Dirofilariasis in Argentina: Historical review and first report of *Dirofilaria immitis* in a natural mosquito population

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Abstract

Argentina is one of the four South American countries where the presence of *Dirofilaria immitis* is currently confirmed. The objective of this study was to review information on dirofilariasis in the country, and to report our recent findings on mosquito vectors. Since the first report of dogs with unidentified microfilariae in 1926, *D. immitis* was found in seven provinces and canine prevalence ranged 0–71% at local scale. National prevalence was 8% by the end of the 1980s and current information is available only for Buenos Aires Province. Four pulmonary human infections of *D. immitis* and one subcutaneous of *Dirofilaria* sp. were documented. The common coati was the only wild host found, and natural infection in mosquitoes was not previously reported in the country. In our recent mosquito survey in Greater Buenos Aires, we captured and dissected 2380 mosquitoes belonging to 20 species. According to a minimum temperature of 14 °C, the potential transmission period (PTP) for *D. immitis* in Buenos Aires covers 6 months, and the most favourable period (mean temperature above 20 °C) takes place from the middle of November to the beginning of April. To identify potential vectors of the parasite, we assessed weekly abundances of mosquito species during those PTP estimated previously. We found two specimens of *Culex pipiens* and one of *Aedes aegypti* carrying non-infective stages of *D. immitis*. These two highly anthropophilic mosquitoes may enhance the role of *D. immitis* as zoonotic agent in temperate Argentina.

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1. Introduction

Dirofilariasis is a disease caused by filarial worms of the genus *Dirofilaria* transmitted by mosquitoes. This genus consists of 27 valid species, and 15 species of questionable validity (Canestri Trotti et al., 1997). Definitive hosts are mammals, mainly primates and carnivores, and adult worms occur in subcutaneous tissues or in the heart. The intermediate hosts and vectors are usually mosquitoes (*D. ursi* is transmitted by simuliids), and larval development generally takes place in the Malpighian tubules (*D. corynodes* develops in the fat body) (Anderson, 2000). Although dirofilariasis was originally considered a disease of strict veterinary importance, it has been recognized as an emerging zoonosis by several authors (Robinson et al., 1977; Simón et al., 1991; Pampiglione et al., 2001; Pampiglione and Rivasi, 2001). Humans are dead-end hosts, and over one thousand cases (295 pulmonary and 780 subcutaneous/ocular) were reported throughout the world (Simón et al., 2005).

Two *Dirofilaria* species, *D. repens* and *D. immitis*, are of special interest to humans because of their harmful effect on our company pets (dogs and cats) and potential zoonotic role (Orihel and Eberhard, 1998; Genchi et al., 2005a). The former is only present in the Old World (Europe, Asia and Africa), where it is the etiologic agent of most subcutaneous/ocular human cases, whereas *D. immitis* is cosmopolitan and responsible for human pulmonary dirofilariasis (Soulsby, 1987; Canestri Trotti et al., 1997; Simón et al., 2005). In regard to canine dirofilariasis, *D. repens* and *D. immitis* have been studied exhaustively, showing great regional and local variations in their prevalences worldwide; e.g. see Genchi et al. (2005b) for a recent review of canine dirofilariasis in Europe, and Labarthe and Guerrero (2005) in South America and Mexico.

In Argentina, many studies about this disease have been conducted since the first finding of microfilariae in the peripheral blood of dogs from northwest provinces in 1926. Unfortunately, the bulk of information is scattered through journals and proceedings of local interest, thus remaining unknown to the international scientific community. This study summarized information available from Argentina on canine and human dirofilariasis, and on *D. immitis* wild hosts and mosquito vectors. We also included results of our recent studies on mosquitoes aiming at identifying potential vectors of *D. immitis* in the country.

2. A historical perspective of dirofilariasis in Argentina

2.1. The regional context

From the thirteen South American countries, current information on the presence or absence of dogs infested with *D. immitis* is available only for Argentina, Brazil, Peru, Colombia, and Chile (Labarthe and Guerrero, 2005). There are some records of canine dirofilariasis in Venezuela, Surinam, Paraguay, and Guiana before 1980, and to our knowledge, no data on the disease have been reported in Bolivia, Ecuador, French Guiana, and Uruguay.

Since the first South American report of canine dirofilariasis in 1875, most surveys on canine heartworm have been conducted in Brazil. Labarthe and Guerrero (2005), who recently summarized the large amount of information on canine prevalence published in this country, showed a decreasing trend in prevalence from 7.9% in 1998 to 2% in 2001, being local values as high as 52.5% in the southeast region. In Peru, the parasite was first documented in 1945 (Acuña and Chávez, 2002), and recent studies by ELISA reported a canine prevalence ranging between 0 and 12.8% (Acuña and Chávez, 2002; Bravo et al., 2002; Chipana et al., 2002; Adrianzén et al., 2003). In Colombia, the first records of canine filariasis due to *D. immitis* were reported in 1964 and 1967, with 1 case/400 dogs and 8 cases/109 dogs, respectively (Little et al., 1968), and the overall prevalence registered by 1990 was 4.8–8.4% (Labarthe and Guerrero, 2005). In Chile, *D. immitis* was not found in two studies conducted during 1976–1979 and 1994, among a total of 1281 dogs surveyed (Alcaíno et al., 1984, 1995). Thus, Chile remains as the only South American country where *D. immitis* was searched but not found.

