Vector Competence of *Culex quinquefasciatus* Say from Different Regions of Brazil to *Dirofilaria immitis*

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The vector competence of Culex quinquefasciatus *from five localities in Brazil to* Dirofilaria immitis *was evaluated experimentally. Females from each locality were fed on an infected dog (~ 6 microfilariae/*µl *blood). A sample of blood fed mosquitoes were dissected approximately 1 h after blood meal. These results demonstrated that all had ingested microfilariae (mean, 4.8 to 24.6 microfilariae/mosquito). Fifteen days after the infected blood meal, the infection and infective rates were low in all populations of* Cx. quinquefasciatus. *The mean number of infective larvae detected in the head and proboscis of these mosquitoes was 1-1.5. The vector efficiency, the number of microfilariae ingested/ number of infective larvae, was low for all populations of* Cx. quinquefasciatus. *However, the survival rate for all populations was high (range 50-75%). The survival rate of* Aedes aegypti *assayed simultaneously for comparison was low (24.7%), while the vector efficiency was much higher than for* Cx. quinquefasciatus. *These data suggest that the vector competence of all assayed populations of* Cx. quinquefasciatus *to* D. immitis *in Brazil is similar and that this species is a secondary vector due to its low susceptibility. Nevertheless, vector capacity may vary between populations due to differences in biting frequency on dogs that has been reported in Brazil.*

Keys words: *Dirofilaria immitis* - *Culex quinquefasciatus* - mosquito - vector competence - Culicidae - Brazil

Dirofilaria immitis (Leidy) is a nematode parasite that infects the right ventricle and pulmonary artery of dogs and other carnivores, and is transmitted by mosquitoes (Diptera, Culicidae). The parasite has a wide geographical distribution, with dogs serving as the principle host, although infection has been reported in other animals and occasionally in man (Robinson et al. 1977, Kasai et al. 1981). The complete development of microfilariae has been reported in species of *Culex*, *Aedes*, *Anopheles*, *Mansonia*, *Psorophora* and *Coquillettidia* (Ludlam et al. 1970).

In some insects there exist biochemical products in the hemolymph and other tissues, including the Malpighian tubule cells and thoracic muscle cells, that may block the development of the parasite by mechanisms comprising sequestration, encapsulation and melanization. The intensity of these reactions varies according to the susceptibility of the mosquito host, resulting in high survival rates of both the parasite and mosquitoes of susceptible populations (Christensen 1981, Christensen & Tracy 1989, Talluri & Cancrini 1994). The migration and development of large numbers of *D. immitis* larvae within the mosquito can produce elevated levels of host mortality (Nayar & Sauerman 1975). Thus, melanization can act as an important mechanism for vector survival by limiting the number of larvae that complete development and thereby guaranteeing its own survival (Christensen 1981, Christensen & Forton 1986, Christensen & Tracy 1989). In addition, some mosquito species have well developed cibarial armature, the teeth of which, when numerous and/or developed, injuring ingested microfilariae and reducing their survival potential (McGreevy et al. 1978, Coluzzi et al. 1982).

The importance of *Cx. quinquefasciatus* Say as an intermediate host for *Wuchereria bancrofti* is well known. In 1901, Bancroft had already noted the development of *D. immitis* in *Cx. pipiens fatigans* (= *Cx. quinquefasciatus*) (*apud* Villavaso & Steelman 1970). The vector competence of nocturnal domestic mosquitoes (*Cx. quinquefasciatus* and *Cx. pipiens* and their subspecies) has been discussed by various researchers worldwide demonstrating the various levels of susceptibility to infection according to geographical location (Ludlam

