Mosquito Infection Studies with *Aotus* Monkeys and Humans Infected with the Chesson Strain of *Plasmodium vivax*

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Abstract. Oocyst counts were compared between mosquitoes that fed on humans versus mosquitoes that fed on *Aotus* monkeys, both of which were infected with the Chesson strain of *Plasmodium vivax*. Oocyst counts obtained from mosquitoes fed on humans were almost 10-fold higher in number. Mosquitoes were more likely to be infected and with a higher rate of infection when they fed on monkeys before the peak in the asexual parasite count. Mosquitoes that fed on humans were more likely to be more heavily infected when fed after the peak in the asexual count. Of several species of owl monkeys, *Aotus vociferans* was infected at a higher frequency. On the basis of oocyst counts, *Anopheles dirus* were the most susceptible and *An. maculatus* were the least susceptible of the mosquito species tested.

INTRODUCTION

In 2009,¹ we described a comparison of parasite levels that can occur between four different species of South American *Aotus* monkeys (*A. lemurinus griseimembra*, *A. nancymae*, *A. vociferans*, and *A. azarae boliviensis*) after infection with the Chesson strain of *Plasmodium vivax*, and compared these levels with those that occurred in humans as obtained from archived records. The number of humans and monkeys that supported the infection of mosquitoes, the different species of mosquitoes that were fed, and the species of mosquitoes that were used for transmission were presented. It was suggested that splenectomized *Aotus* monkeys, particularly, *A. lemurinus griseimembra*, could mimic the course of the Chesson strain of *P. vivax* malaria in humans regarding parasitemia and infectivity to mosquitoes.

Subsequently, studies were made to determine when during infection in monkeys and humans infectivity to mosquitoes was more likely to occur, and to compare the results of the monkey and the human studies. In addition, further observations were made on the comparative receptivity of the available laboratory host mosquitoes for the Chesson strain of *P. vivax*. We present the results of these studies.

MATERIALS AND METHODS

Mosquitoes. *Anopheles freeborni* (F-1 strain originally from California), *An. dirus* (from Thailand), *An. stephensi* (from Delhi, India), *An. maculatus* (from Malaysia), *An. culicifacies* (from India), *An. gambiae* (from The Gambia), and *An. quadrimaculatus* (from the southeastern United States) were laboratory-reared and maintained at the insectaries at the Division of Parasitic Diseases, Centers for Disease Control and Prevention in Chamblee, Georgia.

During periods of highest asexual parasite count, mosquitoes of different species were allowed to feed on tranquilized monkeys. Mosquitoes were contained in pint-sized ice cream

carton cages and allowed to feed directly through netting upon the shaved belly of the animal until engorged to repletion. After feeding, mosquitoes were held in an incubator at 25°C until examined one week later for oocysts on their midguts. If oocysts were present, mosquitoes were kept until sporozoites were present in the salivary glands, usually after 14 or more days of extrinsic incubation. The results presented are based on the oocyst examinations.

All feedings for the human studies were made with laboratory-reared *An. quadrimaculatus* mosquitoes either in the U.S. Public Health Laboratories in Columbia, South Carolina, or in Milledgeville, Georgia, during 1946–1963. After feeding on the patients, the mosquitoes were held under similar condition during the extrinsic incubation period.

Monkeys. *Aotus lemurinus griseimembra* and *A. azarae boliviensis* were wild caught and imported from Colombia and Bolivia, respectively, during periods when export was allowed from those countries. After the ban on exportation by these two countries, animals were vivarium born. *Aotus nancymae* and *A. vociferans* monkeys were largely imported from Peru, and a limited number of these animals were vivarium born.