In Venezuela, Surinam, Guiana, and Paraguay there is no information on canine dirofilariasis for the last two decades, but some historical records suggest the presence of *D. immitis* in these countries. In this sense,

Venezuela represents the most notorious example, with at least eight reports of *D. immitis* from its first finding in 1934 through 1970, covering seven of the twenty-three estates; these studies, summarized by D'Alessandro (1971), revealed a canine prevalence of 4–29%. In Surinam, dogs infected with *D. immitis* were registered in 1920, 1938, and 1956 (Panday et al., 1981). Rep and Heinemann (1976) estimated a prevalence of 22% among 124 street dogs from seven localities, and a few years later Panday et al. (1981) recorded a prevalence of 26% (*n* = 521) in the Capital City. In Guiana, Orihel (1964) found a prevalence of 14.1% among 2135 inspected dogs, with values along the rural coast higher than in urban areas. Finally, in Paraguay, Masi Pallares et al. (1967) reported 8 *D. immitis*-infected dogs among 200 necropsies of street animals.

Feline dirofilariasis in South America was reported only in Venezuela and Brazil (Labarthe and Guerrero, 2005). Generally, infected cats are amicrofilaremic and their prevalence parallels that in dogs but at a lower rate; for example, in Rio de Janeiro feline prevalence was 20 times lower than canine prevalence (Labarthe et al., 1997). On this basis, it is highly probable that veterinarian practitioners would not detect infected cats in other countries where canine dirofilariasis occurs.

The first case of human pulmonary dirofilariasis in the world was reported by De Magalhães in 1887 from a child of Rio de Janeiro, Brazil (Shah, 1999). Since then, 50 new cases were documented in Brazil, mainly from Rio de Janeiro, São Paulo, and Florianópolis (Milanez de Campos et al., 1997; Cavallazzi et al., 2002; Rodrigues-Silva et al., 2004). In the remaining South American countries, human cases of pulmonary dirofilariasis due to *Dirofilaria* spp. have rarely been reported: one case from Venezuela (Salfelder et al., 1976), one from Colombia (Beaver et al., 1990), and a few from Argentina compiled in the present study. In addition, Vieira et al. (2000) detected human dirofilariasis in different geographical and climatic areas of Colombia by means of ELISA test. Human infections with *D. immitis* are found wherever the parasite is enzootic (Orihel and Eberhard, 1998), and therefore the disease is likely to remain undiagnosed in several South American countries, as is the case with feline dirofilariasis.

In regard to research on mosquito vectors of *Dirofilaria* in South America, Brazil has the lead over

the other countries, just like in other issues concerning dirofilariasis. At least eight mosquito species were found infected with *D. immitis* larvae in this country. The occurrence of last stage L$_3$ was recorded in natural populations of *Aedes taeniorhynchus*, *Ochlerotatus scapularis*, and *Culex quinquefasciatus* (Lourenço de Oliveira and Deane, 1995; Labarthe et al., 1998; Ahid and Lourenço de Oliveira, 1999). Experimental infections using brazilian mosquito strains were successful in *Oc. fluviatilis*, *Oc. scapularis*, *Cx. quinquefasciatus*, and *Ae. aegypti* (Kasai and Williams, 1986; Macedo et al., 1998; Brito et al., 1999; Ahid et al., 2000; Serrão et al., 2001). On the other hand, *Cx. declarator*, *Cx. saltanensis*, and *Wyeomyia bourrouli* were found naturally infected, with non-infective L$_1$–L$_2$ larvae only (Labarthe et al., 1998). Except for Brazil and Argentina, no information on *D. immitis* vectors is available in the continent, and data collected from the latter country are provided in Section 2.5.

2.2. Canine heartworm

In Argentina, there were only verbal references about dogs infected with *D. immitis* until 1926. In this year, Mazza and Rosenbusch (1926) documented microfilariae in the peripheral blood of 34.5% dogs (*n* = 55) from northwest provinces of the country (Salta, Jujuy, and Tucumán; Fig. 1a). No adult worms were found in the four infected dogs that were necropsied, and based on length measures of the microfilariae and the relative location of some reference points (Giemsa stain method), they concluded that the parasite did not match with *D. immitis* or *D. repens*. In the same year, two surveys conducted in Buenos Aires City and its outskirts revealed dogs hosting unidentified microfilariae (Mazza et al., 1926; Bacigalupo, 1941). In this area, blood microfilariae were again detected in four out of twenty dogs (Antequeda, 1929). Two years later, Mazza and Romaña (1931) found two infected dogs in the north of Santa Fe Province, one harbouring *D. immitis* and *D. repens* (microfilariae and adults of both species), and the other *D. repens* (microfilariae and adults). This reference is considered for all subsequent researchers as the first citation of *D. immitis* in the country; the finding of *D. repens* will be discussed later. Between 1941 and 1949, 13 microfilaremic dogs from Buenos
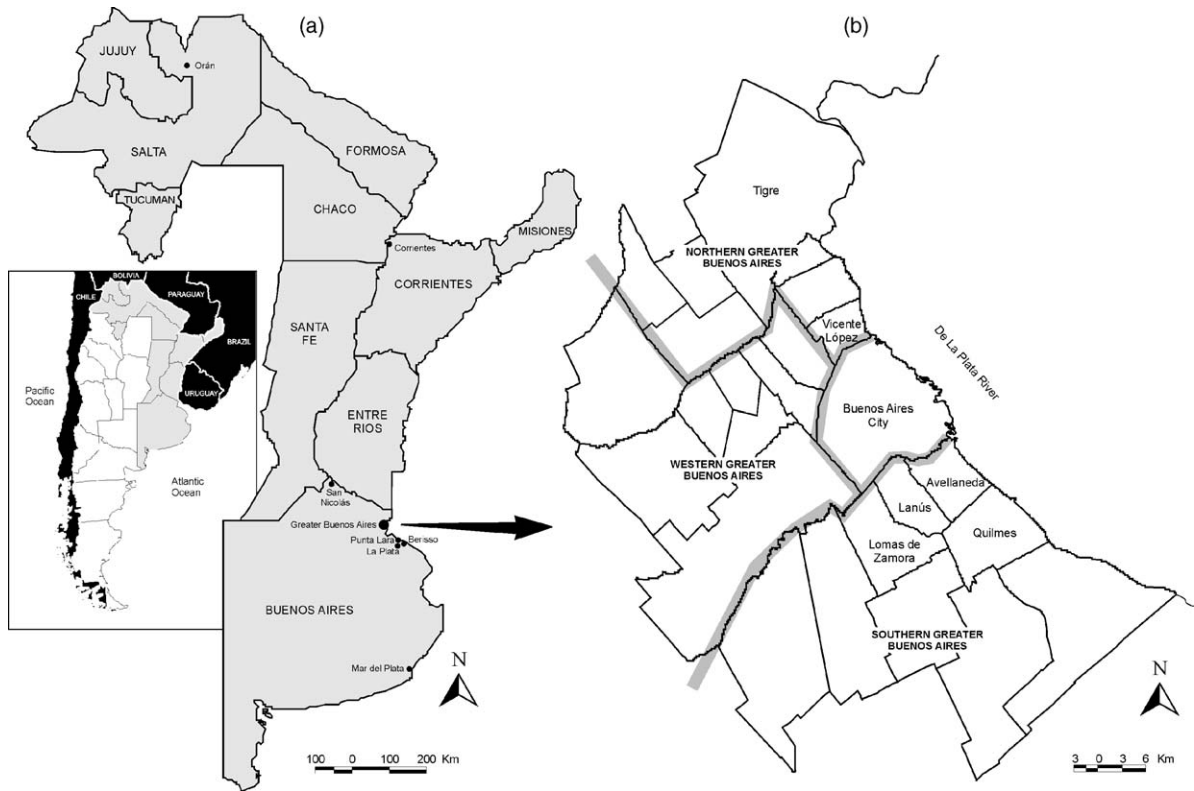
Fig. 1. Maps of Argentina and Greater Buenos Aires. (a) Geographic location of Argentine provinces and cities mentioned in the text. (b) Details of Greater Buenos Aires areas. Only those municipalities mentioned in the text are indicated.

