The prepatent period was 8 days and the mosquito feedings commenced 12 days after inoculation. The results of these feedings are shown in Table 3. The *A. freeborni* and *A. quadrimaculatus* were fed on 12 different days; the *A. labranchiae atroparvus* and *A. sundaicus* on seven of these days. The number of oocysts found in the *A. freeborni* was approximately 2.9 times that found in the *A. quadrimaculatus*. The *A. quadrimaculatus* had approximately 3 times as many oocysts as were found on the *A. labranchiae atroparvus* and approximately 10 times the number on the *A. sundaicus*. The susceptibility of these four species, based on oocyst counts alone, can therefore be arranged as follows: *A. freeborni* >*A. quadrimaculatus* >*A. labranchiae atroparvus* >*A. sundaicus*.

Our previous studies had shown that *P. gonderi* sporozoites were produced in *A. freeborni* and *A. quadrimaculatus*. However, in the present trials, by the 15th day many of the oocysts had failed to develop to maturity and appeared to have degenerated. The reason for the failure

TABLE 3.—Summary of comparative infectivity feedings with *Plasmodium gonderi* using *Anopheles freeborni, A. quadrimaculatus, A. labranchiae atroparvus* and *A. sundaicus*. (*A. quadrimaculatus* used as a standard=100).

Mosquito species comparison	No. of feedings	Oocysts on gut: Pos. guts/no. dis.	Oocysts on gut: Percent inf.	GII*	GII ratio**
A. freeborni	12	206/300	69	1451	289
A. quadrimaculatus	12	115/300	38	502	100
A. freeborni	7	122/175	70	1043	438
A. quadrimaculatus	7	64/175	37	238	100
A. l. atroparvus	7	28/200	14	74	31
A. freeborni	7	113/175	65	1164	337
A. quadrimaculatus	7	48/175	27	345	100
A. sundaicus	7	13/143	9	36	10

* GII=Gut infection index (average number of oocysts per 100 guts).
** GII ratio=ratio of gut infection indexes using *A. quadrimaculatus* as the standard=100.

of the oocysts to complete their development is not known. Sporozoites were found in the salivary glands of the *A. freeborni* mosquitoes only. None of a total of 56 salivary glands examined from *A. quadrimaculatus* was shown to contain sporozoites and the number of oocysts found in *A. labranchiae atroparvus* and *A. sundaicus* were too low to warrant examination of the salivary glands for sporozoites. It had previously been reported by Garnham *et al.* (1958) that sporozoites were produced in *A. maculipennis* var. *atroparvus,* but in limited numbers.

Transmission Studies. For the transmission attempts, a *Macaca mulatta* monkey (W-756) was inoculated with 2.5 ml. of parasitized blood, and a parasitemia was seen daily thereafter in this monkey. The peak parasitemia (254,946/mm^3) was on the 8th day of patency, and mosquitoes were fed on the 19th and 20th days after inoculation when the parasitemia was approaching a second peak.

On day 19, when 8 male and 52 female gametocytes per WBC could be found, *A. freeborni* mosquitoes were allowed to feed. The infection rate for this lot of mosquitoes was 56 percent with a gut infection index of 1156. After 16 days of extrinsic incubation, and when sporozoites were abundant in the salivary glands, 75 of these mosquitoes were fed on *M. mulatta* A-690. Monkey A-690 developed an infection after a prepatent period of 13 days.

On day 20, *A. freeborni* mosquitoes were again fed on monkey W-756. The gametocyte count was 5 males and 17 females per 50 WBC. The infection rate was 70 percent with a gut infection index of 790 and after 15 days of extrinsic incubation, approximately 200 mosquitoes were fed on *M. mulatta* monkey A-689. The prepatent period for this monkey was 14 days.

DISCUSSION. From these studies, it appears that both *A. freeborni* and *A. quadrimaculatus* are susceptible to infection by *P. gonderi* to the extent that sporozoites can be subsequently found in the salivary glands. These two species can therefore be considered potential experimental vectors of *P. gonderi.*

Transmission was in fact obtained on two occasions using *A. freeborni.* This is the third species of simian malaria reported to be transmissible by *A. freeborni,* the others being *P. cynomolgi* (Eyles 1960a and 1960b), and *P. brasilianum* (Contacos *et al.,* 1963). No attempts were made to transmit the infection using *A. quadrimaculatus,* but the presence of sporozoites in the salivary glands on a number of occasions would indicate its potential as a vector.

It is of interest that *Anopheles aztecus, A. freeborni, A. labranchiae atroparvus* and *A. quadrimaculatus* all belong to the subgenus *Anopheles* (*Anopheles*) whereas *A. stephensi* and *A. sundaicus* belong to the subgenus *Anopheles* (*Myzomyia*). Quite possibly, the members of the subgenus *Anopheles* (*Anopheles*) are better able to transmit *P. gonderi* than are members of the subgenus *Anopheles* (*Myzomyia*). Further studies along these lines would appear indicated.