Upon arrival at the facility, all animals were quarantined for a two-month conditioning period, weighed, and tested for tuberculosis. Parasitologic and serologic examination indicated that the animals were free of infection with malaria parasites before inoculation with the Chesson strain, although some of the animals had been previously infected with other species and strains of *Plasmodium*. All monkeys were splenectomized before or during exposure. All surgery was performed in an Association for the Assessment and Accreditation of Laboratory Animal Care, International, Inc., approved surgical suite appropriate for aseptic surgery. Protocols were reviewed and approved by the Centers for Disease Control and Prevention Institutional Animal Care and Use Committee, in accordance with procedures described in the 1986 U.S. Public Health Policy.

Monkeys generally were pair-housed. In some cases, monkeys were single-housed to avoid injuries caused by fighting with cage mates. Space recommendations for laboratory animals were followed as set forth in the NIH *Guide for the Care and Use of Laboratory Animals*. All animals were fed a diet that has been proven to provide adequate nutrition and calories to captive *Aotus* monkeys used in malaria-related research.

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Feed was free of contaminants and freshly prepared. Daily observations of the animals' behavior, appetite, feces, and condition were recorded. Any medical conditions were treated by an attending veterinarian as needed.

Infections were initiated either through the inoculation of sporozoites or of cryopreserved or fresh infected blood intravenously via the femoral vein. Blood-stage parasitemia was monitored and quantified by the daily examination of Giemsa-stained thick and thin blood films by the method of Earle and Perez.²

Human patient management. Consent for any necessary patient treatment was granted by the families of the patients or by the courts when patients were admitted to the hospitals. The decision to infect a neurosyphilitic patient with malaria was made as part of standard patient care by the medical staff of the hospital. Patient care and evaluation of clinical endpoints (e.g., fever) were the responsibility of the medical staff. As reported,³ during infection, hospital personnel routinely monitored the temperature, pulse, and respiration every four hours and during paroxysms (fevers), every hour. During paroxysms, patients were treated symptomatically.

Patients were infected with the Chesson strain of *P. vivax* either by sporozoites or the inoculation of infectious blood from a donor patient.¹ The U.S. Public Health Service personnel provided the parasites for inoculation, monitored the daily parasite counts to determine the course of infection, provided the mosquitoes to be fed on the patients, and performed mosquito dissections and examinations. All patients undergoing malaria therapy were confined in screened wards of the hospital to prevent possible infection of local anopheline mosquitoes.

Treatment. Infections were terminated at the direction of the attending physician. Infections were terminated by treatment with various drugs such as chloroquine, pyrimethamine, quinine, chlorguanide, and primaquine, depending on the route of inoculation.

RESULTS

Owl monkeys. Gametocytes were rarely seen in these studies and feedings were generally performed over many days blindly to increase the prospects of getting infection. For all species of *Aotus* monkeys, during the period between days -12 and +12 of the peak asexual parasite count, no male gametocytes were observed on 65.0% of the 726 days on which gametocyte counts were made. Of the 254 male gametocyte positive days, 138 (54.3%) occurred on day 0 or before and 116 (45.7%) occurred after day 0. The median male gametocyte count before day 0 varied from 30 to 300/ μ L; afterwards, it varied from 60 to 240/ μ L.

Aotus lemurinus griseimembra. Six different species of mosquitoes were fed on 27 *A. l. griseimembra* monkeys infected with Chesson strain of *P. vivax* (Table 1). The median maximum asexual parasite count was 27,100/ μ L. Of the 1,787 lots of mosquitoes fed, 766 (42.9%) were positive for oocysts. For all species of *Anopheles*, 4,157 (8.5%) of the 48,941 mosquitoes dissected and examined were positive.

Aotus azarae boliviensis. Four species of mosquitoes were fed on 15 *A. a. boliviensis* monkeys (Table 1). The median maximum asexual parasite count was 9,299/ μ L. Of the 469 lots of mosquitoes fed, 82 (17.5%) were positive for oocysts. For all species, 205 (1.9%) of the 10,527 mosquitoes dissected and examined were positive. The percentages infected were con-

siderably lower than seen for feedings with *A. l. griseimembra*. In addition, fewer mosquitoes were fed on these animals because, as indicated in the previous report,¹ lower parasite counts occurred in this host and there were fewer indications for mosquito infection.