Aires were dissected but no adult parasite was found and the species remained unidentified (Bacigalupo, 1950). By that time, in the Province of Tucumán, Toranzos (1950) found microfilariae in 18 dogs out of a total of 93 examined, and based on biometric studies of 100 microfilariae from eight dogs, he assigned them to the same parasite species; once again, unfortunately, the species could not be identified.

There are no new records about canine dirofilariasis in Argentina during the period 1951–1983, and in the last two decades all filarial worms in dogs from different regions of the country have been identified as *D. immitis* or *Acanthocheilonema* (formerly *Dipetalonema*) *reconditum*, and none as *D. repens*. This substantial change was obviously due to the use of better diagnostic techniques such as modified Knott and serologic methods, together with an increasing knowledge of the disease and its etiologic agents worldwide.

The first mention of *D. immitis* over the last two decades corresponded to three dogs carrying adult worms out of 100 necropsied in Corrientes City in 1983 (Santa Cruz and Lombardero, 1987). One year later, Ruager et al. (1984) confirmed the presence of *D. immitis* adults in a dog from Southern Greater Buenos Aires (Fig. 1b) among more than 3000 necropsies performed along 17 years. Subsequently, Bulman et al. (1989a,b) conducted the earliest studies on canine dirofilariasis in an attempt to estimate national prevalence, with a broad geographic distribution of sampled dogs. Considering both surveys together, the national prevalence was 7.8% (152 infected dogs out of 1957 inspected). At a local level, they obtained the following results: 0.72–36% in the northwest provinces (Formosa, Chaco, Corrientes, Misiones, Entre Ríos, and Santa Fe), 4–5% in Greater Buenos Aires, 3 cases out of 279 examined dogs in Buenos Aires City, 2 cases out of 26 examined dogs in La Plata City, and

0% in 30 dogs from Mar del Plata City (Fig. 1a). Almost simultaneously, a study carried out on the northern border of the country (Formosa Province) showed a canine prevalence of 41.1% (189/460), and revealed a gradient of prevalence inversely related to the degree of urbanisation (urban: 34%, suburban: 44%, rural: 74%) (Mancebo et al., 1992). In addition, this was the first research in Argentina to evaluate *D. immitis* microfilarial periodicity in dogs, showing an abundance peak between 19 and 24 h.

From 1992 onwards, all studies dealing with canine prevalence were conducted exclusively in Buenos Aires Province. In Greater Buenos Aires (including Buenos Aires City), none of the 320 examined dogs had blood microfilariae (Lightowler et al., 1992). In Northern Greater Buenos Aires (Municipality of Tigre), four out of twenty dogs were infected with *D. immitis* microfilariae (Rosa et al., 1994). In Southern Greater Buenos Aires, local prevalences at municipality scale were highly variable, with values of 8.7% in Lanús and Lomas de Zamora (Labbé et al., 1995), 36% in Lanús, and 41–60% in Avellaneda (Meyer and Milanta, 1997) (Fig. 1b). In two cities located at the south of Greater Buenos Aires, prevalences were 12.7% (Berisso, *n* = 94) and 3.3% (La Plata, *n* = 61) (Arias et al., 1994). Recently, Rosa et al. (2002), who evaluated prevalence by ELISA in dogs of Buenos Aires City and its surroundings, detected infection in the northern (17/96 = 17.7%) and southern areas (23/98 = 23.5%), while dogs from western areas (*n* = 417) and Buenos Aires City (*n* = 171) were uninfected. Finally, Notarnicola (2004) reported an overall prevalence of 2.3% among 265 dogs from two municipalities of Southern Greater Buenos Aires and southern cities of Berisso and La Plata (Fig. 1b).

