Potential Vectors of *Dirofilaria immitis* (Leidy, 1856) in Itacoatiara, Oceanic Region of Niteróí Municipality, State of Rio de Janeiro, Brazil

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*Dirofilaria immitis* is a widespread mosquito-borne parasite that causes dirofilariasis, a commonly diagnosed disease of dogs that is rarely reported in cats and humans. A mosquito survey was conducted in Itacoatiara in the State of Rio de Janeiro, from March 1995 to February 1996, using canine, feline and human baits. A total of 3,667 mosquitoes were dissected for *D. immitis* larvae, representing 19 species and 10 genera. From those, *Ae. scapularis*, *Ae. taeniorhynchus*, *Cx. quinquefasciatus*, *Cx. declarator*, *Cx. saltanensis* and *Wy. bourrouli* were found infected with *D. immitis* parasites, and among those, only the first three harbored infective larvae. The majority of larvae were found in the Malpighian tubules (889/936), and larval melanization was observed in the two *Aedes* species. In descending order, the best vectors were *Ae. scapularis*, *Ae. taeniorhynchus*, and *Cx. quinquefasciatus* which alternate seasonally in importance. *Cx. quinquefasciatus* is suggested to be a vector to cats. The potential transmission of *D. immitis* parasites by these three vectors to man is discussed.

Key words: *Dirofilaria immitis* - mosquitoes - heartworm - potential vector

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*Dirofilaria immitis* (Leidy 1856) is a widespread mosquito-borne nematode parasite of dogs. While canine heartworm is enzootic in many areas (Guerrero et al. 1992a), feline dirofilariasis is much less common. Feline heartworm infections parallels that of dogs in a defined area. While feline dirofilariasis is at a lower infection rate than canine dirofilariasis (Dillon 1988, Elkins & Kadel 1988), it is thought to be increasing in prevalence and distribution (Guerrero et al. 1992b). Heartworm is also a zoonosis (OMS 1979).

Heartworm is common in dogs in Rio de Janeiro, Brazil (21.3%). In the coastal region of the county of Niteróí an even higher prevalence is observed (37.5%), and microfilaremic dogs, the source of mosquito infections, account for 25% of the local population (Labarthe et al. 1997a).

Dogs may have high levels of microfilaremia ($10^3$ to $10^5$/ml) (Lok 1988). Mosquitoes blood-feeding on dogs with even moderate numbers of microfilariae frequently die (Sauerman 1980). Mosquitoes need to survive *D. immitis* infection in order to support the extrinsic cycle of the nematode through development of infective third stage larvae ($L_3$). Vector survival seems to rely on biochemical reactions which limit the nematode burden in the mosquito (Christensen 1977, 1978, 1981). Intrinsic barriers to *D. immitis* development in mosquitoes include: larval damage due to the cibarial armature (Coluzzi & Trabucchi 1968) or to the presence of oxyhaemoglobin crystals formed by bloodmeal coagulation in the midgut (Nayar & Sauerman 1975, Lowrie 1991); trapping of larvae in the coagulated bloodmeal in the mosquito’s midgut (Kartman 1953); lysis of larval cuticle by host cells (Talluri & Cancrini 1994) and other immune responses, i.e., encapsulation and/or melanization of the parasite in the mosquito Malpighian tubules (Lindemann 1977, Christensen 1981, Christensen et al. 1989). Among extrinsic factors, temperature is the most important and has been shown to regulate the duration of parasite development in the mosquito (Kutz & Dobson 1974, Christensen & Hollander 1978).

