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Original article

Mosquito abundance in a *Dirofilaria immitis* hotspot in the eastern state of Rio de Janeiro, Brazil

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ABSTRACT

Coastal lowlands in the state of Rio de Janeiro, Brazil, support high numbers of heartworm-infected dogs. Microfilariae of heartworm need to be ingested by a potencial mosquito vector in order to develop into infective larvae and infect a new host. *Ochlerotatus taeniorhynchus* and *Ochlerotatus scapularis* are the primary vector species in the coastal lowlands of metropolitan Rio de Janeiro; thus, the aim of this study was to investigate whether these two species were abundant enough at the heartworm hotspot in the eastern area of the state to be important to the local parasite's life cycle. The study was conducted at the Massambaba sandbank (22°55′45″S;42°18′51″W), where canine heartworm prevalence was 53.1%. Mosquitoes were captured monthly using two traps with CO_2, as well as eventual human landing. A total of 6118 mosquitoes of 16 species were collected. *Ochlerotatus taeniorhynchus* (37.5%), *Culex quinquefasciatus* (19.3%), *Oc. scapularis* (18.2%), and *Coquillettidia* sp. (10.5%) were the most abundant species. The monthly variation in frequency was marked for *Oc. scapularis* and *Cx. quinquefasciatus* and steadier for *Oc. taeniorhynchus* and *Coquillettidia* sp. The abundance of the two *Ochlerotatus* species reaffirms that they are the primary natural vectors for *D. immitis* in the coastal Rio de Janeiro state lowlands.

1. Introduction

The life-threatening canine nematode *Dirofilaria immitis* (Leidy, 1856) Raillet & Henry, 1911 inhabits the pulmonary arteries and right cardiac chambers, where males and females mate and deliver innumerous microfilariae into the host's blood (Knight, 1987). The coastal lowlands of the state of Rio de Janeiro are known to support high numbers of heartworm-infected dogs. Studies have shown prevalence as high as 52% in the past (Labarthe et al., 1997), and more recently, 44.2% (Labarthe et al., 2014) or 53.1% (Bendas et al., 2007).

For the life cycle to be completed, microfilariae need to be ingested by potential mosquito vectors while they take their blood meal. The microfilariae develop into infective larvae (L3) within the vector, and when the larvae move to the head or mouthparts of the mosquito, they are ready to infect a new host. When the mosquito takes its next blood meal, larvae migrate toward the host's skin and infect the new host. If the new host is susceptible to the infection, the larvae will migrate toward the pulmonary arteries and right heart chambers, develop into adults, and deliver microfilariae in approximately six months (Knight, 1987).

There are at least 70 competent mosquito species (Ludlam et al., 1970; Serrão et al., 2001; Cancrini et al., 2006), although their vectorial capacity varies (Sauerman, 1980). In order to vector *D. immitis* mosquitoes must be susceptible yet resistant to the infection (Christensen, 1977, 1978). During the extrinsic cycle, larvae may kill mosquitoes due to Malpighian tubule cell invasions or to infective larval migration

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toward the head and mouthparts (Kutz and Dobson, 1974; Serrão et al., 2001).

In the coastal lowlands of metropolitan Rio de Janeiro, Brazil, *Ochlerotatus taeniorhynchus* and *Ochlerotatus scapularis* are known to support the development of large numbers of larvae and have been reported as primary *D. immitis* vectors, whereas *Culex quinquefasciatus* is reported to be a secondary vector (Labarthe et al., 1998a,b). Therefore, the aim of this study was to investigate whether the species considered to be the primary vectors of *D. immitis* in the metropolitan area of Rio de Janeiro were frequent enough at the *D. immitis* hotspot in the eastern area of the state to be important to the local parasite's life cycle.

2. Material studied, area descriptions, methods, techniques

The survey was conducted in an active *D. immitis* hotspot at Massambaba sandbank, Arraial do Cabo municipality, RJ, Brazil (22°55′45″S; 42°18′51″W). Mosquitoes were captured from December 2005 through May 2007 in an area where canine heartworm prevalence was known to be 53.1% at the time of mosquito collection (Bendas et al., 2007).

Mosquito capture was performed monthly using two CDC (Centers for Disease Control and Prevention, Atlanta, USA) traps with CO_2 (Bates, 1949) and human landing captures (Buxton, 1928). Traps were lit at dusk and collected 12 h later. While the operators were installing the traps, mosquitoes landing on them were also collected.

The frequency distribution was analyzed using the chi-square in contingency tables with Fisher's exact test (Sampaio, 2002). The monthly number of collected individuals of the most frequent species was normalized using log (x + 1) to analyze serial data (Williams, 1937; Haddow, 1954).

3. Results

A total of 6118 mosquitoes of 16 species were collected. The most abundant species were *Oc. taeniorhynchus* (37.5%), *Cx. quinquefasciatus* (19.3%), *Oc. scapularis* (18.2%) and *Coquillettidia sp.* (10.5%). *Oc. taeniorhynchus* was the most frequent as compared to *Cx. quinquefasciatus* ($x^2 = 498.43$; p = 0.0000), *Oc. scapularis* ($x^2 = 568.97$; p = 0.0000), or *Coquillettidia sp.* ($x^2 = 1225.09$; p = 0.0000). There was no difference between *Cx. quinquefasciatus* and *Oc. scapularis* frequencies ($x^2 = 2.48$; p = 0.1153052) (Table 1).