In regard to *D. repens*, it is widely accepted that this species is present only in the Old World (Canestri Trotti et al., 1997; Pampiglione et al., 2001). There are two historical references on this species in Argentina (Mazza and Romaña, 1931; Bacigalupo, 1950). However, the record of 1950 was invalidated by Bulman et al. (1989a), who suggested that the described species was *A. reconditum* according to morphological characteristics. Likewise, the record of 1931 may involve specimens of *A. reconditum* or wild *Dirofilaria* species.

A. reconditum, transmitted by fleas, is another subcutaneous parasite of dogs (Newton and Wright,

1957). In Argentina it was first identified by 1947 in dogs of the Pampean District, probably within Buenos Aires Province (Roveda and Ringuelet, 1947). Bulman et al. (1989a) detected 2 infected dogs of a total of 162 examined in Buenos Aires City, and Rosa et al. (1994) 6 out of 20 in the Municipality of Tigre (Province of Buenos Aires). In the north of the country, there is a single report from Formosa Province by Mancebo et al. (1992), showing a prevalence of 2.4% (*n* = 460). In this study, microfilariae showed periodicity in the peripheral blood with an abundance peak between 12 and 18 h.

According to the information given above, La Plata is the southernmost city with *D. immitis*-infected dogs, and nearby areas would represent the southern limit of dirofilariasis in Argentina. This disease is probably absent southwards due to temperature constraints for the development of the parasite in the mosquito vector. Despite the large amount of available information, it is difficult to assess the overall canine prevalence in Argentina. The value of 7.8% obtained when considering the two surveys of Bulman et al. (1989a,b) would be the most reliable estimation of national prevalence, at least at the end of the 1980s. The highest prevalences documented in the country were 71% for a rural environment (Mancebo et al., 1992) and 60% for an urban one (Meyer and Milanta, 1997), but the number of examined dogs was not specified. There was great heterogeneity of prevalences in Greater Buenos Aires (e.g. 0, 5, 8.7, 17.7, 23.5, 36, 41, 57, 60%). This could be partially explained by differences in environmental and urban characteristics among municipalities that may affect vector abundances and dog densities. However, some methodological issues such as the low number of sampled dogs should also be considered.

2.3. Human dirofilariasis

The first two cases of human dirofilariasis in Argentina were documented just 11 years ago. These were 54- and 60-year-old men residing in Buenos Aires with a solitary pulmonary nodule due to *D. immitis* (Caballer et al., 1994). These two patients had travelled to countries in South or North America, and it could not be ascertained if infection was acquired in Argentina. The third case was a woman of 61 years living in San Nicolas City (Fig. 1a), at the north of

Buenos Aires Province, who suffered from subcutaneous nodules in thorax and head due to *Dirofilaria* sp. (Abuin et al., 1998). The fourth case was a man aged 53 years from Buenos Aires (Barcat et al., 1999), and the last one was a man aged 35 years from Corrientes City (Fig. 1a) (Riache et al., 2001), both with a solitary pulmonary nodule.

In summary, there have been only five reports of human dirofilariasis in the country, one of them being recorded out of Buenos Aires Province. These findings could be due to the actual occurrence of more human cases, or to a higher level of awareness on pulmonary diseases and more frequent medical attention near De La Plata River (central-east) than in northeast areas of the country. In the Old World, the increased incidence of human dirofilariasis was partially attributed to a higher availability of information that raised awareness about dirofilariasis (Simón et al., 2005). Taking into account that (a) access to information by patients and physicians is easier in Buenos Aires than in the rest of the country and (b) canine prevalence in Buenos Aires is similar to or even lower than that in northern provinces, it can be assumed that the higher number of human cases detected in the Federal District and its surroundings is due to a higher awareness on medical issues.

Although pulmonary dirofilariasis does not pose a significant threat to humans, it is important for the differential diagnosis of other well-defined pulmonary lesions such as tuberculosis, fungal infections, carcinoma, and hamartoma (Narine et al., 1999). This parasitosis is likely to be underdiagnosed worldwide, and in some areas seroprevalence in humans correlates to a certain extent with the prevalence in the canine population (Muro et al., 1999; Simón et al., 2005; Theis, 2005). From this viewpoint, human dirofilariasis in Argentina would be highly underdiagnosed on account of the canine prevalences reported.

2.4. Other hosts of D. immitis

Considering the lack of information about canine dirofilariasis in wide areas of Argentina (e.g. central and northwest provinces) and the few current data outside Buenos Aires Province, it is not surprising that only two reports about wild hosts of *D. immitis* were published in the country. The first one involved a coati (*Nasua* sp.) from Orán (Salta Province) harbouring

microfilariae and adults of *D. nasuae*, a supposed new *Dirofilaria* species (Mazza, 1926), currently considered as *D. immitis* (Canestri Trotti et al., 1997). The second study, conducted in Formosa by Mancebo et al. (1992), comprised 57 individuals of 9 mammal species examined for microfilariae (by modified Knott's method) and adult worms (by necropsy). Animals analysed were as follows: 16 *Dasypus novemcinctus* (nine-banded armadillo), 15 *Nasua solitaria* (common coati), 13 *Lutreolina crassicaudata* (thick-tailed opossum), 5 *Cavia aparea* (guinea pig), 3 *Cerdocyon thous* (crab-eating fox), 2 *Procyon lotor* (raccoon), 1 *Didelphis albiventris* (white-eared opossum), 1 *Didelphis* sp., and 1 mouse. Six individuals of *N. solitaria* were carrying adult *D. immitis*, and five of them microfilariae.

To our knowledge, there are no documented cases of feline dirofilariasis in the country.

