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Development in Mosquitoes of *Dirofilaria immitis* Microfilariae in Dog Blood Refrigerated at 4°C

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Abstract: The development in *Aedes togoi* (Shikimi strain) of the canine heart-worm *Dirofilaria immitis* microfilariae from a domestic dog was observed. Infective fresh and stored dog-blood at 4°C was used to feed the mosquitoes at 37°C through mouse skin. The stored blood varied with storage period in infectivity to the vector. It was found that microfilariae of *D. immitis* can be stored up to 20 days at 4°C for the laboratory infection experiment.

Key words: *Dirofilaria immitis*, *Aedes togoi*, Microfilariae storage, Membrane feeding

INTRODUCTION

Filarial worms of the genus *Dirofilaria* are normally parasitic in dogs, cats and racoons but can occasionally infect man. *Dirofilaria immitis* transmitted by mosquitoes is prevailing among house dogs in all parts of Japan (Ohishi, 1986). The worm is associated with pulmonary dirofilariasis with cases reported from Japan (Yoshimura *et al.*, 1980; Yoshimura, 1985; Makiya *et al.*, 1988), Australia and U.S.A. (Beaver and Orihel, 1965; Pacheco and Schofield, 1968) and Columbia (Beaver *et al.*, 1990).

Mosquitoes of three genera, namely *Anopheles*, *Aedes* and *Culex* are principal experimental vectors of *D. immitis* (Vanderberg and Gwadz, 1980), but they are more or less differently susceptible to *D. immitis*. To make comparative studies on the susceptibility of mosquitoes, blood with the same density of microfilariae has preferably to be fed to the mosquitoes. However, it is difficult to obtain blood with even similar microfilarial density. If the same microfilaraemic blood can be used on successive days, then comparative infection experiments would be much facilitated. For this reason, it was examined how long *D. immitis* microfilariae in dog blood can be stored at 4°C without reducing the infectivity to *Aedes togoi*, which has previously been found to be susceptible to *D. immitis* (Inoue, 1936, 1937; Suenaga, 1972).

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MATERIALS AND METHODS

Ae. togoi larvae and pupae were obtained from seashore rock pools in Shikimi, Nagasaki city by a sampling ladle and reared in an insectary with controlled conditions of 70 % relative humidity maintained by a humidity sensor model HN-02 Chino, 25°C and a photoperiod of 16 : 8 (L:D). The larvae were put in plastic trays of 30cm × 20cm × 4cm with tap water which had stayed overnight in a polyethylene bucket to evaporate chloride. A few small pieces of mouse pellets were provided to the larvae depending on their densities. Water in the trays was changed when necessary during larval development. Pupae were held in transparent 90ml plastic cups and confined to wire-frame cages, 30cm × 20cm × 20cm covered with fine mesh mosquito nets. Sugar cubes (Suenaga *et al.*, 1987) or 2–3% sucrose solution was provided.

Blood was drawn from a naturally infected house-dog by syringe and needle with 2–3 units of heparin per ml to prevent clotting, and stored at 4°C in refrigerator. Infective blood (1.0ml) was diluted with normal saline (4.0ml) and used. Worm density per ml blood was estimated from the number of microfilariae in 30mm³ blood sample before experimental use. Mosquitoes were exposed through hair-removed mouse skin to infective blood with *D. immitis* whose temperature was maintained at 37°C by a thermostatic machine. Engorged adult mosquitoes were maintained routinely until 13–15 days post-feeding then dissected for filarial infection, but some were killed and dissected 8–12 days post-feeding. Mature larvae were measured by a calibrated ocular micrometer.

RESULTS AND DISCUSSION

Microfilarial density in blood before experimental use

Microfilarial densities per ml of diluted infective blood just before experimental use were estimated to 1,000 in each experiment. All worms were moving normally, except some worms stored for 23 and 28 days were rather inactive.

Filaria infection rates in *Ae. togoi*

Results of the dissection of mosquitoes fed on fresh or stored infective dog blood and examined 8–15 days post-feeding are shown in Table 1. Larvae of *D. immitis* were mostly in stage II and III (mature), but it is known that stage II larvae usually develop to stage III in *Ae. togoi* (Suenaga, 1972), therefore total infection rate is given in Table 1 without discriminating the larval stage. However, as will be mentioned later, fewer stage II larvae may develop to stage III in mosquitoes fed on blood stored for a longer period.

At least some *D. immitis* microfilariae stored for up to 21 days could develop in *Ae. togoi*. The infection rates of mosquitoes fed on fresh blood and blood stored for up to 20 days were similar, ranging from 23.4 to 60.0%. Only one (1.3%) out of 79 mosquitoes fed on blood stored for 21 days had filarial larvae. Mosquitoes fed on blood stored for 23 and 28 days were not positive for filarial larvae.

Table 1 Infection rate of *Aedes togoi* fed on dog blood with *Dirofilaria immitis* microfilariae stored for different periods at 4°C

Storage period of microfilariae in days	Number of mosquitoes		% positive
	examined	positive	
0	73	31	42.5
7	45	27	60.0
14	107	25	23.4
16	19	9	47.4
18	34	11	32.4
20	14	6	42.9
21	79	1	1.3
23	39	0	0.0
28	33	0	0.0

Mosquitoes were maintained at 25°C and dissected between 8-15 days post-feeding.

Filarial load and sizes

Infective mature larvae in the proboscis of mosquitoes dissected between 13–15 days post-feeding are shown in Table 2. Infective larvae were found in mosquitoes fed on fresh and stored blood for up to 20 days, but the rate of mosquitoes positive for infective larvae seems to have decreased with the period of storage, when compared with the corresponding rate of mosquitoes positive for stage II and infective larvae (Table 1). This may imply that microfilariae stored for longer period lose, to some extent, their activity to develop to the infective stage in mosquitoes, but further studies would be needed to confirm it.

The number of larvae per positive mosquito was similar, and the mean length and width of the larvae were not different between mosquitoes fed on fresh blood and those on stored blood.

Table 2 Infective *Dirofilaria immitis* larvae in the proboscis of *Aedes togoi* fed on fresh or stored dog-blood with microfilariae

Storage period of mf in days	Number of mosquitoes		% positive	Number of infective larvae			Size of infective larvae (μ)			
	examined	positive		Total	Mean	Range	Length		Width	
							Mean	Range	Mean	Range
0	45	12	26.7	24	2.0	1–4	940.5	700–1,100	28.3	25–30
7	20	14	70.0	22	1.8	1–3	826.4	650–1,100	28.2	25–30
14	92	5	5.4	8	1.6	1–4	900.0	750–1,100	30.0	—
16	8	2	25.0	6	3.0	2–4	956.7	900–1,100	20.0	—
18	19	0	0.0	—	—	—	—	—	—	—
20	10	1	10.0	1	—	—	1,000.0	—	25.0	—

Mosquitoes were maintained at 25°C and dissected between 13–15 days post-feeding.

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