Aotus nancymae. Five species of mosquitoes were fed on 18 *A. nancymae* monkeys (Table 1). The median maximum asexual parasite count was 12,462/ μ L. Of the 374 lots of mosquitoes fed, 125 (33.4%) were positive. For all species of *Anopheles*, 496 (7.0%) of the 7,120 dissected and examined were positive. This value was almost as high as was obtained with *A. l. griseimembra* monkeys with regard to individual mosquito dissection. However, asexual parasite counts were generally lower and mosquitoes were fed on fewer occasions (similar to that of *A. a. boliviensis*).

Aotus vociferans. Four species of mosquitoes were fed on 8 *A. vociferans* monkeys (Table 1). The median asexual parasite count was 21,118/ μ L. Of the 41 lots of mosquitoes fed, 24 (58.5%) were infected. For all species of *Anopheles*, 168 (19.6%) of the 857 dissected and examined were positive. Although this finding is based on limited numbers, of the four species of monkeys infected with the Chesson strain of *P. vivax*, infections in *A. l. griseimembra* and *A. vociferans* appeared to be the best at infecting mosquitoes. The median asexual parasite counts were also higher than in the other monkeys.

An examination of mosquito infection patterns showed that they were associated with peaks in the asexual parasite count. Of the 473 infections in *An. freeborni* fed on *Aotus* monkeys, 384 (81.2%) were obtained during the 12-day period preceding or over the 12-day period after the peak asexual parasite counts. These 384 infections were plotted in relation to the peak in the asexual parasite count (Figure 1). There were 358 negative feedings during this 25-day period; 130 of these negative lots were fed during the 12 days before the peak during which there were also 212 infected lots (infection rate = 62.0%). For the 12-day period after the peak, there were 214 negative lots and 144 infected lots (infection rate = 40.2%) for that 12-day period. The average number of oocysts per positive gut during the period before the peak day was 3.61. On the day of maximum asexual parasite count, the average number of oocysts per positive gut was 2.96. The average number of oocysts per positive gut for the 12-day period after the peak was 2.33. More infectivity, as judged by a higher infection rate, and a greater intensity of infection (oocysts per gut) occurred before the observed peak in the asexual parasite count.

An examination was made of the comparative intensity in infection of the four most frequently fed mosquitoes (*An. freeborni*, *An. maculates*, *An. stephensi*, and *An. dirus*) based on the average number of oocysts per positive gut. Based on 115 comparative feedings, *An. freeborni* had an average of 3.76 oocysts per positive gut versus 2.11 oocysts per positive gut for *An. maculatus* (ratio = 100:56.1). Based on 49 comparative feedings between *An. freeborni* and *An. stephensi*, the mean values were 4.71 versus 4.51 oocysts per positive gut (ratio = 100:96.0). Based on 68 comparative feedings between *An. freeborni* and *An. dirus*, the mean numbers of oocysts per positive gut were 4.01 and 5.51 (ratio = 100:137.4). It was apparent that in general, *An. dirus* were most heavily infected and the *An. maculatus* the least heavily infected when compared with the standard *An. freeborni*. A pooled *t*-test was used to compare the oocyst means for each of the species pairs. There was no statistical difference in any of the paired

TABLE 1

Infection of different species of *Anopheles* mosquitoes on monkeys and humans infected with the Chesson strains of *Plasmodium vivax*

