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Susceptibility of *Armigeres subalbatus* to the Offspring of Periodic *Brugia malayi* Maintained in Jirds

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Abstract: The susceptibility of the Nagasaki strain of *Armigeres subalbatus* to the 1st (G-1), 2nd (G-2), 3rd (G-3) and 4th (G-4) generations of periodic *Brugia malayi* (Che-ju strain, Korea) maintained in jirds was studied. The infection rates in the mosquitoes were 6, 18, 4 and 2% in the G-1, G-2, G-3 and G-4 respectively. The mean number of third stage larvae of *B. malayi* per infected mosquito fed on a jird of G-1, G-2, G-3 and G-4 were 0.08, 0.48, 0.08 and 0.04 respectively. There were no significant differences between the generations of *B. malayi*. The percentages of third stage larvae obtained from the head, thorax and abdomen of mosquitoes were 39.6%, 41.7% and 18.7% respectively.

Key words: Brugia malayi, Armigeres subalbatus, Jird, Offspring, Susceptibility

INTRODUCTION

Since the report that domestic cats were successfully infected with periodic *Brugia malayi* (Che-ju strain, Korea) by Nakajima *et al.* (1976), the susceptibility of mosquitoes to the parasite has been studied by feeding them in microfilaremic cats (Nakajima *et al.*, 1976; Kobayashi *et al.*, 1981). Recently we have succeeded in serial passage of periodic *B. malayi* originated from cats in jirds by using *Aedes aegypti. Armigeres subalbatus,* a common mosquito species in Japan, was reported to be susceptible to *B. pahangi* in jirds (Edeson *et al.*, 1960; Yamamoto *et al.*, 1983) but refractory to the periodic *B. malayi* in cats (Nakajima *et al.*, 1976; Oda and Wada, 1980). There has been, however, no report on the susceptibility of mosquitoes to the periodic *B. malayi* maintained in jirds.

This study describes the susceptibility of *Ar. subalbatus* to periodic *B. malayi* of the 1st, 2nd, 3rd and 4th generations maintained in jirds.

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MATERIALS AND METHODS

The Che-ju (Korea) strain of periodic *B. malayi* was established in domestic cats in our laboratory. The first generation of periodic *B. malayi* in jirds was established as follows. *Aedes aegypti* were fed on a cat infected with the periodic *B. malayi*, and then reared in mosquito cages at 27°C, 70% RH. At 14 days after feeding, the infective larvae from *Ae. aegypti* were inoculated into the inguinal region of male jirds subcutaneously. The infective larvae obtained from *Ae. aegypti*, which were fed on a jird infected with the first generation of *B. malayi*, were inoculated into the inguinal region of jirds to establish the second generation. The third and fourth generations of *B. malayi* were established by the same method.

The susceptibility of *Ar. subalbatus* to each generation of *B. malayi* in jirds was examined. The laboratory-bred *Ar. subalbatus* of Nagasaki strain were fed on jirds for 2 hours. Ten fully engorged mosquitoes were examined immediately for the number of microfilariae in each abdomen at 2 hours after feeding. Another group of 50 mosquitoes, which were fully engorged with microfilariae of each generation of *B. malayi*, were kept in cages at 27°C, 70% RH. The mosquitoes were dissected and examined for the third stage larvae at 14 days after feeding. The same number of mosquitoes were also similarly treated to examine the susceptibility to *B. pahangi*. The level of susceptibility was expressed by the number of third stage larvae per mosquito at 14 days after feeding. Statistical analysis was performed, where appropriate, using the Wilcoxon's rank-sum test or the Scheffe's test.

The animal experiment in this study was performed at The Animal Research Center for Infectious Tropical Diseases, The Institute of Tropical Medicine, Nagasaki University.

RESULTS

Results of the susceptibility test for *Ar. subalbatus*, Nagasaki strain, are shown in Table 1. The mean number of microfilariae per μl blood in G-1, G-2, G-3 and G-4 of jirds as a feeding source were 18.1, 30.3, 19.3 and 30.3 respectively. The number (mean$\pm$SD) of microfilariae detected in abdomens of mosquitoes fed on G-1, G-2, G-3 and G-4 were 30.5 ± 21.6, 57.8 ± 60.5, 28.5 ± 19.6 and 50.5 ± 46.6 respectively. There was a large difference in the number of microfilariae engorged by each mosquito. The mean number of third stage larvae of *B. malayi* per infected mosquito fed on G-1, G-2, G-3 and G—4 was 0.08, 0.48, 0.08 and 0.02 respectively. There were no significant differences between the generations of *B. malayi* ($p>0.01$). The infection rates with third stage larvae in the mosquitoes were 6, 18, 4 and 2% in the G-1, G-2, G-3 and G-4 respectively. However, in the mosquitoes fed on the jird infected with *B. pahangi*, all 50 mosquitoes were infected, and the mean number of third stage larvae of *B. pahangi* per mosquito was significantly larger than that of *B. malayi* ($p<0.01$).

The distribution of third stage larvae in the body of *Ar. subalbatus* is shown in Table 2. In mosquitoes infected with *B. malayi*, the percentage of third stage larvae obtained from the head, thorax and abdomen was 34.3%, 48.6% and 17.1% respectively. Mosquitoes infected with *B. pahangi* showed the percentages of 48.8, 28.9 and 22.3 in the head, thorax and abdomen respectively.

