RESEARCH NOTE

Susceptibility of *Aedes scapularis* (Rondani, 1848) to *Dirofilaria immitis* (Leidy, 1856), an Emerging Zoonosis

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Dirofilaria immitis is the causative agent of canine heartworm, a well known parasitic cardiopulmonary disease of dogs and cats, that can also affect man (NB Robinson et al. 1977 *J Thor Cardiov Surg 74*: 403-408). When this nematode infects man the disease is difficult to diagnose, but even so there are more than 200 cases of human pulmonary dirofilariasis reported throughout the world (CAR Schneider et al. 1986 *Acta Oncol Bras 6*: 125-130, R Rodrigues-Silva et al. 1995 *Rev Inst Med Trop São Paulo 37*: 523-530).

Although the principal mosquito vectors of *D. immitis* have not been determined in all areas, it has been shown that, depending on the geographic region, the nematode can be transmitted by mosquito species belonging to the genera *Culex, Aedes, Anopheles, Mansonia, Psorophora* and *Coquillettidia* (KW Ludlam et al. 1970 *JAVMA 157*: 1354-1359). Investigations to elucidate the potential of some mosquito species as vectors of canine heartworm in southeastern Brazil were prompted by reports of prevalences in dogs, as high as 52.5% at the seashore and by the threat of human infections (N Labarthe et al. 1997 *Mem Inst Oswaldo Cruz 92*: 47-51). Natural infections of one *Ae. scapularis* and one *Ae. taeniorhynchus* (Wiedemann, 1821) with presumed *D. immitis* larvae in their Malpighian tubules were reported previously in a coastal lowland area in the city of Rio de Janeiro (R Lourenço-de-Oliveira & LM Deane 1995 *Mem Inst Oswaldo Cruz 90*: 387-388).

Both mosquito species were later found to be among the most abundant, besides being found harboring filariae, at a canine heartworm focus of similar landscape in the municipality of Niterói, State of Rio de Janeiro (N Labarthe et al. 1998 *Mem Inst Oswaldo Cruz 93: 425-432*). *Ae. taeniorhynchus* has been considered the primary vector of *D. immitis* in areas of the United States (DM Sauerman & JK Nayar 1983 *Mosq News* 43: 222-225). However, the vectorial competence of *Ae. scapularis* for *D. immitis* has never been evaluated. The present study is aimed to evaluate this species as a vector under experimental conditions.

Females of *Ae. scapularis* were captured on human-bait in Itacoatiara, Guaraí and Pedra de Guaratiba, coastal lowlands in the State of Rio de Janeiro. They were bloodfed and subsequently laid eggs on wet filter paper. The eggs were kept wet for 10-15 days and subsequently stored dry in desiccators. Eggs were hatched by submerging them in water. Larvae were fed with a commercial fish food (TetraMin, Tetrawerke Co.). A susceptible strain of *Ae. aegypti* (Linnaeus, 1762) was used as control. It was an *Ae. aegypti* colony started ten years ago from wild females caught in Rio de Janeiro, whose larvae were reared in the same conditions of those of *Ae. scapularis*. Seven-day old females of both species were simultaneously provided with fresh dog blood containing 60-70 microfilariae/20 µl in an artificial feeding apparatus (LC Rutledge et al. 1964 *Mosq News 24*: 407-419). The females of both species were similar in size and it was supposed that they took similar amounts of infected blood. After the infective blood meal, mosquitoes were provided a solution of 10% dextrose and kept at 24-26°C and 65-70% relative humidity. Mosquito dissections were performed weekly for three weeks after the infective blood meal, and mortality rates were recorded. After chloroform anesthesia, the mosquitoes were dissected and the head, mouthparts, midgut, Malpighian tubules, and thorax were thoroughly examined in 0.89% NaCl for worms, and the number at different locations counted. The number of larvae/location was statistically analyzed by median scores and the proportion of infected/dissected mosquitoes analyzed by *chi* square.

The results of dissections of both *Ae. scapularis* (Fig.) and *Ae. aegypti* from 9 to 21 days post-infective blood meal are presented in the Table. One hundred *Ae. aegypti* and 210 *Ae. scapularis* were fed with infected blood. The mortality rate was high

