

AN UPDATE SURVEY OF THE PREVALENCE OF CANINE DIROFILARIASIS IN A FOCUS AREA OF THE CITY OF RIO DE JANEIRO, BRAZIL

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ABSTRACT:- COSTA, R.C.; COUTO-LIMA, D.; SERRÃO, M.L.; LABARTHE, N. **An update survey of the prevalence of Canine Dirofilariasis in a focus area of the city of Rio de Janeiro, Brazil.** [Atualização da prevalência da Dirofilariose Canina em uma área de foco na cidade do Rio de Janeiro, Brasil.] *Revista Brasileira de Parasitologia Veterinária*, v. 13, n. 1, p. 23-28, 2004. Curso de Pós-Graduação em Ciências Veterinárias (CPGCV), Universidade Federal Rural do Rio de Janeiro (UFRRJ), BR-465, Km 7, 23890-000, Seropédica, RJ, Brasil. E-mail: animalia@rjnet.com.br

Dirofilaria immitis is a nematode transmitted by culicids, commonly found all over the world. In the western area of Rio de Janeiro, where in 1990 31% of dogs were parasitized, a survey of the culicid fauna and an update of the prevalence of canine dirofilariasis was carried out in two populations: one of them not assisted and the other one regularly assisted by veterinarians. To evaluate whether the preventive medication recommended by the veterinarian was followed, the number of attended animals was compared with the number of preventive doses sold by the veterinary clinic. Female mosquitoes of the species *Aedes scapularis*, *Culex quinquefasciatus* and *A. taeniorhynchus*, efficient vectors in the region, were captured in low frequencies. The prevalence of canine infection by *D. immitis* detected by an immunoenzymatic test was 1.96% in the population not assisted by veterinarians with no microfilaremic animal and no positive dog was found among those which were regularly attended. Clinical compliance was of 45.5%, a higher percentage than that found in hyperendemic regions in the United States (41%). A decline in the prevalence of canine heartworm disease in the area under study could be noted and observance of the veterinary prescription for preventive medication seems to have contributed to this effect.

KEY WORDS: Vectors, Culicidae, Canine heartworm, Epidemiology, Clinical compliance.

RESUMO

Dirofilaria immitis é um nematódeo transmitido por culicídeos, encontrado em todo o mundo. Na Zona Oeste do Rio de Janeiro onde, em 1990, 31% dos cães estavam parasitados, uma pesquisa da fauna culicídea e atualização da prevalência da dirofilariose canina foi feita em duas populações: uma não assistida por veterinários e outra regularmente assistida por veterinários. Para avaliar se a medicação preventiva recomendada pelos veterinários era seguida, o número

total de animais atendidos foi comparado ao número de doses de preventivos vendidos em uma clínica veterinária da região. Mosquitos fêmeas das espécies *Aedes scapularis*, *Culex quinquefasciatus* e *A. taeniorhynchus*, vetores eficientes na região, foram capturados em baixa frequência. A prevalência da infecção canina por *D. immitis*, detectada por teste imunoenzimático, foi de 1,96% na população não assistida por veterinários e nenhum cão positivo foi encontrado entre aqueles regularmente assistidos. A adesão à prescrição foi de 45,5%, um percentual maior do que o encontrado em regiões hiperendêmicas dos Estados Unidos (41%). O declínio da prevalência de dirofilariose canina na área estudada foi observado e a adesão à prescrição veterinária de medicação preventiva parece ter contribuído para esta diminuição.

PALAVRAS-CHAVE: Vetores, Culicidae, Dirofilariose canina, Epidemiologia, Adesão à prescrição.

INTRODUCTION

Canine dirofilariasis is caused by the parasite *Dirofilaria immitis* (Leidy, 1856), a nematode that, in adult stage, is mainly

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found in the pulmonary arteries and in the right ventricle of the definite host. This parasitosis, which mainly affects domestic dogs but which already has been reported in more than 30 species including man, is considered a zoonosis since 1979 (OMS, 1979). In Brazil, an increasing number of cases of human dirofilariasis have been described in the literature (RODRIGUES-SILVA et al., 1995).

The life cycle of *D. immitis* implies in a passage through its intermediate hosts, the culicids. Although more than seventy species of mosquitoes in different regions of the world already had their vector competence proven, it is accepted that only a few species are responsible for transmitting the parasite under natural conditions (OTTO; JACKOWSKI, 1981). Studies carried out in the State of Rio de Janeiro, Brazil, indicated *Aedes scapularis* (Rondani) and *A. taeniorhynchus* (Wiedman) as primary vectors and *Culex quinquefasciatus* as secondary vector in the transmission of canine heartworm disease (LOURENÇO-DE-OLIVEIRA; DEANE, 1995; LABARTHE et al., 1998b).