Host	No.	Mosquito	No. lots positive/no. fed	% Positive	No. mosquitoes positive/no. examined	% Positive
<i>Aotus l. griseimembra</i>	27	<i>An. freeborni</i>	413/965	42.80	2,095/21,155	9.90
		<i>An. dirus</i>	104/206	50.49	736/3,988	18.46
		<i>An. maculatus</i>	144/420	34.29	629/19,750	3.18
		<i>An. stephensi</i>	69/120	57.50	404/2,396	16.86
		<i>An. quadrimaculatus</i>	17/48	35.42	96/1,130	8.50
<i>A. a. boliviensis</i>	15	<i>An. culicifacies</i>	19/28	67.86	195/522	37.36
		<i>An. freeborni</i>	19/111	17.12	48/2,386	2.01
		<i>An. dirus</i>	18/91	19.78	47/2,162	2.17
		<i>An. maculatus</i>	1/11	9.09	1/200	0.50
		<i>An. stephensi</i>	44/256	17.19	109/5,779	1.89
<i>A. nancymae</i>	18	<i>An. freeborni</i>	35/153	22.88	124/3,148	3.94
		<i>An. dirus</i>	68/156	43.59	317/2,752	11.52
		<i>An. maculatus</i>	0/7	0.00	0/142	0.00
		<i>An. stephensi</i>	18/42	42.86	44/801	5.49
		<i>An. gambiae</i>	4/16	25.00	11/277	3.97
<i>A. vociferans</i>	8	<i>An. freeborni</i>	6/12	50.00	33/251	13.15
		<i>An. dirus</i>	7/12	58.33	74/222	33.33
		<i>An. stephensi</i>	5/8	62.50	19/189	10.05
		<i>An. gambiae</i>	6/9	66.67	42/195	21.54
Totals	68	<i>An. freeborni</i>	473/1,241	38.11	2,300/26,940	8.54
		<i>An. dirus</i>	197/465	42.37	1,174/9,124	12.87
		<i>An. maculatus</i>	145/438	33.11	630/20,092	3.14
		<i>An. stephensi</i>	136/426	31.92	576/9,165	6.26
		<i>An. gambiae</i>	10/25	40.00	53/472	11.23
		<i>An. quadrimaculatus</i>	17/48	35.42	98/1,130	0.71
		<i>An. culicifacies</i>	19/28	67.86	195/522	37.36
		<i>An. quadrimaculatus</i>	211/244	86.48	1,865/3,764	49.55

oocysts counts except for *An. freeborni* versus *An. maculatus* ($P < 0.0001$).

Only nine comparative feedings were conducted between *An. freeborni* and *An. quadrimaculatus*. The mean numbers were 3.66 versus 3.03 (ratio = 100:82.7). Thus, the high oocyst counts obtained in the human studies may have been even higher if *An. freeborni* had been available. *Anopheles culicifacies* and *An. gambiae* were not fed comparatively with *An. freeborni*.

Humans. *Anopheles quadrimaculatus* mosquitoes were fed on 47 patients infected with the Chesson strain (Table 1). The median asexual parasite count was 22,065/ μ L. Of the 244 lots examined, 211 (86.5%) were infected. Of the 3,764 mosquitoes dissected and examined, 1,865 were shown to have oocysts; average of 70.11 oocysts per positive gut. Of the 211 positive lots, 166 occurred during the period of 8 days before ($n = 37$) and 12 days after ($n = 110$) the peak in the asexual parasite

FIGURE 1. Lots of *Anopheles freeborni* mosquitoes infected and the average number of oocysts per positive gut before and after the peak in the asexual parasite count when fed on *Aotus* monkeys infected with the Chesson strain of *Plasmodium vivax*. Pos. Lots = positive lots.

FIGURE 2. Lots of *Anopheles quadrimaculatus* mosquitoes infected and the average number of oocysts per positive gut before and after the peak in the asexual parasite count when fed on humans infected with the Chesson strain of *Plasmodium vivax*. Infect. = infection.

count (Figure 2). Forty-seven (22.3%) positive lots occurred during the 8 days before the peak parasite count, and 140 lots were positive (66.3%) after the day of peak parasite count. Thus, in contrast to the findings with the monkey feedings, the highest number of lots infected and the greater number of oocysts per positive gut were obtained after the peak asexual parasite count. The overall intensity of mosquito infection was markedly higher with humans than seen in any of the monkey species fed on by any of the *Anopheles* species.