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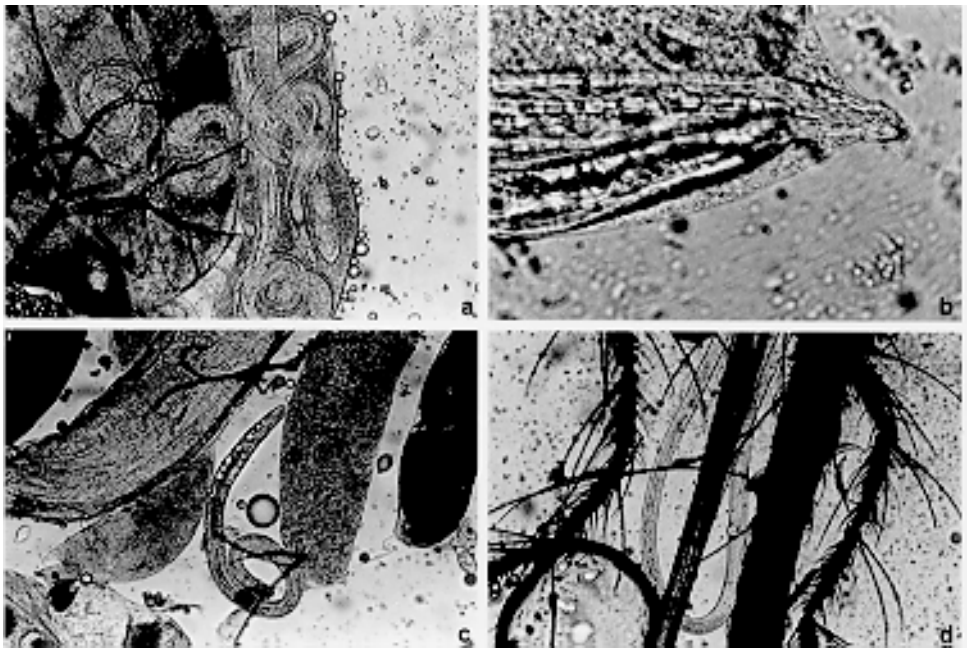
in both species: 71% *Ae. aegypti* and 79% *Ae. scapularis*. Since there were no comparisons with controls fed uninfected blood, these mortality rates may not be attributable to nematode burden.

The larval number in the Malpighian tubules/ infected mosquito was nearly the same for both mosquito species (p=0.4561), while at two and three weeks post-infection a larger proportion of larvae reached the infective stage in the head and proboscis of *Ae. aegypti* than in *Ae. scapularis* (p = 0.0833, Table). Larval development in *Ae. scapularis* appears to be slower than in *Ae. aegypti*.

The mean number of larvae per infected mosquito was 8.4 in *Ae. aegypti* and 6.9 in *Ae. scapularis*. A larger proportion of mosquitoes was found infected in *Ae. scapularis* (79.5%) than in



Dirofilaria immitis larvae developing in *Aedes scapularis* under experimental conditions. a: larvae (L_2 and L_3) in Malpighian tubules (200x); b: third stage larva trying to leave the distal tip of the Malpighian tubule (400x); c: free L_3 larva in the hemolymph, just after leaving the Malpighian tubule (200x); d: infective larva at the mouthparts (200x).

TABLE

Aedes scapularis and *Ae. aegypti* artificially infected by *Dirofilaria immitis*

	Ae. scapularis			*Ae. aegypti*		
	No. infected/ dissected	Local	No. larvae	No. infected/ dissected	Local	No. larvae
9 days PI	1/1	MT	15	1/1	MT	17
2 weeks PI	22/26	MT	167	4/21	MT	13
		HD/PB	05		TX	07
					HD/PB	09
3 weeks PI	12/17	MT	43	3/7	MT/AB	03
		TX	01		HD/PB	18
		HD/PB	11			
Total	35/44	MT	225[a]	8/29	MT/AB	33[b]
		TX	01		TX	07
		HD/PB	16		HD/PB	27

PI: post infection; MT: Malpighian tubules; TX: thorax; HD: head; PB: proboscis; *a:* 28% of the larvae were melanized; *b*: no melanization was observed.

Ae. aegypti (27.6%) ($p<0.0001$, Table). Although mosquito mortality after infection was high in both species, those figures suggest that infected *Ae. scapularis* may show a higher capacity to support the infection than *Ae. aegypti*, perhaps arresting some larvae development (L Kartman 1953 *Exp Parasitol* 2: 27-78, BM Christensen 1981 *Trans R Soc Trop Med 75*: 439-443). Accordingly, only some larvae complete their cycle in *Ae. scapularis* and so, by means of controlling such infection, this mosquito species enhances its own survival. Dogs frequently demonstrate high microfilaremia (JB Lok et al. 1988 *J Helminthal 62*: 175-180) and mosquitoes may die after ingesting blood with a high density of microfilariae (DM Sauerman 1980 *Mechanisms in Mosquitoes Responsible for Variation in Susceptibility to Infection by* D. immitis *(Leidy), Etiologic Agent of Canine Heartworm Disease,* PhD Thesis, University of Florida, 168 pp.). To be efficient vectors of *D. immitis*, mosquitoes must limit the number of developing parasites (Christensen 1981 *loc. cit.*). *Ae. scapularis* mosquitoes apparently controlled their larval burden (28% larval melanization, Table) but did not suppress their maturation and migration of non-melanized larvae to the head and proboscis. This provides additional evidence that *Ae. scapularis* is a suitable vector for *D. immitis.*

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