During many years diagnosis has relied on identification of microfilariae in the blood, the diagnostic technique most commonly used at present however is detection of antigens of the adult parasite, a method also capable of detecting occult forms of the infection (McTIER et al., 1995; McCALL et al., 2001; COURTNEY, 2001; LABARTHE et al., 2003).

The prevalence of canine infection by *D. immitis* in Brazil varies considerably from region to region. In the State of Rio de Janeiro, studies carried out between 1936 and 1992 indicate variations ranging from 4.9% (PINTO; LUZ, 1936) to 34% (HATSCHBACH et al., 1976) of infected dogs. Recently, in a study carried out in 2002, the prevalence

found was of 3.8% (LABARTHE et al., 2003). In the western area of Rio de Janeiro, in a district called Itanhangá, studies carried out in the course of the last two decades reported infection rates between 31% in 1990 (LABARTHE et al., 1997) and 100% in 1981 (ALMEIDA, 1981) among the investigated dogs.

The aim of this study was to provide an updated mapping of the prevalence of canine infection by *D. immitis* and of the culicid fauna in the region, once the number of infected dogs is decreasing in the region during the last decade, in order to explain the variations among the results obtained in this work and in previous studies.

Since preventive medication was first launched in Brazil in 1992 (ALMOSNY, 2002) the clinical compliance could be one of the reasons for the decline, therefore, owners compliance was evaluated.

MATERIAL AND METHODS

Area of survey

The study was carried out in a district named Itanhangá, situated in the western area of the city of Rio de Janeiro, Brazil (Fig. 1), which together with eight other districts composes the administrative region of Barra da Tijuca. The estimated human population is of 200,000 inhabitants (CARVALHO HOSKEN, 1996) and the canine population of 20,000 (10% of the human population) according to the WHO's estimative (OMS/WSPA, 1990). Besides extensive preserved areas and biological reservations, there are lakes in the interior of the region and the Atlantic Ocean bathes the beaches of its littoral.

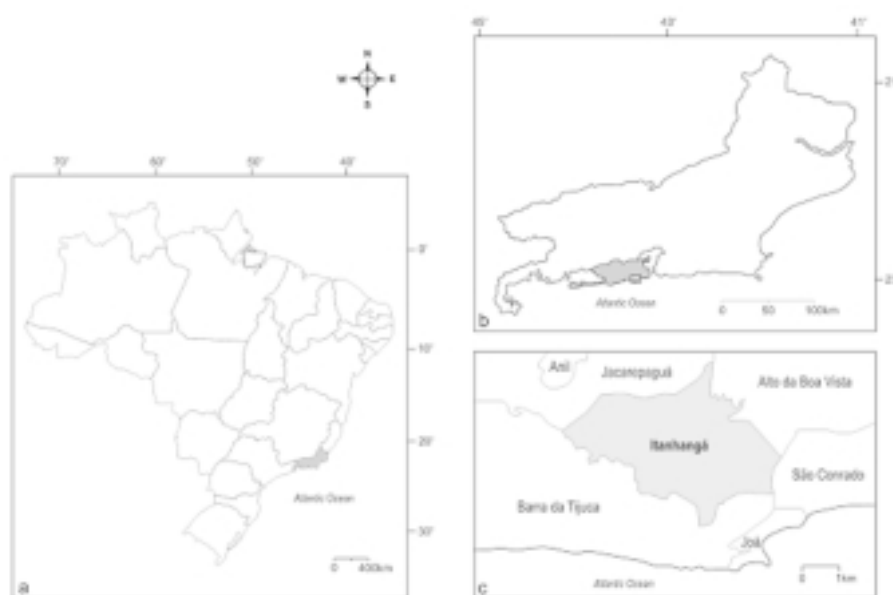


Figure 1. Map showing the location where mosquitoes were collected from August 2000 to July 2001: a) Map of Brazil, state of Rio de Janeiro (detail); b) Map of the state of Rio de Janeiro, city of Rio de Janeiro (detail); c) Map of western section of the city of Rio de Janeiro, neighborhood of Itanhangá (detail).