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et al. 1970, Lowrie 1991, Loftin et al. 1995). In Rio de Janeiro *Cx. quinquefasciatus* was considered a secondary vector of *D. immitis* (Labarthe et al. 1998a, b). In São Luís (State of Maranhão), where the prevalence of canine dirofilariasis varies from 11.3 to 52.5% depending on the origin of the dogs and the proximity to the sea shore, only 0.5% of the *Ae. taeniorhynchus* and 0.1% of *Cx. quinquefasciatus* examined were found to be naturally infected. *Cx. quinquefasciatus* accounted for 54% of the total mosquitoes collected (man and dogs) and 96.7% of the total captured in dog baited traps (Ahid et al. 1999, Ahid & Lourenço-de-Oliveira 1999). The marked difference noted in the frequency of *Cx. quinquefasciatus* collection using live dog baited traps in Maranhão and that seen in Rio de Janeiro could reflect differences in the vector capacity of this species in these two locations. Moreover, there have been reports of vector competence differences between *Cx. quinquefasciatus* populations isolated from regions with various levels of prevalence of bancroftian filariasis (Janousek & Lowrie 1989, Lowrie et al 1989, Brito et al. 1997, Calheiros et al. 1998). Could *Cx. quinquefasciatus* from Maranhão show a different degree of susceptibility to infection with *D. immitis* in comparison to other parts of Brazil?

In the present study, the vector competence of *Cx. quinquefasciatus* in relation to *D. immitis* was evaluated under laboratory conditions using mosquitoes originating from five distinct Brazilian populations.

MATERIALS AND METHODS

The experiments were performed using female *Cx. quinquefasciatus* derived from 32 to 107 wild collected females from each of five test locations: Recife, Pernambuco (PE), the city of Rio de Janeiro (RJ), Porto Velho, Rondônia (RO), São Luís, Maranhão (MA), and Florianópolis, Santa Catarina (SC). Transmission of *D. immitis* was previously reported in all sample locations (Alves et al. 1993, Labarthe 1997, Ahid et al. 1999), except Porto Velho, where the examination of 45 dogs for the presence of the parasite were negative (Lima et al. 1996). The progenies from each location were reared separately, but simultaneously under the same conditions with regard to feeding, temperature and illumination. Larvae were reared in groups of approximately 400 in 40 x 25 cm pans and provided with fish food (TetraMin^{Tm}) mixed with cat food (Whiskas^{Tm}). Pupae were transferred daily (in containers filled with tap water) to screened cages (40 x 40 x 40 cm) where adults emerged. Adult mosquitoes were daily provided with 10% sucrose solution. Three to five days-old nuliparous (F_7) females were used for experimental infections with *D. immitis*. Twenty-four hours prior to blood-feeding on the infected blood source, sucrose was removed. The colonies were maintained and experimental infections were conducted in the laboratory at 29ºC ± 1ºC and 70% ± 10% relative humidity (RH).

To identify different mosquito populations following feeding, the females from each location were marked with luminous powder of different colors (Luminous Powder-Bioquip products) a few minutes prior to the infection experiment. Females were separated by observing the color of adhering luminous powder when illuminated with a black fluorescent light source. Approximately 1,500 female *Cx. quinquefasciatus* (approximately 300 from each population) and 300 *Ae. aegypti* (São Luís strain, as control) reared under the same conditions as *Cx. quinquefasciatus*, were placed simultaneously in a covered cage (120 x 100 x 80 cm) along with a previously anesthetized, infected dog. All the mosquitoes were allowed to feed on the dog from 23.00 h to 3.00 h in total darkness. During the first hour of exposure to the dog five engorged females from each population were collected using an aspirator and immediately dissected (as described below) to determine the number of ingested microfilariae.

A nine years old, male Labrador with a natural *D. immitis* infection (approximately six microfilariae per µl of blood) was used as the source of infection for the test mosquitoes. The dog was anaesthetized with acepramazine (0.2 ml/kg) during the entire exposure period. To determine the average number of circulating microfilariae, six 20 µl blood samples were collected from the dog and examined by Giemsa staining. Three of the samples were collected 24 h prior to exposure to the mosquitoes while the other three were collected 1 h pre-exposure.

At the end of the 4 h exposure period, engorged females from each population were collected and transferred to a screened circular cage (8 cm diameter), held in an incubator at 28ºC ± 1ºC and 70% (RH) and provided with 10% sucrose. Partially engorged females were excluded from the experiment. Moribund females were removed and dissected daily to determine the progress of the infection. At 15 days post infection all surviving females were anaesthetized in ethyl acetate vapor, their wings and legs removed, and then dissected in sterile saline (0.89% NaCl). The head, thorax, stomach and Malpighian tubules were dissected with the help of needles and examined individually for the presence of larval forms (Lourenço-de Oliveira & Deane 1995). The larvae encountered were classified on the basis of their larval development stage as described by Taylor (1960).