Laboratory and field data show that many mosquito species, from different geographic areas worldwide, are susceptible and yield infective larvae under field or laboratory conditions. Although more than 60 mosquito species have been identified as potential vectors of *D. immitis* worldwide (Ludlam et al. 1970), their vectorial capacity varies (Sauerman 1980). In the Americas, mosquito
species belonging to the subgenus *Ochlerotatus* of *Aedes* are considered the best vectors of heartworm (Yen 1938, Kershaw et al. 1953, Ludlam et al. 1970, Christensen 1977, Arnott & Edman 1978, Buxton & Mullen 1980, Walters & Lavoipierre 1982, Sauerman & Nayar 1983, Ernst & Slocombe 1984, Hribar & Gerhardt 1985, Roberts et al. 1985, Johnson & Harrell 1986, Parker 1986, 1993, Scoles et al. 1993, Loftin et al. 1995). In Brazil, there are two reports: one under experimental conditions with *Ae. fluviatilis* (Lutz) that suggested that although infective larvae have been found in some individuals, this species is not likely to be an efficient vector in nature (Kasai & Williams 1986) and another in which *Ae. taeniorhynchus* (Wiedemann) and *Ae. scapularis* (Rondani) were shown to be suitable natural potential vectors of heartworms in Rio de Janeiro (Lourenço-de-Oliveira & Deane 1995).

In nature, the complete life cycle of the mosquito must be considered before concluding either that a given species or strain is a probable vector of *D. immitis*. The present study examines infection rates of mosquito species with *D. immitis* at Niterói in relation to their biology (feeding behavior and seasonal biting frequency).

**MATERIALS AND METHODS**

Mosquitoes were collected in Itacoatiara, State of Rio de Janeiro, where the average prevalence of canine microfilarial heartworm is 31.7% (unpublished data). Data on the collection sites and methods are available in Labarthe et al. (1998). Briefly, mosquitoes were captured four days each month using a dog, a cat and two human volunteers as baits, from March 1995 until February 1996.

Mosquitoes were kept in cylindrical cages of 8.5 cm diameter at 28°C, 80% RH and provided with a 10% glucose solution. Dissection of live females was initiated immediately after collections, and all mosquitoes were dissected within five days of collection. After chloroform anesthesia, each mosquito was identified using taxonomic keys of Lane (1953) and Consoli and Lourenço-de-Oliveira (1994). Subsequently, the head was placed in a small saline droplet on a microscope slide, and the alimentary tract and Malpighian tubules were drawn from the body into another saline droplet by gently pulling the terminal segments backwards with hypodermic needles. The thorax was teased apart in a third droplet of saline. All preparations were examined for the presence of worms under microscopic magnification after placement of a coverslip.

Larvae found in the mosquitoes were identified as *D. immitis* based on: morphological characteristics previously described (Taylor 1960) and those observed in experimental infections (Macêdo et al. 1998); the Malpighian tubule developmental site of worms, known only among species of the genus *Dirofilaria* of Onchocercidae (Symes 1960, Walters & Lavoipierre 1982, Sauerman & Nayar 1983); the only *Dirofilaria* species known from the lowlands of Rio de Janeiro is *D. immitis* (Lourenço-de-Oliveira & Deane 1995); and the mosquito collecting site is an active *D. immitis* transmission focus (Labarthe et al. 1997a).

The infection rate was determined as the percentage of numbers of a species infected with any stage larvae (Chandra et al. 1996). The transmission capacity of each species was determined by the annual transmission potential (ATP) that corresponds to the sum of the monthly transmission potentials (MTP) (WHO 1987), where:

\[
MTP = \frac{\text{no. of mosquitoes caught}}{\text{no. of mosquitoes dissected}} \times \frac{\text{no. of } L_3 \text{ in the head and proboscis}}{\text{no. of days in the month}} \times \frac{\text{no. of } L_3 \text{ in head and proboscis}}{\text{no. of days}}
\]

The frequencies of infected and infective mosquitoes (with *L* _3_ in head and proboscis) were analyzed by chi-square or when values were less than 5, Fisher’s exact test was used (Mood & Graybill 1963, Rodrigues 1993).

