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Research Note

Susceptibility of *Anopheles stephensi* and Other *Anopheles* Strains to *Plasmodium yoelii nigeriensis*

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The rodent malaria *Plasmodium yoelii nigeriensis* is widely used as a laboratory model for studies of malaria (Bruce-Chwatt, 1980) and shows considerable variability in levels of infectivity to different species and strains of *Anopheles* mosquitoes. In the course of laboratory studies of malara transmission, it is necessary to find the most efficient mosquito strain for use as a vector. Paired experiments were conducted to compare the susceptibility of four strains of *An.stephensi* and single strains of *An.gambiae, An.arabiensis* and *An.dirus.* The results of these comparisons are presented here.

The mosquitoes used are all maintained at the London school of Hygiene and Tropical Medicine. BEECH strain of *An.stephensi* was originally derived from Delhi, India in 1947 and had been kept for several years by the Beecham Company. DELHI strain was collected from Delhi, India in 1947, LASS strain from Lahore, Pakistan in 1978, and IRAQ strain from Iraq in 1982. *An.gambiae* 16cSS strain was collected in Lagos, Nigeria in 1950. *An.arabiensis* SENN strain was obtained from Sennar, Sudan in 1982 where it had been maintained in a laboratory for 10 years. *An.dirus* BALM strain was originally isolated in Malaysia and colonized in 1978. All these mosquitoes are easy to maintain in the laboratory, and insectary conditions and details of mosquito rearing techniques are given elsewhere (Ichimori 1987).

N67 strain of *P.y.nigeriensis* in liquid nitrogen was recovered in a TO mouse (Theiler's original). Blood from the donor mouse with about 5 to 20% parasitaemia was mixed with heparinised PBS so that 10^7 parasitized red cells were in 0.2 ml. This volume was inoculated intra-peritoneally into each of two or more mice. On day 3 after inoculation, an infected mouse was chosen for mosquito feeding.

Mosquitoes were infected when 2 to 5 days old. On a day previous to the feeding, 20 to 50 female mosquitoes were placed in a 300 ml paper cup covered with a mesh screen. The glucose feeder which was usually kept on the top of the cups was removed 6 to 12 hours before the feed. This made the mosquitoes hungrier and also ensured that the abdomen was clear of glucose allowing complete engorgement to take place. Mosquitoes fed readily within 5 to 10 minutes on anaesthetized mice placed on top of cups containing the mosquitoes. In paired feeding experiments, BEECH strain of *An.stephensi* from India, as a standard, was fed simultaneously on the same mouse whenever another species or strain was fed.

After the blood meal, mosquitoes were released into a 20 cm cube cage and only fully fed females were removed into new paper cups. The mosquitoes were then maintained at 24+1°C and at a relative humidity of 80 to 90%. On day 7 or 8 after feeding, 10 to 20 stomachs were dissected out in PBS and examined at low power

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Table 1 Comparison of strain/species of *Anopheles* mosquitoes with BEECH strain of *Anopheles stephensi* for susceptibility to infection with *Plasmodium yoelii nigeriensis* (N67)

STRAINS COMPARED (SPECIES)	NUMBER OF PAIRED EXPERIMENTS	POSITIVE MOSQUITOES/ NUMBER OF DISSECTED (%)		WILLIAM'S MEAN OOCYST NUMBER/GUT	
		BEECH	OTHER	BEECH	OTHER
BEECH: DELHI (*STEPHENSI*)	5	93/101(92.1)	41/52(78.8)	59.28	21.61*
BEECH: LASS (*STEPHENSI*)	5	70/101(69.3)	79/144(54.9)	11.61	16.73*
BEECH: IRAQ (*STEPHENSI*)	6	95/101(94.1)	76/88(86.4)	34.07	28.26*
BEECH: 16CSS (*GAMBIAE*)	5	84/101(83.1)	4/84(4.8)	31.58	0.04*
BEECH: SENN (*ARABIENSIS*)	3	35/63(55.6)	11/50(22.0)	16.21	0.64
BEECH: BALM (*DIRUS*)	4	63/75(84.0)	6/68(8.8)	20.55	0.08

* $P > 0.05$

($\times 100$). Oocysts on both sides of the stomach wall were counted by focussing up and down. In order to obtain a better representation of the results, William's mean (geometric mean) oocyst number was calculated (William, 1937).

Six paired comparisons have been made. The data for all replicates of each species/strain are summarized in Table 1. *An.arabiensis* (SENN), *An.gambiae* (16cSS) and *An.dirus* (BALM) were much less susceptible than the BEECH strain. Among geographic strains of *An.stephensi* DELHI, LASS and IRAQ strains did not differ significantly from BEECH (paired t test: DELHI, $t = 2.21$, $df = 4$, $P > 0.05$; LASS, $t = 1.55$, $df = 4$, $P > 0.05$; IRAQ, $t = 2.24$, $df = 5$, $P > 0.05$).

BEECH strain has been used as a control in rodent malaria experiments (*P.berghei* by Al-Mashhadani *et al.*, 1980; *P.y.nigeriensis* by Prasittisuk, 1979; and Graves and Curtis, 1982). In this study, the paired feeding experiments showed that the BEECH strain was more susceptible than all the other strains tested and confirmed that this strain was a stable and suitable vector of N67 strain of *P.y.nigeriensis*.

The strains used in this study have been maintained in the laboratory insectary for a long period of time. Therefore the susceptibility of these populations probably does not reflect the susceptibility of mosquitoes from the places where these strains were first collected, due to inbreeding and genetic drift.

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