In this study we refer to "infected mosquitoes" as those in which larvae of *D. immitis* were observed at any stage of development. The term "infective mosquitoes" refers to mosquitoes that had third stage larvae in their head and/or proboscis (H/p). The analysis of the average number of microfilariae ingested soon after engorgement was performed using the Duncan test in order to transform the data into a logarithmic form for analysis of variance at the 5% level. Other variables were analyzed using the χ^2 test for non parametric data at the 5% level.

RESULTS AND DISCUSSION

Among the total female *Cx. quinquefasciatus* (approximately 300 per population) placed in contact with the infected dog, 858 engorged. A variable percentage of engorged females was observed, ranging from 40 to 98% for different populations (Table I). In the case of *Ae. aegypti* 57% of the females engorged. All *Cx. quinquefasciatus* dissected immediately after feeding contained microfilariae in the midgut, with rates that varied from 24 to 123 microfilariae (mf) per mosquito (mosq). The average numbers of mf/mosq were 4.8 ± 4.3 to 24.6 ± 23.3 (Tables I, II). Statistical analysis (Duncan Test) revealed a significant difference in the average number of mf/mosq for PE and RO populations of *Cx. quinquefasciatus (*24.6 ± 23.3; 4.8 ± 4.3, respectively). No significant differences were observed between the average number of mf/mosq in *Cx. quinquefasciatus* from PE and *Ae. aegypti* (26.6 ± 11.5) or between RO population and the other populations of *Cx. quinquefasciatus* (Tables I, II). Calheiros et al. (1998) infected *Cx. quinquefasciatus* with *W. bancrofti* and encountered three to 102 larvae (average 19.8 ± 19.5) in the midgut of 97% of mosquitoes dissected immediately after feeding on infected blood. Loftin et al. (1995) reported average levels of mf/mosq of 34.2 ± 6.3, 29 ± 6.1 and 39.3 ± 11 in *Cx. quinquefasciatus*, *Cx. tarsalis* and *Ae. vexans* respectively, that fed on a dog infected with *D. immitis*. In our experiments all *Ae. aegypti* were found to be infected with an average mf/mosq level of 26.6 ± 11.5 (Tables I, II).

We observed a rapid coagulation of midgut contents in female *Cx. quinquefasciatus* which were dissected within 1 h of feeding. Here, the formation of crystals, a considerable reduction in the

TABLE I

Distribution of infected and infective mosquitoes, number of microfilariae (mf) ingested by female *Culex quinquefasciatus* of different origin and by *Aedes aegypti*, fed on a naturally infected dog (~6 mf/20 µl of blood)

Mosquitoes species/ Origins	Total of mosquitoes		Mosquitoes examined 1 h after feeding		Mosquitoes examined from the 1st to 10th day after feeding[a]	Mosquitoes with L_3 (infective larvae) between the 11th to 15th day after feeding[b]	
	Engorged (%)	Dissected	No. of infected mosq./dissec. (%)	mf/mosq. dissected X ± S	No. of infected mosq./ dissected (%)	$\overline{X}$ L_3/dissected mosq.	$\overline{X}$ L_3/infected mosq.
Cx. quinquefasciatus							
Pernambuco	295 (98)	172	5/5 (100)	24.6 ± 23.3	4/17 (23.5)	0.1	1.4
Rio de Janeiro	121 (40)	90	5/5 (100)	8.6 ± 4.5	6/16 (37.5)	<0.1	1
Rondônia	136 (45)	111	5/5 (100)	4.8 ± 4.3	12/19 (63.2)	0.1	1.5
Maranhão	164 (55)	142	5/5 (100)	9.8 ± 8.9	6/14 (42.8)	0.1	1.2
Santa Catarina	142 (47)	107	5/5 (100)	6.8 ± 6.6	6/22 (27.3)	<0.1	1.5
Ae. aegypti	170 (57)	134	5/5 (100)	26.6 ± 11.5	84/87 (96.5)	4	4.1

a: larvae encountered solely in the Malpighian tubules; *b*: total counts for L_3 observed in the Malphigian tubules, head and mouth parts, in accordance with the scheme of Kartman (1953, 1954).