**RESULTS**

A total of 3,667 mosquitoes belonging to 19 species and 10 genera was captured and dissected. From those, six species were found naturally infected with various larval stages. Only *Ae. taeniorhynchus*, *Cx. quinquefasciatus* and *Ae. scapularis* harbored infective larvae in the head and proboscis, and so were rated as infective (Table 1). Traditional statistical analysis crossing species versus infective and infected mosquitoes showed little or no relevance to the objective of the present study as chi-squares and Fisher tests became less statistically significant as relevant species were segregated and crossed among each other. When the number of uninfected *Ae. taeniorhynchus*, *Cx. quinquefasciatus*, *Ae. scapularis* and *Cx. declator* captured were analyzed versus their infected members, *Ae. taeniorhynchus* was the most infected, followed by *Ae. scapularis*, *Cx. quinquefasciatus* and *Cx. declator* (chi-square 19.1, p<0.01). When infectivity was analyzed, Fisher test showed p>0.07 for all combinations among the three species found infective.

A total of 936 larvae was found among the dissected mosquitoes. The majority of larvae (889/936) was found in the Malpighian tubules (Fig. a) and half of the infective mosquitoes (4/8) still har-
bored either L2 or L3 larvae in the tubules. Twenty eight infective larvae were found in the head and proboscis of mosquitoes: eighteen were found in *Ae. scapularis* (Fig. b) in January 1996; four in April and three in July 1995 in *Ae. taeniorhynchus* (Fig. c); and two in August and one in November 1995 in *Cx. quinquefasciatus*. Melanization of larvae was observed only in the two *Aedes* species. In *Ae. taeniorhynchus* and *Ae. scapularis*, 9.5% and 16.7% of the harbored larvae were melanized, respectively (Table I). All *Ae. taeniorhynchus* with some melanized larvae had at least 45 larvae, except for one with 55 larvae in the cells of the Malpighian tubules, none of which were melanized. Among *Ae. scapularis*, melanization was observed even when the mosquito had as few as eight larvae. However, melanization of larvae was not observed in six *Ae. scapularis* harboring 9-20 larvae.

When the ATP was considered, the best vector in the surveyed area was *Ae. scapularis*, followed by *Ae. taeniorhynchus* and *Cx. quinquefasciatus* (Table I).

Infected mosquitoes were found in every month of the year, but February, while infective mosquitoes were found sparsely throughout the year (Table II).

**DISCUSSION**

To vector *D. immitis*, mosquitoes must live long enough to allow complete filarial development. Multivoltine species would probably make better vectors than univoltine species, and a specie’s flight range and host seeking preference can influence the importance of a species as a vector (Ludlam et al. 1970, Otto & Jachowski 1980). In the present survey, *Ae. taeniorhynchus*, *Ae. scapularis* and *Cx. quinquefasciatus* were found to fulfill these prerequisites and to be natural vectors of *D. immitis* in Itacoatiara. *Ae. taeniorhynchus* and *Cx. quinquefasciatus* have been known as natural vectors of *D. immitis* in other areas (Villavaso & Steelman 1970, Sauerman & Nayar 1983, Russell 1985, Parker 1986, 1993). In Rio de Janeiro, both *Ae. taeniorhynchus* and *Ae. scapularis* have already been found naturally infected with presumed *D. immitis* larvae and considered to be potential vectors of the parasite (Lourenço-de-Oliveira & Deane 1995). In the surveyed area the annual transmission potential (ATP) for *Ae. scapularis* was approximately three times that of *Ae. taeniorhynchus* and six times more than that of *Cx. quinquefasciatus*. The differences in ATP values is related to variations in mosquito population density, biting frequency and distribution throughout the year (Labarthe et al. 1998), showing that *Ae. scapularis* is the most important vector in Itacoatiara, followed closely by *Ae. taeniorhynchus*. *Cx. quinquefasciatus* is a secondary vector. In localities in the State of Rio de Janeiro (FEEMA 1983) where the hemisynanthropic primary vectors are abundant, canine heartworm frequency is high while where the endophilic secondary vector predominates, canine heartworm frequency is low (Labarthe et al. 1992, Souza 1992).