Table 1
Number of collected mosquitoes in a heartworm hotspot at the Massambaba sandbank, Arraial do Cabo municipality, RJ, Brazil by species from December 2005 to May 2007.

Species	Mosquitoes	
	Frequency	%
Ochlerotatus taeniorhynchus (Widemann, 1841)	2297	37.5[a]
Culex quinquefasciatus (Say, 1823)	1182	19.3[b]
Ochlerotatus scapularis (Rondani, 1848)	1113	18.2[b]
Coquillettidia sp. (Dyar, 1905)	642	10.5[c]
Culex (Melanoconion) sp. (Theobald, 1903)	265	4.3[d]
Culex declarator (Dyar & Knab, 1906)	179	2.9[e]
Anopheles albitarsis (Lynch Arribalzaga, 1878)	153	2.5[e]
Limatus sp. (Theobald, 1901)	105	1.7[f]
Culex coronator (Dyar & Knab, 1906)	73	1.2[g]
Culex (Culex) sp. (Linnaeus, 1758)	41	0.7[h]
Aedes sp. (Meigen, 1818)	24	0.4[i]
Mansonia sp. (Blanchard, 1901)	18	0.3[i]
Culex nigripalpus (Theobald, 1901)	16	0.3[i]
Anopheles aquasalis (Curry, 1932)	5	0.08[j]
Phoniomyia sp. (Theobald, 1903)	4	0.07[j]
Anopheles sp. (Meigen, 1818)	1	0.02[j]
Total	6118	100

Different superscript letters indicate statistical difference at 5%.

The monthly variation in frequency among the known heartworm potential vectors in the Rio de Janeiro State lowlands showed that variations in *Oc. scapularis* (CV = 221.3%) and *Cx. quinquefasciatus* (CV = 226.9%) were marked and that the frequencies of *Oc. taeniorhynchus* (CV = 98.4%) and *Coquillettidia sp.* (CV = 99.9%) were steadier (Table 2). The month with the highest total number of captures was January 2007 (967 mosquitoes), and the lowest numbers were in March (19 mosquitoes) and April (17 mosquitoes) of the same year (Fig. 1, Table 2).

4. Discussion

Among the four most frequently collected mosquito species were the three known competent heartworm vectors registered approximately 10 years before at a similar area of the state of Rio de Janeiro. The former study was carried out at an area (22°55′S; 43°03′W) approximately 84 km from the present study area, where there were preserved patches of Atlantic forest, lagoons formed by sandbars, and lowland mostly covered by the sandbank vegetation (Labarthe et al., 1998a; Labarthe et al., 1998b). Although the present study did not focus on determining the mosquitoes' infectiveness, when considering the local number of infected dogs and the most frequent mosquito species, it can be suggested that the local primary vectors are *Oc. taeniorhynchus* and *Oc. scapularis* and the secondary vector *Cx. quinquefasciatus*, as determined previously (Labarthe et al., 1998b). The genus *Coquillettidia* was not previously registered as a vector in Brazilian coastal lowlands; even so, it cannot be neglected since the genus has been listed as a *D. immitis* larvae-harboring culicide in Italy (Cancrini et al., 2006).

The high monthly variation in frequency of *Oc. scapularis* (CV = 221.3%) is to be expected since its breeding sites are shallow and transient and depend on clean water that can be supplied by rain or river overflow, among others (Forattini, 2002). The high CV confirmed the multivoltine pattern that is thought to make mosquitoes long-lived enough to transmit *D. immitis*. Although *Oc. scapularis* was not the most frequent species collected, it might display good vectorial capacity due to its lack of cibarial armature, a foregut structure that can damage the worms as they are taken with the blood meal (McGreevy et al., 1978). As a matter of fact, its annual transmission potential (ATP) has been graded as 147.6 (Labarthe et al., 1998b). The fact that the species *Oc. taeniorhynchus* was collected in 17 of the 18 months of the survey must reflect the availability of brackish water breeding sites since the poorly drained peaty soil allowed permanent flooding. Since it is abundant, lacks cibarial armature, and supports larval development to the infective stage (ATP = 57.2) (Labarthe et al., 1998b), it may be inferred that in Massambaba, as well as in Niterói, it is a potentially competent *D. immitis* vector.

Although *Cx. quinquefasciatus* was also shown to be long-lived (CV = 226.9%), the species is known to harbor few infective larvae, and is therefore considered a secondary vector due to its low ATP (ATP = 23.4) (Labarthe et al., 1998b). Furthermore, *Culex* species have the larvae-damaging structure that *Ochlerotatus* lack, the cibarial armature that can injure microfilariae (McGreevy et al., 1978). The importance of the cibarial armature has been reinforced by the fact that microfilarial density in *Cx. quinquefasciatus* blood meal does not influence its vectorial capacity (Carvalho et al., 2008).