movement of the microfilariae and the presence of dead or injured larvae were observed. Similar observations were reported by Nayar and Sauerman (1975) and Lowrie (1991), who suggested that the presence of crystals of oxyhemoglobin, resulting from the lysis of blood cells, was the principle factor responsible for the reduced larval activity and death of *D. immitis* following ingestion by *Cx. quinquefasciatus*. In addition, Lowrie (1991) in a study with *Cx. quinquefasciatus* found that 12% of the mf of *D. immitis* had been damaged by the action of the cibarial armature. In the present study, the rapid coagulation and formation of crystals was not observed in *Ae. aegypti*, instead the microfilariae were active and moving freely in the midgut soon after their ingestion.

Regarding the number of larvae encountered in female *Cx. quinquefasciatus* at different sampling times, no significant differences were noted between females dissected 48 h post engorgement ($\chi^2_{\alpha 5} = 2.15$) and those sampled at intervals between three to seven days ($\chi^2_{\alpha 5} = 3.437$) or between 11 to 15 days ($\chi^2_{\alpha 5} = 2.956$) (Table II). Similarly, differences were not observed between the mean larval development/mosquito for different test populations of *Cx. quinquefasciatus* ($\chi^2_{\alpha 5}$ =3.84). During the 8th to 10th day post feeding, moribund female *Cx. quinquefasciatus* were dissected but none were found to be infected (Table II). Macêdo et al. (1998) encountered averages of 6.9 and 8.4 larvae/mosquito in *Ae. scapularis* and *Ae. aegypti* fed using an apparatus containing blood infected with *D. immitis* (60 to 70mf/20 µl). In our experiments the mean numbers of mf/mosq recorded in *Ae. aegypti* were greater than those observed in *Cx. quinquefasciatus* at all sampling times (Table II). More female *Ae. aegypti* maintained larvae in the Malpighian tubules than *Cx. quinquefasciatus*, irrespective of the origin of the population (23.5 to 63.2% of infected mosquitoes) (Table I).

The data indicate that the infection during the first 48-h feeding period for *Cx. quinquefasciatus* was not sufficient to induce an elevated level of mortality in those mosquitoes: MA 1.46% (2/137), PE 2.39% (4/167), SC 2.94% (3/102), RO 5.66% (6/106) and RJ 7.06% (6/85). Significant differences were not noted ($\chi^2_{\alpha 5} = 5.7$) when we compared the mortality levels of different populations of *Cx. quinquefasciatus*. However, populations of *Cx. quinquefasciatus* and *Ae. aegypti* showed a marked difference. During this period the level of mortality observed for *Ae. aegypti* was elevated (31%). These results are similar to those reported by Serrão (1998), i.e. 24.7 and 35.7% for females fed on blood with moderate microfilaraemia (3,000 to 5,000 mf/ml). Although the female *Cx. quinquefasciatus* from PE had ingested a significantly larger number of mf than had any other population, this did not result in a higher mortality rate in the two days following the infected blood meal. In the case of *Ae. aegypti* there was a correlation between the number of mf ingested and mortality rate. These data support the hypothesis that fewer live mf reach the Malpighian tubules in *Cx. quinquefasciatus* than in *Ae. aegypti*, owing to

TABLE II

Number of *Dirofilaria immitis* larvae observed over a 15 day period in female *Culex quinquefasciatus* from different regions of Brazil and in *Aedes aegypti*, that fed on an infected dog (~6 microfilariae/20 µl blood). Average in parenthesis

Mosquito species/	No. of larvae encountered/No. of mosquitoes dissected					
Origin	1 h	until 48 h	3-7 days	8-10 days	11-15 days	Total
Cx. quinquefasciatus						
Pernambuco	123/5 (24.6)	24/4 (6)	37/6 (6.2)	0/7 (0)	22/150 (0.2)	206/172 (1.2)
Rio de Janeiro	43/5 (8.6)	73/6 (12.2)	47/5 (9.4)	0/5 (0)	2/69 (<0.1)	165/90 (1.8)
Rondônia	24/5 (4.8)	61/6 (10.2)	37/10 (3.7)	0/3 (0)	8/87 (0.1)	130/111 (1.2)
Maranhão	49/5 (9.8)	15/2 (7.5)	52/8 (6.5)	0/4 (0)	13/123 (0.1)	129/142 (0.9)
Santa Catarina	34/5 (6.8)	38/3 (12.7)	26/10 (2.6)	0/9 (0)	3/80 (<0.1)	101/107 (0.9)
Ae. aegypti	133/5 (26.6)	1046/48 (21.8)	486/38 (12.8)	3/1 (3.0)	221/42 (5.3)	1889/134 (14.1)

