MOSQUITO SIZE AND MULTIPLE TRANSMISSION OF AVIAN MALARIA IN THE LABORATORY[1]

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ABSTRACT. Probing mosquitoes salivate before ingesting blood, and malaria sporozoites are transmitted during this phase of feeding. Large and small *Aedes aegypti* infected with *Plasmodium gallinaceum* were allowed to probe briefly on a series of 3 naive chicks. Large mosquitoes were more infective overall, but there was no difference in the ability of either size class to infect the first host. Large mosquitoes were more likely than small mosquitoes to infect more than one host during serial feeding.

INTRODUCTION

Larval temperature, crowding and nutrition can affect the body size of adult mosquitoes (Hare and Nasci 1986). Female body size has been shown to affect survival and viral infection rate (DeFoliart et al. 1987, Paulson and Hawley 1991) but not the transmission rate of mosquito-borne protozoan parasites. Salivary gland size and body size appear to be positively correlated (Mellink et al. 1982), and the number of malaria sporozoites available for transmission during blood feeding may depend on salivary gland size (Janzen and Wright 1971). The number of sporozoites injected into the host is important because it directly affects the course and severity of the resulting infection (James et al. 1936).

Previously, we demonstrated that malaria-infected *Aedes aegypti* (Linn.) can infect up to 3 hosts during rapid serial feeding (Kelly and Edman 1992). The objective of this study was to compare the ability of both large- and small-bodied *Ae. aegypti* to serially infect multiple hosts with avian malaria.

MATERIALS AND METHODS

Aedes aegypti Rockefeller strain, an efficient vector of avian malaria (Huff and Coulston 1944), was used in this study. Mosquitoes were maintained at 26 ± 0.25°C, 85 ± 5% RH and a 14:10 (L:D) photoperiod. Larvae were reared on 2 different regimes in order to produce 2 different size classes of adult mosquitoes. One day after hatching, larvae were placed into 30 × 25 × 5 mm white plastic trays containing 1.5 liters of distilled water. For regime 1 (large adults) and regime 2 (small adults), respectively, 200 larvae and 500 larvae were placed in each tray. Larvae were fed a diet of lactalbumin and brewer's yeast (1:1) according to the schedules in Table 1. Pupae from each regime were placed into water-filled containers within separate cages and allowed to emerge. Females and males were housed together to ensure that mating occurred, and were provided a 10% sucrose solution.

Adult size was estimated using wing length measurements taken from the axillary incision to the apical margin, not including the fringe of scales (Anderson 1989).[2] The right wing of each female mosquito was removed, placed on a slide, covered with a cover slip, and measured at 60× on a dissecting microscope equipped with an ocular micrometer. The mean wing length of large and small mosquitoes was determined for each experimental group. Mean wing lengths were tested for significance by analysis of variance (ANOVA). The mean wing length for the large mosquitoes was 3.07 ± 0.36 mm and for the small mosquitoes was 2.60 ± 0.27 mm.

Plasmodium gallinaceum was obtained from the American Type Culture Collection (lot #30218). Blood (0.5 ml) from this stock culture was injected intravenously into 1-wk-old chicks. Host parasitemia was determined by examination of Giemsa-stained thin blood smears. Five-day-old mosquitoes that were not previously blood fed were fed on infected chicks just prior to peak gametocyte production, which is approximately 2–4 days after gametocyte production is first observed. Mosquitoes generally became infective (i.e., sporozoites were seen in the salivary glands) within 12 days after bloodfeeding on an infected chick. Twelve days after an infective blood meal each potentially infective mosquito was allowed to probe on 3 naive 1-wk-old chicks for 10 sec, with a 1-min period between birds. This 10-sec period corresponds to the approximate time that *Ae. aegypti* were observed to

[1] Winning manuscript from the 1992 Student Paper Competition at the Annual Meeting of the American Mosquito Control Association.

[2] Anderson, R. 1989. Body size variation of and multiple blood feeding by *Culiseta melanura* (Coquillett) in southeastern Massachusetts. M.S. Thesis, University of Massachusetts, Amherst, MA.

Table 1. Larvae feeding schedule.

Day	Regime 1 (200 larvae/pan)	Regime 2 (500 larvae/pan)
1	50 mg	50 mg
2	50	0
3	50	0
4	100	100
5	150	0
6	200	0
7	150	50 (replaced H_2O)
8	100	0
9	50	50
10	50	50

probe before desisting or beginning to ingest blood (Mellink et al. 1982). Mosquitoes were dissected immediately after the 3-probe sequence to determine the presence of sporozoites in the salivary glands. If sporozoites were seen within the mosquito's salivary glands upon dissection, the group of birds was retained and blood smears were taken every day for 3–4 weeks. Groups were scored as: 0, uninfected; 1, one bird infected; 2, two birds infected; or 3, three birds infected.