Teble 1. Susceptibility of *Armigeres subalbatus* to *Brugia malayi* of 4 generations and *B. pahangi*

Generation	Microfilarial density of jirds (per μl blood)	Mean No. of larvae/mosquito dissected immediately after feeding (Mean ± SD)*	No. of infected mosquitoes**	Mean No. of third stage larvae/ mosquito
G-1	18.1	30.5±21.6	3 (6%)	0.08
G-2	30.3	57.8±60.5	9 (18%)	0.48
G-3	19.3	28.5±19.6	2 (4%)	0.08
G-4	30.3	50.5±46.6	1 (2%)	0.04
B. pahangi	34.1	24.1±29.7	50 (100%)	14.68

* Ten fully engorged mosquitoes were examined
** Fifty mosquitoes were examined at 14 days after feeding

Table 2. Distribution of third stage larvae in the head, thorax and abdomen of *Armigeres subalbatus*

Generation	No. of mosquitoes dissected	No. of third stage larvae detected	Number and percentage of third stage larvae in		
			Head	Thorax	Abdomen
G-1	50	4	2 (50%)	1 (25%)	1 (25%)
G-2	50	25	9 (36%)	12 (48%)	4 (16%)
G-3	50	4	1 (25%)	3 (75%)	0 (0%)
G-4	50	2	0 (0%)	1 (50%)	1 (50%)
B. pahangi	50	734	358 (48.8%)	212 (28.9%)	164 (22.3%)

DISCUSSION

The susceptibility of *Ar. subalbatus* to G-1, G-2, G-3 and G-4 of *B. malayi* maintained in jirds was studied. It has been reported that *Ar. subalbatus* was refractory to the periodic *B. malayi* maintained in cats (Nakajima *et al.*, 1976; Oda and Wada, 1980; Kobayashi *et al.*, 1981). However, the susceptibility of *Ar. subalbatus* to the periodic *B. malayi* maintained in jirds has not been investigated. In our study, third stage larvae were obtained from *Ar. subalbatus* fed on G-1, G-2, G-3 and G-4, though the number was very small. On the other hand, no infective larvae were recovered from *Ar. subalbatus* fed on a

cat infected with *B. malayi* on the 9th day dissection (Kobayashi *et al.*, 1981) and at 13 days after feeding (Nakajima *et al.*, 1976). This discrepancy might be caused by different number of microfilariae ingested. In our study, the number of microfilariae per μl blood was 18.1, 30.3, 19.3 and 30.3 in G-1, G-2, G-3 and G-4 respectively. These microfilarial densities were much higher than those of cats (1.6 per μl average) reported by Kobayashi *et al.* (1981). Wada *et al.* (1977) reported that 0.1 infected larva per mosquito was obtained from an *Ar. subalbatus* fed on a cat infected with subperiodic *B. malayi*. It is interesting that this result is similar to ours obtained from jirds infected with the periodic *B. malayi*. In contrast to the results with *B. malayi*, *Ar. subalbatus* was reported to be very susceptible to *B. pahangi* (Edeson *et al.*, 1960; Nakajima *et al.*, 1976; Kobayashi *et al.*, 1981; Yamamoto *et al.*, 1985). Our study confirms their results. This difference of susceptibility between *B. malayi* and *B. pahangi* will be useful in distinguishing these two filarial parasites.

In this study, the three parts of a mosquito - head, thorax and abdomen - were examined for the third stage larvae. The total number of third stage larvae in the head, thorax and abdomen of *Ar. subalbatus* infected with periodic *B. malayi* was 12 (34.3%), 17 (48.6%) and 6 (17.1%) respectively. On the other hand, in mosquitoes infected with *B. pahangi* the rates of third stage larvae in the head, thorax and abdomen were 48.8%, 28.9% and 22.3% respectively. It has been reported that the percentage of third stage larvae in the head decreases when a large number of larvae are present in *Culex pipiens quinquefasciatus* (as *fatigans*) infected with *Wuchereria bancrofti* (Omori, 1958; de Meillon *et al.*, 1967), in *Aedes togoi* infected with *B. pahangi* (Lavoipierre and Ho, 1960) and in *Ae. togoi* infected with *Dirofilaria immitis* (Zielke, 1973). In this study, the rate of third stage larvae was higher in the head of mosquitoes infected with *B. pahangi* than with *B. malayi*, though only a small number of larvae of *B. malayi* was recovered in total. This seems to indicate that the low rate in the head in *B. malayi* is not due to the extrication of infective larvae from mosquitoes as reported by Wada *et al.* (1977) but to the result of low susceptibility.

This study indicated that there was no significant difference in the susceptibility of *Ar. subalbatus* to successive generations of the periodic *B. malayi* maintained in jirds.

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オオクロヤブカの定期出現型マレー糸状虫（済州島産）の各世代に対する感受性について

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ジャードによって継代維持された韓国済州島産の定期出現型マレー糸状虫の初代，2代目，3代目，4代目及び *B. pahangi* に対する長崎産オオクロヤブカの感受性について検討した．
各世代におけるオオクロヤブカ1匹当りの第Ⅲ期幼虫の平均虫体数は初代で0.08，2代目で0.48，3代目で0.08そして4代目で0.04と非常に低い値であった．これに対して *B. pahangi* では蚊1匹当り14.68と非常に高い感受性を示した．本実験では少数のマレー糸状虫Ⅲ期幼虫しか得られなかったが，その蚊体内での分布を見ると胸部に多く存在した．以上のことよりオオクロヤブカのマレー糸状虫に対する感受性は低く，ジャードによって継代維持された4代目までの世代間に大きな違いは認められなかった．

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