the barriers encountered in the digestive tract. These barriers may include the action of the cibarial armature, blockage by rapid coagulation of the ingested blood and the presence of crystals (Nayar & Sauerman 1975, McGreevy et al 1978, Lowrie 1991, Loftin et al. 1995).

At the end of the 15-day post feeding period no significant difference in survival was observed among the test *Cx. quinquefasciatus* populations ($\chi^2_{\alpha 5} = 6.66$), with a variation of between 50.8% to 75% (Table III). Levels of survival were greater than those reported by Brito et al. (1999), who only recorded a 30.6% survival in *Cx. quinquefasciatus* from Alagoas (AL) infected with *D. immitis*. Moreover, our data differ from the values of 17 and 63% survival reported by Lowrie (1991) using two different strains of *Cx. quinquefasciatus* (Haiti and USA). Nevertheless, our values are similar to those observed by Calheiros et al. (1998) who evaluated the experimental infection of a Brazilian strain of *Cx. quinquefasciatus* with *W. bancrofti* and reported 66% survival rate. A higher level of survival was observed for Brazilian populations of *Cx. quinquefasciatus* in this study than *Ae. aegypti* (24.7%) (Table III).

Third stage larvae were encountered in the Malpighian tubules only on day 11 in *Cx. quinquefasciatus* from PE and MA and only on day 13 in specimens from RJ, RO and SC. Infective larvae L_3 were observed in the head and proboscis (H/p) from day 13 in *Cx. quinquefasciatus* from MA and on day 14 in females from PE, RJ and SC. Females from RO were the only population that infective stage larvae were not observed in the H/p within the 15 day observation period, despite the observation of live L_3 (0.1 L_3/mosq) in the Malpighian tubules (Table I). These findings are in agreement with studies on *Cx. quinquefasciatus* from other locations (Kartman 1953, 1954, Villavaso & Steelman 1970, Lowrie 1991, Loftin et al. 1995, Brito et al. 1999). In the case of *Ae. aegypti*, L_3 were detected in the Malphigian tubules on day 11 and in the H/p on day 12 after feeding on the infected dog. In general terms, three-four times as many L_3 were detected in *Ae. aegypti* than were found in *Cx. quinquefasciatus* test populations (1 to 1.5 L_3/infected mosquito) (Table I).

The level of infection ranged from 12 to 20.7% in the populations of *Cx. quinquefasciatus* (Table III), although no significant difference was observed ($\chi^2_{\alpha 5} = 2.66$). This level of infection was lower than that reported by Loftin et al. (1995), who observed the presence of L_3 in 40.6% of infected *Cx. quinquefasciatus*. The infection rate found in our study with *Ae. aegypti* was very high (96.3%) (Table I), greatly surpassing the values of 27.6% for this species and 79.5% for *Ae. scapularis* noted by Macêdo et al. (1998).

TABLE III

Results of infection with *Dirofilaria immitis* in *Culex quinquefasciatus* from five distinct regions of Brazil and in *Aedes aegypti* after 15 days observation