SUMMARY. It has been shown that *Anopheles freeborni, A. quadrimaculatus, A. labranchiae atroparvus,* and *A. sundaicus* are susceptible to infection by *Plasmodium gonderi.* Sporozoites have been demonstrated in the salivary glands of the first two species and transmission of *P. gonderi* to monkeys has been accomplished on two occasions using *A. freeborni* mosquitoes.

ACKNOWLEDGMENTS. The authors wish to acknowledge the technical assistance of Miss Peggy Stanfill, Mr. Ernest Guy and Mr. Thomas Kearse, Biological Laboratory Technicians.

References

CONTACOS, P. G., LUND, J. S., COATNEY, G. R., KILPATRCK, J. W., and JONES, F. E. 1963. Quartan-type malaria parasites of new world monkeys transmissible to man. Science 142:676.

EYLES, D. E. 1960a. *Anopheles freeborni*

and *Anopheles quadrimaculatus* as experimental vectors of *Plasmodium cynomolgi* and *P. inui*. J. Parasit. 46:540.

———. 1960b. The exo-erythrocytic cycle of *Plasmodium cynomolgi* and *P. cynomolgi bastianellii* in the rhesus monkey. Am. J. Trop. Med. Hyg. 9:543–555.

GARNHAM, P. C. C. 1957. Biological aspects of the transmission of disease. Edinburgh and London: Oliver and Boyd, 103–104.

———, LAINSON, R., and COOPER, W. 1958. The complete life cycle of a new strain of *Plasmodium gonderi* from the drill (*Mandrillus leucophaeus*) including its sporogony in *Anopheles aztecus* and its pre-erythrocytic schizogony in the rhesus monkey. Trans. Roy. Soc. Trop. Med. and Hyg., 52(6):509–517.

GONDER, R., and BERENBERG-GOSSLER, H. VON. 1909. Malaria. 1:47.

RODHAIN, J., and VAN HOFF, T. 1940. Contribution à l'étude des plasmodiums des singes africains. Le compartement different des *Pl. gonderi* et *Pl. kochi* chez les moustiques. Bull. Soc. Path. Exot. 33:107–113.

———, and VAN DEN BERGHE, L. 1936. Contribution à l'étude des plasmodiums des singes africains. Ann. Soc. Belge Med. Trop. 16:521–531.

PRELIMINARY OBSERVATIONS OF FALL SWARMS OF *CULEX PIPIENS* L.[1]

WILLIAM C. FROHNE[2]

INTRODUCTION. This paper is a discussion of the natural history of male swarming of *Culex pipiens* L. in autumn in northern Ohio during and after the fall of the leaves of deciduous trees. This is a season of transition: temperatures are dropping, windiness increasing, mosquito breeding waning; the females are retiring into hibernation and the males inevitably aging as winter sets in. What happens from day to day at male swarming sites? Indeed, is the swarming phenomenon a purely summer exuberance to which full stop is put by autumn's frost, fall of the leaves, and windy threats of winter? For a period of 64 days, September 25–November 27, 1963, the swarming behavior of this species was kept under surveillance in an area of its abundance. More than a thousand swarms were looked at superficially and certain representative swarming sites were observed intensively, usually for 15 or 30 minutes of swarming. To answer, if only sketchily, what may be seen taking place at the swarming sites, I checked the size of swarms and the shifting locations of big aggregations relative to wind and sun and especially to loss of leaves of trees. I determined that mating goes on about as late in the season as swarming itself and noted such male habits at swarms as interference with mating pairs by supernumerary males, and grappling between males.

Swarming of *C. pipiens,* one of the best known mosquitoes, is a phenomenon reported of old from Europe and in the classical American mosquito literature, e.g., Howard, Dyar & Knab (1912–1917). Still, even in our day, as the recent invaluable review of mosquito swarming literature for which we are indebted to Nielsen & Haeger (1960) demonstrates, there has been no great leap forward to understanding the significance of the swarming habit. Chiang (1963), hinting we need more facts and less speculation, complains justifiably that public health students of crepuscular Diptera ignore the diurnal swarming of other than blood-sucking Nematocera. Drs.

[1] Presented at the annual meeting of the American Mosquito Control Association, March 1–5, 1964, Chicago, Illinois.

[2] Formerly Director of Laboratories, Lake Erie Field Station, Cleveland, Ohio, Great Lakes-Illinois River Basins Project, Division of Water Supply & Pollution Control, Public Health Service, Department of Health, Education, and Welfare, Region V, Chicago, Illinois, now at Alaska Methodist University, Anchorage, Alaska.