Capture of mosquitoes

Mosquitoes were captured weekly between the months August 2000 and July 2001, using light traps type CDC (Communicable Disease Center, Atlanta, EUA). The traps were set at two previously determined points at a distance of 1.2 km from each other at the district of Itanhangá, Rio de Janeiro, Brazil (22° e 99°S 43°29'W e 22° e 99°S 43° e 31'W). Captures were started 30 minutes before sunset and took a total of 12 hours. The traps were set at one meter above the soil.

During the same period, in one of the previously described points (22° e 99°S 43°29'W) the mosquitoes trying to feed on the human volunteers were captured during 60 minutes using manual aspirators (BUXTON, 1928). All captured insects were identified to species according to the taxonomic keys of Lane (1953) and Consoli and Lourenço-de-Oliveira (1994), using binocular stereoscopic microscopes.

Dog populations

Two different populations were studied with authorization of the owners. One group of animals, which had been assisted by veterinarians and brought to the clinic at least two times a year and another, which had not received veterinary assistance. The owners of the latter group are people with low income and the animals use to receive veterinary assistance only on a charity basis and anti-rabies vaccine during the vaccination campaign provided by the municipality of the city of Rio de Janeiro.

Population not assisted by veterinarians – During the anti-rabies vaccination campaign promoted by the municipality of Rio de Janeiro in the year 2000, 861 dogs were vaccinated in Itanhangá. From this total, 204 animals (23.7%) with more than 6 months of age and which, according to their owners, lived in the neighborhood since they were born were identified in individual records. From each animal a blood sample (3 ml) was taken for detection of *Dirofilaria immitis* antigens by an immunoenzymatic test (Witness Dirofilaria, Symbiotics) and, for detection of microfilariae, a Knott's modified test (1 ml) (KNOTT, 1939; NEWTON; WRIGHT, 1987) was performed.

Population assisted by veterinarians – During the period of October 2001 to September 2002, 756 dogs were attended for different procedures at the clinic. An ELISA test (SNAP 3Dx, IDEXX Laboratories) for detection of *D. immitis* antigen was recommended for all animals with more than 6 months of age at the first visit. A total of 86 (11.4%) dogs had a blood sample drawn (2ml) and tested. Monthly use of preventive medication against dirofilariosis was recommended for all antigen-negative animals. For asymptomatic dogs, whose owners had declined from the testing, preventive medication was also prescribed.

Two different tests were used for detection of *D. immitis* antigens, Witness Dirofilaria and SNAP 3Dx. The antigen tests were changed because Witness Dirofilaria was no longer available in the Brazilian market and because the overall accuracy of SNAP 3Dx is higher than Witness Dirofilarias' (McCALL et al., 2001; COURTNEY, 2001).

Evaluation of clinical compliance

During the period of October 2001 to September 2002, the number of sold doses for monthly prevention of dirofilariosis

was compared with the number of animals attended in the clinic, whose owners had been alerted to the need for prevention. The data were obtained directly from the records provided by the management of the clinic. The comparison between doses sold and doses necessary for monthly prevention for all dogs attended during 12 months indicated the percentage of compliance with the veterinary recommendation (CUMMINGS et al., 1995).

Statistical analysis

The relation mosquito density/distribution per month was calculated using the mean of Williams (WILLIAMS, 1937; HADDOW, 1954).

RESULTS

A total of 858 mosquitoes of 13 species were captured. The most frequently captured species (frequency more than 1%) were in descending order: *A. scapularis*; *C. quinquefasciatus*; *A. albopictus* (Skuse); *A. aegypti* (Linnaeus); *Wyeomyia confusa* (Lutz) and *A. taeniorhynchus* (Table I). Only two species were present during all months of the year: *A.*

Table 1. Density of culicids captured in the district of Itanhangá, Rio de Janeiro, using traps type CDC and attracted by volunteers during the period August 2000 to July 2001.

Species	CDC	Volunteers	CDC+ Volunteers
<i>Aedes scapularis</i> (Rondani)	124 41,3%	378 67,9%	502 58,5%
<i>Culex quinquefasciatus</i> Say	146 48,7%	17 3%	163 18,9%
<i>A. albopictus</i> (Skuse)	14 4,7%	94 16,8%	108 12,5%
<i>A. aegypti</i> (Linnaeus)	2 0,7%	22 3,9%	24 2,8%
<i>Wyeomyia confusa</i> (Lutz)	0 -	24 4,3%	24 2,8%
<i>A. taeniorhynchus</i> (Wiedmann)	0 -	10 1,8%	10 1,2%
<i>Culex</i> sp.	7 2,3%	0 -	7 0,8%
<i>Phoniomyia</i> sp.	0 -	7 1,3%	7 0,8%
<i>Limatus durhami</i> Theobald	0 -	3 0,5%	3 0,4%
<i>Mansonia titillans</i> (Walker)	3 1%	0 -	3 0,4%
<i>Runchomyia</i> sp.	0 -	3 0,5%	3 0,4%
<i>Uranotaenia lowi</i> Lynch-Arribalzaga	3 1%	0 -	3 0,4%
<i>Mansonia</i> sp.	1 0,3%	0 -	1 0,1%
Total	300 100%	558 100%	858 100