5. Conclusion

Since *Oc. scapularis* and *Oc. taeniorhynchus* population densities showed that the Massambaba sandbank had the characteristics needed to support an abundance of both mosquito species, it may be reaffirmed that along the coastal Rio de Janeiro state lowlands they are the main natural potencial vectors for *D. immitis*.

Table 2
Monthly distribution of the most frequent mosquito species captured in the Massambaba sandbank, Arraial do Cabo, RJ, Brazil.

Species		2005	2006												2007					Total
		Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	
Ae. taeniorhynchus (Widemann, 1841)	Freq	90	109	244	341	288	63	121	13	3	66	8	60	248	247	364	0	3	29	2297
	log(x + 1)	1.96	2.04	2.39	2.53	2.46	1.81	2.09	1.15	0.60	1.83	0.95	1.79	2.40	2.39	2.56	000	0.60	1.48	
Cx. quinquefasciatus Say, 1823	Freq	17	2	6	10	13	540	392	82	6	10	7	11	10	36	24	5	7	4	1182
	log(x + 1)	1.26	0.48	0.85	1.04	1.15	2.73	2.59	1.92	0.85	1.04	0.90	1.08	1.04	1.57	1.40	0.78	0.90	0.70	
Oc. scapularis (Rondani, 1848)	Freq	9	93	36	27	157	10	24	1	3	2	0	1	77	582	80	0	0	11	1113
	log(x + 1)	1.00	1.97	1.57	1.45	2.20	1.04	1.40	0.30	0.60	0.48	0.00	0.30	1.89	2.77	1.91	0.00	0.00	1.08	
Coquillettidia sp. Dyar, 1905	Freq	29	56	18	38	110	12	14	24	18	133	41	2	48	33	50	10	3	3	642
	log(x + 1)	1.48	1.76	1.28	1.59	2.05	1.11	1.18	1.40	1.28	2.13	1.62	0.48	1.69	1.53	1.71	1.04	0.60	0.60	
Other species[a]	Freq	73	103	52	78	73	109	97	77	21	8	12	53	20	69	18	4	4	13	884
	log(x + 1)	1.0.87	2.0.02	1.72	1.90	1.87	2.04	1.99	1.89	1.34	0.95	1.11	1.73	1.32	1.85	1.28	0.70	0.70	1.15	
Total	Freq	218	363	356	494	641	734	648	197	51	219	68	127	403	967	536	19	17	60	6118
	log(x + 1)	2.0.34	2.0.56	2.55	2.69	2.81	2.87	2.81	2.30	1.72	2.34	1.84	2.11	2.61	2.99	2.73	1.30	1.26	1.79	

Freq: Absolute number.
log (x + 1): logarithm base 10 of the absolute number of mosquitoes +1 according to Williams, 1937 and Haddow, 1954.
[a] *Culex* (*Melanoconion*) sp., *Culex declarator*, *Anopheles albitarsis*, *Limatus* sp., *Culex coronator*, *Culex* (*Culex*) sp., *Aedes* sp., *Mansonia* sp., *Culex nigripalpus*, *Anopheles aquasalis*, *Phoniomyia* sp. e *Anopheles* sp.

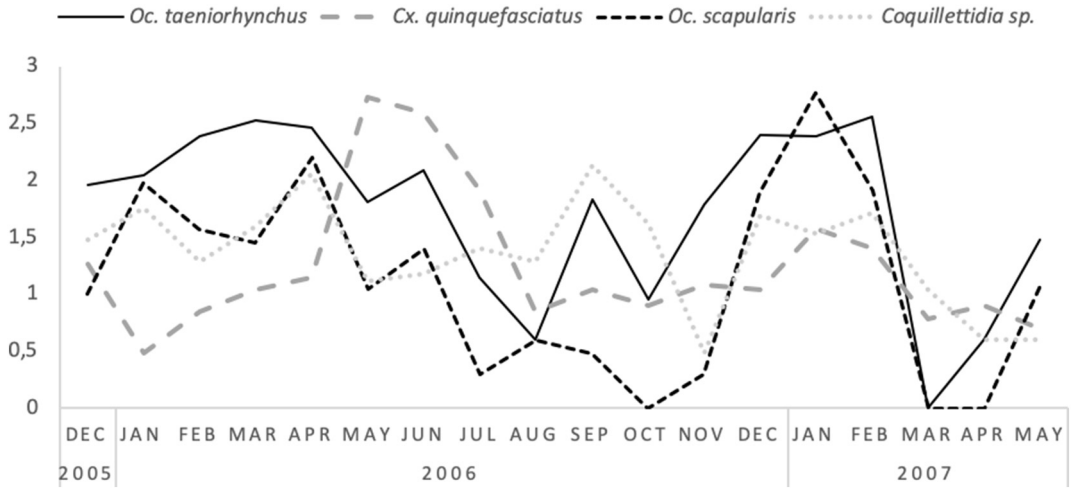

Fig. 1. Monthly variation in the number of the four most frequently collected mosquito species at the Massambaba sandbank, Arraial do Cabo, Brazil.

Declarations of Competing Interests

None.

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