An examination showed that male gametocyte counts recorded for humans were much higher than those recorded for *Aotus* monkeys. Between day -8 and day +12, there were 387 recordings of male gametocytes and no males were observed on 63 (16.3%) of the days; 32 (25%) of 128 in the period from day -8 through day 0, and 31 of 259 in the period from day +1 through day +12. The mean male gametocyte counts ranged from 13.1 to 111.3/100 leukocytes.

DISCUSSION

The Chesson strain is one of the classic parasites that has been studied for many years in humans and during the past several decades in monkeys and chimpanzees.^{1,4-15} During recent times, many isolates of this parasite have been stored frozen and subsequently passed through humans, monkeys, and chimpanzees. Whether it is now the same parasite as was studied in humans during the 1940s through 1960s is questionable. A molecular examination of preserved human material from that early era would indicate whether the present day parasites are similar to or the same as the one used in the early human studies. At the present time, we assume that we are working with Chesson stain of *P. vivax* based only on its genealogy.

It was apparent from an examination of the data from feeding on infected *Aotus* monkeys that mosquito infection most often occurred before peaks in the asexual blood parasite count.

Gametocytes were rarely observed on the blood films and could not be used as predictors of mosquito infection or the intensity of infection. In the monkeys, higher levels of infection occurred in the period preceding the peak in the asexual parasite count than occurred after the peak (Figure 1). This finding contrasts with observations from archival data from human studies (Figure 2), in which a higher percentage of mosquito infection occurred after the peak in the asexual blood parasite count.

In the human studies, the intensity of mosquito infection, (based on oocysts count) was at least 10-fold higher than that obtained by feeding on monkeys. In humans, gametocytes were often observed, although the number of gametocytes present was not always a predictor of the number of oocysts that would be found in the mosquitoes. This high level of infection had also been observed in previous studies with *P. vivax* in chimpanzees.⁴ It is apparent that splenectomized *Aotus* monkeys do not support the production of infective gametocytes at the same high density levels as do humans and splenectomized chimpanzees. In addition, it appears that in *Aotus* monkeys, mosquito infection decreases once the peak in the asexual parasite count is reached, whereas in humans, mosquito infection appears to continue or increase beyond the peak in the asexual parasite count.

The *Aotus* monkeys, particularly splenectomized *A. lemurinus griseimembra*, mimic the human host for studies with the Chesson strain of *P. vivax* with regard to asexual parasitemia, susceptibility to infection by sporozoite inoculation, and recrudescences. However, these animals are apparently unable to produce infective gametocytes at levels approaching that of humans, which results in a much reduced level in the intensity of mosquito infection (oocysts per positive gut). In particular, microgametocytes are rarely seen during *P. vivax* infections in *Aotus* monkeys. Therefore, experimental feeding must be scheduled in relation to the anticipated peak in the asexual

parasite count. The frequency of infection is reduced or aborted soon after the peak in the asexual count. This finding contrasts with archival data from human studies in which mosquito infection continues after the peak in the asexual parasite count.

This reduced intensity of mosquito infection and the limitation in periods of infectivity define the usefulness of the *Aotus* monkeys for studies with the Chesson strain where mosquito infection is a vital component. Currently, only *A. nancymae* and *A. vociferans* are available in sufficient numbers for extensive studies. The higher median asexual parasite counts that were obtained in the *A. vociferans* and the higher level mosquito infection over that of *A. nancymae* indicates that *A. vociferans* would be the preferred host for studies with this parasite. Of the laboratory mosquitoes tested for susceptibility to infection with Chesson strain, *An. dirus* was the most heavily infected. Thus, at the present time, studies with Chesson strain could best be conducted using *A. vociferans* monkeys and *An. dirus* as the vector mosquito.

Received April 26, 2011. Accepted for publication December 9, 2011.

Acknowledgments: We thank the staff of the Animal Resources Branch, the National Center for Infectious Diseases, for the care of the animals.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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