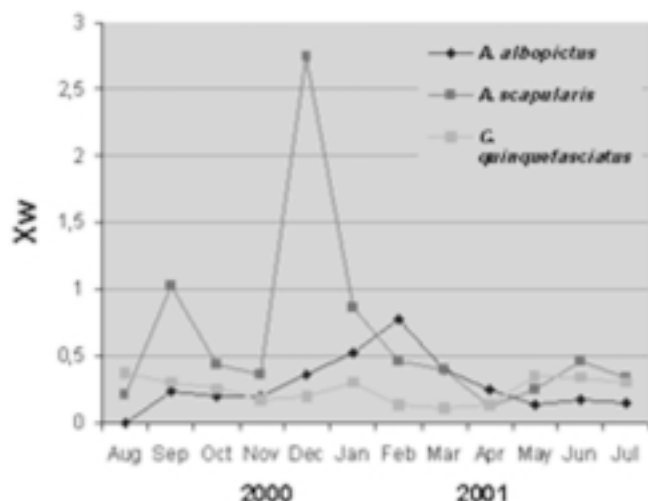


Figure 2. Monthly Williams' means (Xw) of the three most frequently collected mosquito species in Itanhangá, Rio de Janeiro, Brazil, from August 2000 to July 2001.

scapularis and *C. quinquefasciatus* (Fig. 2). The first was mainly captured when trying to feed on the volunteers, the second using traps type CDC.

Four (1.96%) out of the 204 blood samples from dogs whose owners belong to the population with low income presented antigens to *D. immitis* (immunoenzymatic) and none of them showed microfilariae.

Among the 86 blood samples from dogs attended in the clinic, none showed detectable *D. immitis* antigens (ELISA).

Seven hundred and fifty six dogs were attended in the veterinary clinic and 4132 doses for monthly prevention were sold during the same period revealing that 45.5% of owners followed the veterinary recommendation for preventive medication.

DISCUSSION

Observing the distribution of different mosquito species with regard to the month of capture in the course of the year it could be noted that only *A. scapularis* (primary vector in Rio de Janeiro) and *C. quinquefasciatus* (secondary vector for dogs and primary vector for cats in Rio de Janeiro) (LABARTHE et al., 1998b) were present in all months. *A. scapularis* showed two populational peaks while the population of *C. quinquefasciatus* kept stable with only small fluctuations. Given that this phenomenon was observed in two localities where canine dirofilariosis is enzootic, the lowland of Jacarepaguá, city of Rio de Janeiro (LOURENÇO-DE-OLIVEIRA et al., 1985), and the oceanic region of Niterói, state of Rio de Janeiro (LABARTHE et al., 1998a), this behavior seems to be representative for these two species in the State of Rio de Janeiro. Interestingly, the occurrence of *A. taeniorhynchus*, primary vector in Rio de Janeiro, was low (1.2%) in comparison to the oceanic region (30%) (LABARTHE et al., 1998a), but similar to that found in the lowland of Jacarepaguá (0.7%) (LOURENÇO-DE-OLIVEIRA et al., 1985).

Among the six most frequently captured species were *A. albopictus* which, although in other countries considered an important vector for *D. immitis* (SULAIMAN; JEFFERY, 1986; KONISHI, 1989; SCOLES; DICKSON, 1995; NAYAR; KNIGHT, 1999), has not yet been described as such in Brazil. The population of this species, recently introduced to the country (FORATTINI, 1986), seems to be expanding. In 1981-1982 no exemplar was captured in the lowland of Jacarepaguá (LOURENÇO-DE-OLIVEIRA et al., 1985) and in the oceanic region the species only represented 2.8% of captured culicids (LABARTHE et al., 1998a).