*Cx. declarator*, *Cx. saltanensis* and *Wy. bourrousii* did not harbor infective larvae and have never been described as potential vectors of *D. immitis*. Therefore, their infections are thought to be a dead end and if so, have no epidemiological
TABLE I

Infection rate, infective mosquitoes, larvae melanization and annual transmission potential (ATP) of mosquitoes infected with *Dirofilaria immitis*, captured in Itacoatiara, State of Rio de Janeiro, Brazil with human, canine and feline baits

<table>
<thead>
<tr>
<th>Species</th>
<th>Infection rate (a)</th>
<th>Infective mosquitoes (b)</th>
<th>Larvae melanization (c)</th>
<th>ATP (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aedes taeniorhynchus</em></td>
<td>31/1037 (3%)</td>
<td>2/31</td>
<td>60/630 (9.5%)</td>
<td>57.2</td>
</tr>
<tr>
<td><em>Ae. scapularis</em></td>
<td>13/715 (1.8%)</td>
<td>3/13</td>
<td>44/264 (16.7%)</td>
<td>147.6</td>
</tr>
<tr>
<td><em>Culex quinquefasciatus</em></td>
<td>8/865 (0.9%)</td>
<td>3/8</td>
<td>0/28</td>
<td>23.4</td>
</tr>
<tr>
<td><em>Cx. declarator</em></td>
<td>2/644 (0.3%)</td>
<td>0</td>
<td>0/12</td>
<td>0</td>
</tr>
<tr>
<td><em>Wyeomyia bourrouli</em></td>
<td>1/43 (2.3%)</td>
<td>0</td>
<td>0/1</td>
<td>0</td>
</tr>
<tr>
<td><em>Cx. saltanensis</em></td>
<td>1/17 (5.9%)</td>
<td>0</td>
<td>0/1</td>
<td>0</td>
</tr>
<tr>
<td>Other species(e)</td>
<td>0/346</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Total 56/3667 8/52 104/936 228.2

\(a\): no. of female mosquitoes found harboring any larval stage/total no. of dissected mosquitoes; \(b\): no. of mosquitoes found with L3 in head and/or proboscis/no. of infected mosquitoes; \(c\): total no. of melanized larvae found/total no. of larvae; \(d\): the sum of the monthly transmission potentials (WHO 1987); \(e\): other captured species in Labarthe et al. (1998).

Importance of heartworm transmission in the surveyed area.

Melanization of larvae seems to be an important survival reaction of *Aedes* mosquitoes to *D. immitis* infection (Lindemann 1977, Christensen 1977, 1978, 1981, Christensen et al. 1989). Melanized larvae were seen in heavily infected *Ae. taeniorhynchus* mosquitoes but one, which had all 55 larvae in the cells of the Malpighian tubules, suggesting that the infection was too recent for melanization to have taken place. *Ae. scapularis* mosquitoes melanized a larger proportion of larvae than *Ae. taeniorhynchus*, and melanization did not seem to be related to the number of larvae. Since *Cx. quinquefasciatus* mosquitoes were never observed with melanized larvae but always had smaller numbers of larvae than *Ae. scapularis* and *Ae. taeniorhynchus*, it seems that they control larval burden by other mechanisms, such as by reducing the number of larvae by the cibarial armature (Coluzzi & Trabucchi 1968).

Transmission at Itacoatiara can potentially occur throughout the year, since infected and infective mosquitoes were found, respectively, in 11 and 5 months of the year. Environmental temperature directly influences the rate of larval development in the mosquito (Kutz & Dobson 1974, Christensen & Hollander 1978) so, transmission potential can change seasonally with temperature. Different species of mosquitoes were infective during different months: *Ae. taeniorhynchus* in autumn and early winter, *Cx. quinquefasciatus* in winter and spring and *Ae. scapularis* in the summer. These data strongly suggest that in the surveyed area, monthly heartworm chemoprophylaxis should be given to dogs year-round, in contrast with northern temperate latitudes, where chemoprophylaxis is recommended only during some months, depending mainly on the recorded temperatures of each region (Knight & Lok 1995, Slocombe et al. 1995).