2.5. Vectors of D. immitis

There is little information on *Dirofilaria* vectors in Argentina, as is the case for wild and human hosts. In the decade of the 1940s, Bacigalupo (1941, 1945) performed a series of experiments to study the development of microfilariae commonly found among dogs from Buenos Aires in some mosquito species, but without knowing the filarial species he was dealing with. Using infected dogs as microfilariae source, the author infected successfully three mosquito species, namely *Mansonia titillans*, *Oc. albifasciatus*, and *Psorophora cyanescens*. In these species, microfilariae reached the L_3 infective stage in 12 days ranging 1–1.2 mm in length. The daily measures reported by Bacigalupo mismatched with those by Taylor (1960) for *D. immitis* infecting *Ae. aegypti* at 26 °C and 80% RH, but the former author did not specify the experimental conditions used. Bacigalupo (1941) also referred to an attempt to infect *Cx. pipiens*, but none of the mosquitoes fed on the microfilaremic dog. In addition, he did not find any filarial larvae among over 400 mosquitoes captured along the river coast of Buenos Aires.

In the period 1989–1991, some comprehensive surveys of mosquitoes for the assessment of their natural enemies were conducted in Punta Lara (Buenos Aires Province), a natural reserve located in an unurbanised area characterised by marginal

forest. Among the parasite species recorded in female mosquitoes, larvae of Onchocercidae were found in the hemocoele of *Oc. albifasciatus*, *Oc. crinifer*, *Cx. dolosus*, and *Ps. ferox* (García et al., 1994; Campos et al., 1995; Maciá et al., 1995). Onchocercidae larvae were also observed in the Malpighian tubules of a few *Oc. crinifer* specimens (Maciá et al., 1995); these could belong to some wild *Dirofilaria* species.

Recently, Notarnicola (2004) captured in the Municipality of Quilmes and in La Plata City female mosquitoes of *Oc. albifasciatus*, *Cx. pipiens*, *Ps. albigens*, *Ae. aegypti*, and *Oc. crinifer*. All 412 mosquitoes dissected were negative for filarial forms. The same author performed two experiments to infect *Cx. pipiens* using a microfilaremic dog as infection source. In the first trial (*Cx. pipiens* laboratory strain) mosquitoes failed to feed, and in the second one (field population) none of the engorged mosquitoes became infected.

Until the present work, natural infection with *D. immitis* in mosquitoes has not been reported from Argentina. Among the eight potential vectors described from Brazil, *Ae. aegypti*, *Oc. fluviatilis*, *Oc. scapularis*, *Cx. quinquefasciatus*, and *Cx. saltanensis* are present in Argentina (Campos and Maciá, 1998); on this basis, these species could be considered a priori as potential vectors of *D. immitis* in the country. Experimental research undertaken by Bacigalupo (1941, 1945) suggests that *Ma. titillans*, *Oc. albifasciatus*, and *Ps. cyanescens* could also be regarded as potential vectors.

3. Mosquito survey in Greater Buenos Aires, Argentina

We evaluated the occurrence of potential mosquito vectors of *D. immitis* in Buenos Aires according to two different approaches. The first one dealt with the finding of mosquitoes infected with parasite larvae. The second approach was to identify mosquito species being highly abundant during the theoretically favourable period for the development of *D. immitis* within the vector (potential transmission period). A climate that provides adequate temperature to allow maturation of ingested microfilariae to infective larvae within the vector is a pivotal prerequisite for heartworm transmission to occur (McCall et al., 2004).

Consequently, climate dictates the seasonal occurrence of heartworm transmission in temperate latitudes, and the temperature threshold below which development will not proceed is approximately 14 °C (Genchi et al., 2005b).

3.1. Materials and methods

3.1.1. Study area

Greater Buenos Aires is the most crowded urban centre of Argentina. This megalopolis of 11 million inhabitants includes the Federal District (Buenos Aires City; 34°35′S 58°29′W) and a surrounding urban belt composed of 24 municipalities (INDEC, 2003). The climate is temperate humid with four seasons, mean annual rainfall is 1076 mm and mean annual temperature is 17.4 °C (IGM, 1998).

Mosquito surveys were carried out in Northern Greater Buenos Aires (municipalities of Vicente López and Tigre), Southern Greater Buenos Aires (municipalities of Quilmes and Lomas de Zamora), and Buenos Aires City (Fig. 1b). Although the whole Greater Buenos Aires is an urban area, the landscape pattern in northern and southern municipalities ranges from suburban to highly urbanised small centres, whereas the Federal District is almost entirely urbanised.

3.1.2. Methodology

Mosquito captures were performed at least weekly between December 2003–May 2004 and September 2004–May 2005, and in the time band from 7 a.m. to midnight. Places visited were private gardens, inside of houses, public pavements, an university campus, and a public trail along the river. To increase the chance of finding infected mosquitoes, all capture sites were inhabited by dogs in a radius smaller than 100 m. Specimens were collected with oral aspirators and sweep nets by disturbing vegetation and nearby places. The collection included mosquitoes in a host-seeking behaviour and those resting on natural or man-made structures. Collections of resting mosquitoes usually provide more representative samples of the population as a whole than most other methods, with the additional advantage of catching blood-fed and gravid females (Service, 1976).

The mosquitoes were transported alive to the laboratory and maintained at 5 °C until taxonomic

identification and dissection within 48 h; dead mosquitoes were removed. The specimens were anesthetized with ethanol vapour and identified by using complementarily the keys to Argentine mosquitoes (Darsie, 1985), Buenos Aires mosquitoes (Rossi et al., 2002), and neotropical mosquitoes (Forattini, 2002). Considering that the two principal species of the *Cx. pipiens* complex, i.e. *Cx. pipiens* s.str. and *Cx. quinquefasciatus*, are sympatric in Buenos Aires (Mitchell and Darsie, 1985; Rossi, 2000; Forattini, 2002) and that intermediate forms can be found where their ranges overlap (Almirón et al., 1995), these species were not distinguished from each other and were referred to as *Cx. pipiens*.