The absolute number of captured mosquitoes was relatively low (858) compared to the results of captures made during twelve months on other occasions in the oceanic region of Niterói (3,888) (LABARTHE et al., 1998a) and in the lowland of Jacarepaguá (11,230) (LOURENÇO-DE-OLIVEIRA et al., 1985). This fact may be due to the intense urbanization, which is modifying the landscape of the region in addition to the sanitary campaigns against the vectors of Dengue fever virus.

Vectors of *D. immitis* need approximately two weeks to get infected and turn infective (L_3 in the head and proboscis) (TAYLOR, 1960), consequently the mosquitoes have not only to support the parasite load but also to survive long enough for being able to transmit the parasite. Thus, the absolute number of a vector species in a region seems to be important for granting transmission of the parasite, once part of it dies due to the infection. Furthermore, the greater the number of dogs carrying microfilariae in the blood the greater are the chances for a susceptible culicid to get infected (WALTERS, 1995).

The prevalence of canine dirofilariosis is decreasing in Brazil and this trend is coinciding with the availability of preventive medication and diagnostic tests in the country (LABARTHE et al., 2003). In 1998, the nationwide prevalence was of 7.9% (GUERRERO et al., 1992) and in 2001 2% (LABARTHE et al., 2003). In the State of Rio de Janeiro, the prevalence decreased from 16% (GUERRERO et al., 1992) to 3.8% (LABARTHE et al., 2003).

The prevalence in Itanhangá (1.96%) shows that the number of infected dogs seems to be decreasing similarly to other regions of the State of Rio de Janeiro. In a region with characteristics resembling those found in the studied area (Niterói), the same decreasing was noted. The prevalence observed in Niterói, which was of 37.5% in 1990 decreased to 15% in 1997 (LABARTHE et al., 1997), and reached 0.8% in 2001 (unpublished data). Thus, the decrease from 31% (LABARTHE et al., 1997) to 1.96% observed in Itanhangá follows the trend observed in other regions of the state.

Among the 204 blood samples collected from examined dogs belonging to the poor population, only 4 (1.96%) presented antigens to the adult parasite and no microfilariae was detected. The absence of microfilariae circulating in the blood hampers the infection of mosquitoes by *D. immitis*, and this could be one the factors that contribute to the low prevalence of infected animals. On the other hand, the presence

of antigens in the blood of dogs, which according to their owners lived in the region since they were born, indicates circulation of the worm among these animals. One of the reasons for this phenomenon is probably the increasing indiscriminate use by veterinarians and owners of injectable medication against microfilariae as endo and ectoparasiticide (LABARTHE et al., 1997), which influences the presence and/or concentration of microfilariae in the circulation. Correctly used however, these drugs are efficient prophylactics, capable of reducing the number of parasitized dogs (CUMMINGS et al., 1995).

Since 1990, the prevalence of the occult form of infection in Barra de Tijuca (LABARTHE et al., 1997) was higher (40%) than described in the literature (up to 30%) (RAWLINGS et al., 1982; GRIEVE et al., 1986). Now, 10 years later, it could be shown that all infected dogs owned by the poor population presented the occult form of infection (4/4 – 100%). This fact reinforces the need for epidemiological survey by veterinarians already pointed out before, through use of diagnostic tests capable of detecting the occult form of this parasitosis (LABARTHE et al., 1997).

Clinical compliance with the veterinarian prescription by 45.5% of the owners of dogs regularly attended by veterinarians, although similar, was higher than that found in areas of the United States (41% of clinical compliance) where canine heartworm disease is enzootic (CUMMINGS et al., 1995). The rate of clinical compliance associated to the absence of detectable *D. immitis* antigens among dogs regularly attended by veterinarians indicates that monthly prevention, even so in half of the animals (45.5%), contributed to a decrease in the circulation of the worm in that dog population.

Although in the district of Itanhangá a culicid fauna capable of transmitting the helminth, dogs parasitized by the nematode as well as susceptible dogs could be found, transmission seems to occur discretely. This phenomenon is possibly due to the interaction of different factors: 1) indiscriminate use of microfilaricide drugs; 2) correct use of preventive drugs; 3) low population density of culicids and 4) low population density of the primary vector *A. taeniorhynchus*.

The low number of primary vectors and parasitized dogs found in the region notwithstanding, sanitary control and prophylactic measures should be maintained, principally as it is known that small modifications in the environment can cause great differences in the culicid populations. Furthermore, if *A. albopictus* is an efficient vector in our environment the observed growth of its population may signify the reappearance of a focus, which could possibly result in an increase of the number of cases of human pulmonary dirofilariosis.

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