Cats, in spite of being susceptible to *D. immitis* (McCall et al. 1992), are rarely found naturally infected. For instance, in urbanizing sections of Rio de Janeiro, where the canine heartworm prevalence is high (30.9%) (Labarthe et al. 1997a), feline heartworm prevalence is 1.6% (Labarthe et al. 1997b). This expressive difference in prevalence may be explained by the fact that the primary vectors of the parasite, *Ae. scapularis* and *Ae. taeniorhynchus*, are hemisynanthropic mosquito species which seek cats almost accidentally (Labarthe et al. 1998). *Cx. quinquefasciatus*, herein considered a secondary vector, is the only vector species commonly associated with cats (Genchi et al. 1992, Labarthe et al. 1998). That is, the potential mosquito vector for cats is a modest *D. immitis* vector in Rio de Janeiro. Furthermore, both cats and *Cx. quinquefasciatus* show marked nocturnal behavior; free cats are active at the time when the vectors are seeking blood meals, making it more difficult for mosquitoes to feed on them. Although somehow protected from mosquitoes, 33 to 36% of heartworm positive cats are indoors (Atkins 1997). *Cx. quinquefasciatus* is an endophilic species (Deane 1951, Rachou 1956), therefore, in places where their density is high, once an infective mosquito comes in the house, it can feed on cat and infect it.

Since *D. immitis* is infective to man (OMS 1979), it is frequently diagnosed among Brazilians in the three natural vectors (*Ae. scapularis*, *Ae. taeniorhynchus* and *Cx. quinquefasciatus*) have been shown to seek humans in the studied heartworm focus (Labarthe et al. 1998), human cases of dirofilariasis may be expected in
TABLE II
Monthly frequency of mosquitoes infected with *Dirofilaria immitis* captured in Itacoatiara, State of Rio de Janeiro, Brazil, with human, canine and feline baits and number of live larvae and of infective mosquitoes

<table>
<thead>
<tr>
<th>Species</th>
<th>1995 Frequency</th>
<th>1996 Frequency</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mar</td>
<td>Apr</td>
<td>May</td>
</tr>
<tr>
<td><em>Aedes taeniorhynchus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of live larvae</td>
<td>2/50</td>
<td>4/393</td>
<td>7/162</td>
</tr>
<tr>
<td><em>Ae. scapularis</em></td>
<td>0/8</td>
<td>0/35</td>
<td>2/38</td>
</tr>
<tr>
<td>No. of live larvae</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td><em>Culex quinquefasciatus</em></td>
<td>0/73</td>
<td>0/77</td>
<td>0/34</td>
</tr>
<tr>
<td>No. of live larvae</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Cx. declarator</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No. of live larvae</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Wyeomyia bourrouli</em></td>
<td>0/5</td>
<td>1/9</td>
<td>0/10</td>
</tr>
<tr>
<td>No. of live larvae</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>Cx. saltanensis</em></td>
<td>-</td>
<td>1/1</td>
<td>0/3</td>
</tr>
<tr>
<td>No. of live larvae</td>
<td>-</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

| No. of live larvae       | 2     | 84    | 154  | 236    | 9      | 76    | 2     | 2     | 152    | 25   | 90      | 0     | 832           |

*a*: infected mosquitoes/total dissected; *b*: one mosquito with infective larvae in head and/or mouthparts; *c*: three mosquitoes with infective larvae in head and/or mouthparts; *d*: two mosquitoes with infective larvae in head and/or mouthparts.
the study area. Also, since both the canine and culicid fauna are similar along the lowland areas in the State of Rio de Janeiro (Labarthe et al. 1997a, Lourenço-de-Oliveira 1985a,b), health professionals should more seriously consider dirofilariasis among the many possible causes of solitary lesions of the human lung (Levinson et al. 1979, Campos et al. 1997).

ACKNOWLEDGMENTS
To Carlos Alberto Coimbra and Antônio Bernardo da Costa for discussing data. To LP Lounibos for the critical reading of the manuscript. To Genilton Vieira and Heloisa MN Dinis for assistance with images.

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