Immediately after mosquito identification, each specimen was dissected. The head, thorax, Malpighian tubules and alimentary tract, and abdomen remnant were placed separately in a saline droplet. Compression was accomplished by pressing over with a coverslip to detect nematode larvae under light microscope. Larvae found were identified considering the following criteria adopted by Lourenço de Oliveira and Deane (1995) and Labarthe et al. (1998): (a) general morphological characteristics of filarioids described by Taylor (1960), (b) only species of the genus *Dirofilaria* are known to develop within Malpighian tubules, (c) *D. immitis* is the only species belonging to genus *Dirofilaria* reported in the study area, and (d) the study area is an active *D. immitis* transmission focus. Larval stages were classified according to length and width measures, and to the specific location within the mosquito (Taylor, 1960).

3.1.3. Data analysis

Considering the thermal threshold of 14 °C, we estimated the potential transmission period (PTP) of *D. immitis* in Buenos Aires. We calculated the weekly mean and minimum temperatures throughout the year using daily mean and minimum temperatures of 2002, 2003, 2004, and first semester of 2005 (National Meteorology Service). The period estimated by mean temperature (PTPmean14) represents the part of the year when parasite larvae can develop at least during some hours a day. The period estimated by minimum temperature (PTPmin14) represents the part of the year when parasite larvae can develop during the whole day. In addition, we defined the most favourable

period based on a mean temperature above 20 °C (PTPmean20).

To assess potential mosquito vectors, we described the weekly presence and relative abundance of mosquito species captured during those PTP previously estimated.

3.2. Results

A total of 2380 mosquitoes belonging to 20 species were identified (Table 1). Of these, eight species registered more than 100 specimens and encompassed almost 97% of the mosquitoes captured. The three most frequent species were *Oc. albifasciatus*, *Cx. pipiens*, and *Ae. aegypti*.

Out of the 2380 mosquitoes dissected, *D. immitis* larvae were found in two individuals of *Cx. pipiens* and one of *Ae. aegypti*, all from Southern Greater Buenos Aires (Table 2).

The favourable periods for the development of *D. immitis* larvae within the mosquito are shown in Fig. 2. The period calculated using minimum temperature (PTPmin14) lasted for about 6 months, from the

Table 1

Number of mosquitoes captured in Greater Buenos Aires (Argentina) during the period January 2004–May 2005

Species	Northern Buenos Aires	Southern Buenos Aires	Buenos Aires City	Total
Oc. albifasciatus	125	216	263	604
Cx. pipiens	56	281	261	598
Ae. aegypti	4	253	138	395
Cx. maxi	45	121	46	212
Cx. apicinus	0	114	49	163
Oc. crinifer	60	54	3	117
Oc. scapularis	13	71	24	108
Ma. titillans	1	0	101	102
Cx. eduardoi	0	14	11	25
Cx. dolosus	2	15	0	17
Ps. ferox	9	1	1	11
Cx. lahillei	0	3	6	9
Cx. chidesteri	0	2	4	6
Cx. bidens	0	2	1	3
Cx. acharistus	0	2	0	2
Ps. albigenu	1	1	0	2
Ps. varinervis	0	1	1	2
Is. paranensis	0	0	2	2
Ur. pulcherrima	0	0	1	1
An. albitarsis	0	0	1	1
Total	327	1151	902	2380

Table 2
Detailed account of mosquitoes infected with *Dirofilaria immitis* in Southern Greater Buenos Aires (Argentina) during the period January 2004–May 2005

Species	Date/municipality	Description of the infection
Cx. pipiens	April 2004, 2nd week/Quilmes	Nine first stage larvae of 4th day within cells of Malpighian tubules
Ae. aegypti	December 2004, 2nd week/Quilmes	Fifteen first stage larvae of 2nd day within cells of Malpighian tubules
Cx. pipiens	March 2005, 3rd week/Lomas de Zamora	One first stage larvae of 7th or 8th day in the lumen of Malpighian tubules

second week of October to the third week of April. The PTPmean14 extended 2 months the suitable period (one at the beginning and one at the end), thus covering between the first week of September and the first week of June. The most restricted period (PTPmean20) lasted from the middle of November to the beginning of April (Fig. 2).