Mosquito species/ Origin	Indexes (%)				Vector efficiency VE (%)
	Infection rates[a]	Infectivity rate[b]	Intensity of infection[c]	Survival rate[d]	
Cx. quinquefasciatus					
Pernambuco	26/172 (15.1%)	13/150 8.7	10/13 6.66	150/295 50.8	0.49
Rio de Janeiro	13/90 (14.4%)	2/69 2.9	1/2 1.45	69/121 57	0.34
Rondônia	23/111 (20.7%)	4/87 4.6	0/4 0	87/136 63.9	1.44
Maranhão	21/142 (14.8%)	9/123 7.3	4/9 3.25	123/164 75	0.91
Santa Catarina	13/107 (12%)	2/80 2.5	1/2 1.25	80/142 56.3	0.55
Ae. aegypti	129/134 (96.3%)	27/42 64.3	20/27 47.6	42/170 24.7	15.31

a: number of mosquitoes infected/number of mosquitoes dissected; *b*: L_3 in the head and proboscis (H/p) and Malpighian tubules/number surviving mosquitoes; *c*: number of mosquitoes with L_3 in the (H/p)/number of mosquitoes with L_3; *d*: number of surviving mosquitoes/number of engorged mosquitoes; VE (%): X number of L_3 x 100/X number of mf ingested among dissected mosquitoes; in according to Kartman (1953, 1954), Ramachandan (1970) and Brito et al. (1997).

The level of infectivity fluctuated (2.5 to 8.7%) depending on the *Cx. quinquefasciatus* population (Table III). The intensity of the infection [mosq with L_3 in the H/p/mosq with L_3 in any other location] evaluated on day 15 post feeding also varied in relation to population origin i.e. 6.7% in PE to 1.2% in SC, without including *Cx. quinquefasciatus* from RO, since no L_3 were encountered in the H/p at the conclusion of the experiment. In the case of *Ae. aegypti*, the level of infection was 96.3% and the intensity of infection reached 47.6%.

The vector efficiency (VE) was low (0.3 to 1.4%) for different populations of *Cx. quinquefasciatus*, with no significant differences between populations ($\chi^2_{\alpha 5} = 1.79$). These values were similar to those obtained by Kartman (1953) of 0.8% and by Lowrie (1991) who reported values of 0.3 to 1.6%. In both studies *Cx. quinquefasciatus* females were fed under conditions of microfilaraemia similar to those in our experiment. However, our values are slightly higher than that reported by Brito et al. (1999) (i.e. VE = 0.0%) and lower than that reported by Loftin et al. (1995), who determined a VE of 2.7% for the AL strain of *Cx. quinquefasciatus* used in their study. In the case of *Ae. aegypti*, the VE in our study was 15.3%, very similar to the value of 15.8% determined for this species by Brito et al. (1999).

Different Brazilian populations of *Cx. quinquefasciatus* are capable of supporting the development of *D. immitis* until the infective stage, demonstrating that this species is susceptible to infection and has vector potential. Nevertheless, this susceptibility was limited for the specimens derived from five different population suggesting that *Cx. quinquefasciatus* is of secondary importance in the transmission of *D. immitis* in Brazil. Coincidentally, L_3 were not detected in the H/p of *Cx. quinquefasciatus* from RO, the only location where *D. immitis* infection of this species of mosquito has yet to be reported. Possibly, this particular population may be more refractory than the other populations examined, with the infective cycle of the helminth being retarded in mosquitoes from RO. Irrespective of the population origin, the level of survival shown by *Cx. quinquefasciatus* infected with *D. immitis* is higher than that seen for other vectors including *Ae. scapularis, Ae. aegypti, Ae. taeniorhynchus* and *Ae. fluviatilis* (Nayar & Sauerman 1975, Kasai & Williams 1986, Lowrie 1991, Macêdo et al. 1998). This suggests that in areas where transmission is maintained by primary vectors of greater susceptibility and where the microfilaraemia is high among canine populations, *Cx. quinquefasciatus* could play an important role in maintaining the transmission of *D. immitis*. This is, in part, because *Cx. quinquefasciatus* is present during the entire year (Labarthe et al. 1998a, Ahid & Lourenço-de-Oliveira 1999) and the majority of the mosquitoes survive infection by *D. immitis*. In MA, where the incidence of biting by *Cx. quinquefasciatus* in dog populations was much higher than in other localities such as in RJ, the vector capacity of *Cx. quinquefasciatus* for *D. immitis* may be elevated (Labarthe et al. 1998a, Ahid & Lourenço-de-Oliveira 1999). Finally, given that *Cx. quinquefasciatus* is anthropophilic and occurs at high frequencies in areas where the prevalence of canine dirofilariasis is high, it seems possible that man will have an increased probability of becoming an occasional host for this parasite.

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