RESULTS AND DISCUSSION

Fifty-eight groups of birds, with 3 chicks in each, were used in this study. Among the 22 groups fed on by large infective mosquitoes, one bird was infected in 9 groups (41%), two birds were infected in 5 groups (23%), three birds were infected in one group (5%), and no birds were infected in 7 groups (32%) (Fig. 1). Among the 36 groups fed on by small mosquitoes, one bird was infected in 7 groups (19%), two birds were infected in 3 groups (8%), three birds were infected in one group (3%), and no birds were infected in 25 groups (69%) (Fig. 1). The percentage of uninfected birds was quite high, but this was undoubtedly related to the restricted host contact time. Tests for probability (Daniel 1987; Table 2) indicated that large mosquitoes were more likely infect a host ($\alpha = 0.01$), and were more likely to infect more than one host ($\alpha = 0.06$), during serial feeding than small mosquitoes.

When infection rate was sorted according to pattern, the order of infection was not always reflected by order of probing. For single infections, large mosquitoes showed no difference in their ability to infect the 1st, 2nd or 3rd birds. Small mosquitoes were most likely to infect the 1st bird, but this difference was not statistically different from the ability of large mosquitoes to infect the 1st bird. Small mosquitoes were less likely than large mosquitoes to infect the 2nd or 3rd birds. Among multiple infections, the most common pattern seen among large mosquitoes was both 2nd and 3rd birds infected. Among small mosquitoes both 1st and 3rd birds infected was most common. While these results are too limited for statistical testing, they do indicate that the greatest release of sporozoites may not necessarily occur immediately. It is possible that there may be a correlation between blood meal size, sporozoite counts and serial infection rates (Rosenberg et al. 1990), although there is some evidence to the contrary (Ponnudurai et al. 1991). Blood meal size, oocyst number and sporozoite number were not obtained for these experiments, but future work is planned that will include measurement of these parameters.

Our data show that there are differences in the ability of large and small mosquitoes to transmit malaria parasites. Size confers no difference in a mosquito's ability to infect the 1st host, but does affect the ability to infect subsequent hosts in rapid succession, so that large mosquitoes were able to infect more sets of birds than small mosquitoes. These data further reaffirm that multiple host contact, especially by large mosquitoes, provides opportunity for multiple transmission of malaria parasites within a single gonotrophic cycle.

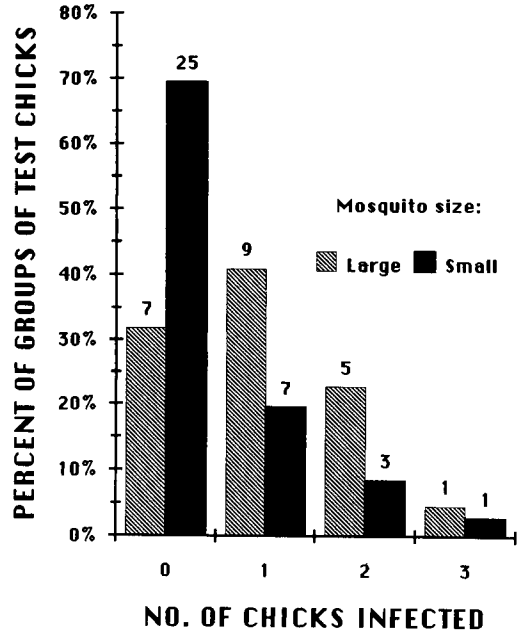

Fig. 1. Serial malaria infection rates in groups of 3 chicks probed by sporozoite-positive *Aedes aegypti* of 2 different size classes. The number over each column indicates the number of groups of test chicks.

Table 2. Test for probability data.

Chicks infected	Infected subsets		All sets of chicks			
	Large	Small	Large	Small	z*	α
0	7	25	21	36	2.65	0.01
1	9	7	22	36	1.775	0.04
2	5	3	22	36	1.543	0.06
3	1	1	22	36	0.358	NS
1, 2 or 3	15	11	22	36	2.796	0.01
2 or 3	6	4	22	36	1.581	0.06
1st only	6	8	22	36	0.436	NS
2nd only	8	4	22	36	2.304	0.01
3rd only	8	4	22	36	2.304	0.01

* $z = \frac{[(p1 - p2) - 0]}{se}$

p1 = x1/n1

p2 = x2/n2

se = ((p·(1 − p)/n1) + (p·(1 − p)/n2)) ^ 0.05

p = x1 + x2/n1 + n2

Where:

x1 = no. groups of birds infected by large mosquitoes

x2 = no. groups of birds infected by small mosquitoes

n1 = group exposed to large mosquitoes

n2 = group exposed to small mosquitoes

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