The eight mosquito species most frequently captured occurred within the potential transmission periods (Fig. 3). *Cx. pipiens*, *Cx. maxi*, and *Ae. aegypti* showed a similar temporal abundance pattern with highest values in March, which was included within the hottest period (PTPmean20). Although *Ma. titillans* also showed an abundance peak at the end of PTPmean20, it was present for a very short period. The *Ochlerotatus* species were mainly captured at the end of the PTPmean14, previous to the beginning of winter. Finally, *Cx. apicinus* was the only species registering higher abundances in October–November, when temperature becomes more appropriate for parasite development.
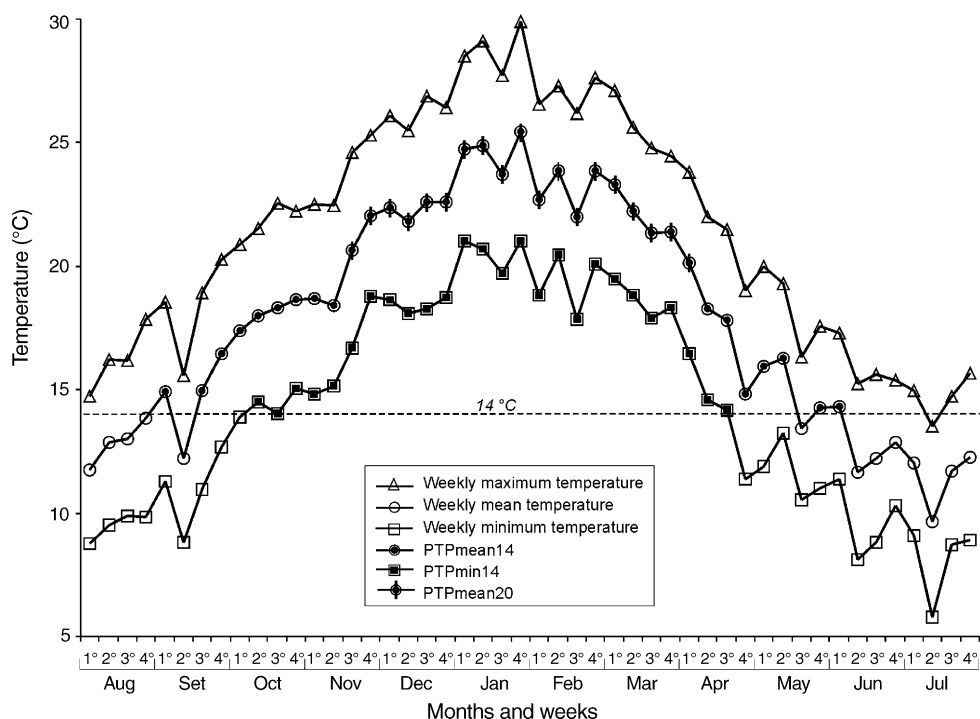

Fig. 2. Weekly temperatures through the year in Buenos Aires (Argentina). Potential transmission periods (PTP) of *Dirofilaria immitis* are indicated. PTPmean14 and PTPmean20 were estimated with mean temperatures above 14 and 20 °C, respectively, and PTPmin14 with a minimum temperature above 14 °C.
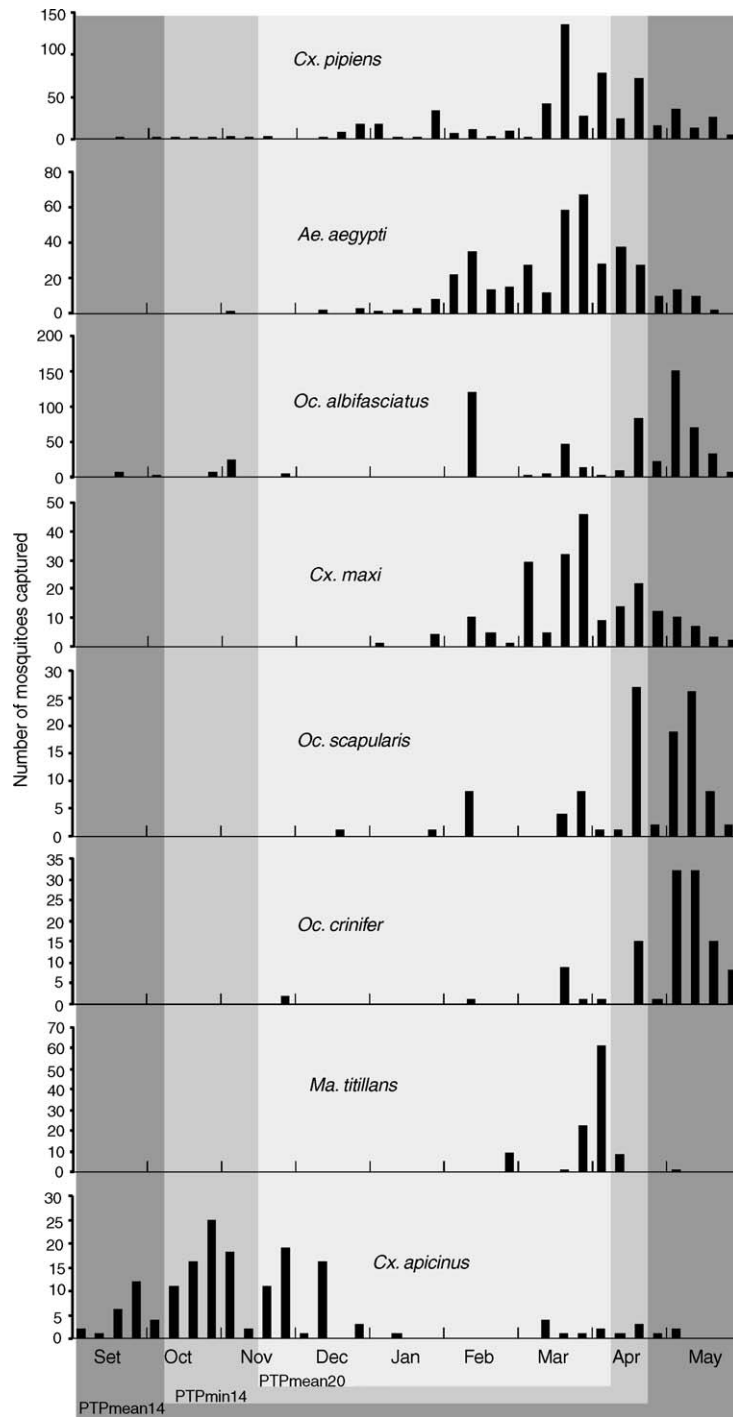
Fig. 3. Weekly number of mosquitoes captured in Greater Buenos Aires (Argentina) from January 2004 to May 2005. Only the most abundant eight mosquito species are shown. Potential transmission periods (PTP) of *Dirofilaria immitis* are shown with different grey shades. PTPmean14: mean temperature above 14 °C, PTPmin14: minimum temperature above 14 °C, and PTPmean20: mean temperature above 20 °C.

3.3. Discussion

To our knowledge, this is the first report of *D. immitis* infection in natural mosquito populations from Argentina. The finding of *Cx. pipiens* and *Ae. aegypti* hosting the parasite confirms that both mosquito species can acquire the infection under natural conditions. However, their role as true vectors in Buenos Aires remains unclear because only non-infective L_1 larvae were found.

According to the temperature analysis, the period of potential transmission based on the thermal threshold of 14 °C (PTPmin14) may indicate that the canine population of Buenos Aires is at risk of acquiring heartworms during 6 months. The most favourable period for larvae development (i.e. mean temperature above 20 °C) takes place from the middle of November to early April. On the other hand, temperature seems to be suitable for some hours a day during 1 month before and 1 month after PTPmin14. Nevertheless, mosquitoes would not live enough to allow parasite maturity to the infective stage because larval development is retarded. Based on these results, a preventive treatment such as monthly prophylactic doses of ivermectin should be administered to dogs at least from October to April. It is also worthwhile mentioning that in the Northern Hemisphere, the peaks of heartworm transmission are observed in July and August and, like for Buenos Aires, transmission would be limited to 6 months near the 37th parallel (McCall et al., 2004).

Among the mosquito species found, *Ae. aegypti*, *Cx. pipiens* complex, and *Cx. maxi*, were highly abundant during the PTPmean20. The two former species have been reported as potential vectors of *D. immitis* in Brazil (see the end of Section 2.1). *Cx. maxi* was found to feed exclusively on chickens and is not considered of medical importance (Mitchell et al., 1987; Almirón and Brewer, 1995; Almirón and Harbach, 1996). *Cx. apicinus* was the only species showing a high abundance at the beginning of the PTPmean20, but it is also known to feed on birds (Almirón and Brewer, 1995). Although two *Ochlerotatus* species, *Oc. albifasciatus* and *Oc. scapularis*, could be considered a priori as potential vectors (see Section 2.5), in Buenos Aires they were abundant at the end of PTPmean14, when temperature became unsuitable for parasite development. Finally, *Ma.*

titillans could be seen as a potential vector because of its occurrence within the PTPmean20, but it was restricted to a short period and was mainly caught in a trail along the river, instead of in typical domestic environments as a backyard.

The 20 mosquito species found have already been registered in the Province of Buenos Aires (Rossi, 2000). Despite the fact that our mosquito survey was not specifically designed to evaluate species abundance or seasonal patterns of adult populations of mosquitoes, previous studies performed in Buenos Aires support our observations. For example, see Campos and Maciá (1996) and Vezzani et al. (2004) for *Ae. aegypti*, Maciá et al. (1995) for *Oc. albifasciatus*, and García et al. (1995) for *Ma. titillans*.

Some important issues of *Cx. pipiens* complex and *Ae. aegypti* in Buenos Aires can be highlighted: (a) they were some of the most abundant mosquito species, (b) their abundances during the PTPmean20 were the highest, and (c) they harboured L_1 larvae. Furthermore, natural populations of *Cx. quinquefasciatus* have been found carrying infective L_3 larvae in Brazil (Labarthe et al., 1998), and natural transmission of *D. immitis* by *Ae. aegypti* was demonstrated (Hendrix et al., 1986). For the reasons mentioned above, these species are the most likely candidates for transmitting *D. immitis* in Buenos Aires. Both species breed in man-made containers and frequently feed on humans (Rossi and Almirón, 2004), and their high anthropophily enhances the role of this parasite as zoonotic agent in temperate Argentina.

4. Conclusions and future perspectives

The leading position of Brazil on dirofilariasis in South America has been stressed throughout the present work. To perform a future comprehensive analysis of this disease at a regional scale, it is necessary to evaluate the presence of *D. immitis* in countries providing only historical records (Venezuela, Surinam, Paraguay, and Guiana), as well as in those where information is not available (Bolivia, Uruguay, Ecuador, and French Guyana).

In Argentina, canine heartworm is widespread from temperate cities in Buenos Aires Province to subtropical cities close to the northern border of the country, covering at least seven provinces. The

national prevalence was approximately 8% at the end of the 80s, and local values range between 0 and 71%. Indeed, no data were recorded from Northwest Argentina since the finding of dogs harbouring unidentified microfilariae, more than 50 years ago. Some crucial questions are pending on canine dirofilariasis at country scale. First, which is the epidemiological situation in the centre and northwest of the country? And second, which is the current national prevalence? Answering these questions will allow to determine if canine heartworm shows a temporal trend, either downward or upward, in Argentina.

Knowledge on human dirofilariasis, as well as on wild hosts and mosquito vectors of *D. immitis* is certainly at an early stage in Argentina. Potential *D. immitis* vectors should be evaluated in different areas because actual vectors can vary throughout the country due to climate heterogeneity. Different vector control strategies should be used depending on the breeding sites of mosquito species (e.g. artificial containers, temporary pools, swamps). It is also necessary to expand the knowledge of wild mammals serving as hosts of *Dirofilaria* species; the common coati is the only one found infected in the country so far. Despite the fact that at least six wild *Dirofilaria* species have been reported from South America (Canestri Trotti et al., 1997), there is no information about *Dirofilaria* species other than *D. immitis* in Argentina.

Human pulmonary dirofilariasis due to *D. immitis* is known to exist in Argentina based on the few but well documented cases since 1994, and therefore clinicians, radiologists and pathologists should be aware of this zoonotic infection. In the last few years, several authors mainly from United States and Europe, provided strong evidence supporting the highly underdiagnosed condition of human dirofilariasis, and assumed that seroprevalence in humans could correlate with canine prevalence. In this sense, human seroprevalence surveys conducted in endemic areas of canine heartworm in Argentina would weigh up how frequent is the contact between humans and infective larvae of *D. immitis*.

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