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Epidemiology of canine heartworm in its southern distribution limit in South America: Risk factors, inter-annual trend and spatial patterns

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ABSTRACT

This study was aimed at understanding some aspects of the canine heartworm epidemiology in the southern distribution limit of the parasite in South America. With this objective, 19,298 blood samples of owned dogs from 65 localities of 13 municipalities of Buenos Aires Province were tested for *Dirofilaria immitis* circulating microfilariae and/or female antigens. The overall heartworm prevalence was 1.63% by microhematocrit tube technique ($n = 19{,}136$), 3.65% by modified Knott ($n = 713$), and 14.41% by antigen test kit ($n = 118$). Microfilaremic dogs showed a median of 1933 microfilariae per millilitre ($q1 = 375$, $q3 = 5625$, $n = 100$). Male dogs belonging to breeds of short hair and large size recorded significantly higher prevalences than the other categories. Also, the prevalence increased significantly with the age and only dogs younger than 12 months were not found infected. A clear decreasing trend of the annual prevalence was observed during the whole study period, from 3.91% in 2001 to 1.17% in 2006. *D. immitis*-infected dogs were detected in 32 localities of 9 municipalities (prevalence range: 0.2–6.7%). Generalized linear models were used to assess associations between heartworm prevalence and environmental variables. The resulting significant models were univariate and included variables related with soil cover and human population density. The best model predicted maximum heartworm prevalences around middle values of bare soil cover, and lower at high and low covers. According to our analyses, canine heartworm infection in urban temperate Argentina could be described as relatively low, endemic, and spatially heterogeneous. Host and environmental factors affecting heartworm transmission at local level were identified and discussed.

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1. Introduction

The nematode *Dirofilaria immitis* (Spirurida: Onchocercidae) is the causative agent of dirofilariasis, a mosquito-borne disease reported from tropical to temperate regions. This parasite, commonly known as the canine heartworm, has elicited a voluminous literature mainly because of its importance as a dog pathogen (Anderson, 2000). Among

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the earliest reports of the parasite, infections in dogs, humans and cats belong to Brazil (Labarthe and Guerrero, 2005). However, current knowledge about its epidemiology in South America is scarce in comparison with North America and Europe (see Genchi et al., 2007a; McCall et al., 2008). The identification of local factors affecting heartworm transmission and of the spatial and temporal patterns of the prevalence is essential to improve disease control in the canine population.

In Argentina, since the first detection of *D. immitis* in 1931, canine dirofilariasis was registered in seven out of 23 provinces, being the southernmost records in the Province of Buenos Aires (Vezzani et al., 2006). According to theoretical models based on thermal regime and potential vectors, about one-third of the country would be suitable for heartworm transmission and Buenos Aires has transmission risk during half of the year (Vezzani and Carbajo, 2006). From 1992 onwards several studies estimated the heartworm prevalence in different local dog populations from this area (Lightowler et al., 1992; Arias et al., 1994; Meyer and Milanta, 1997; Rosa et al., 2002; Notarnicola and Navone, 2007). The bulk of information suggests a spatially heterogeneous infection pattern, but until the present there was no attempt either to model it or to look for variables associated with this presumed pattern.

The objective of this research was to contribute to the understanding of *D. immitis* epidemiology in the southern distribution limit of the parasite. With this aim, we assessed the association of canine heartworm prevalence with some host factors, and analysed the inter-annual trend and the spatial distribution patterns of the infection in urban temperate Argentina.

2. Materials and methods

2.1. Study area

A total of 65 localities belonging to 13 municipalities of Buenos Aires Province were included in the study. The area included in the survey (254,000 ha) embraces the Federal District (Buenos Aires City), La Plata City with its surroundings and the municipalities of the Southern Greater Buenos Aires (Fig. 1). This megalopolis contains areas with different degrees of urbanization, from cities highly urbanized with 3 million people (Federal District) to small localities of less than 2000 inhabitants (El Peligro). The climate is temperate humid with a mean annual RH of 76% and a mean annual temperature of 15.8 °C (Anonymous, 1992).

2.2. Blood samples and techniques

During the period 2001–2006, a total of 19,298 blood samples of owned dogs were submitted by 298 veterinary practitioners to DIAP laboratory (Diagnóstico en Animales Pequeños) for different diagnostic purposes. Whole blood samples collected with EDTA were stored at 4 °C for microfilariae examination within 48 h. Samples were examined to detect circulating microfilariae in the buffy coat interface after centrifugation of the microhematocrit tube during 5 min at 10,000 × *g*. This technique allows the concentration of microfilariae from a small amount of blood (80 μL). Although filarial identification is uncertain by this procedure, it permit to see the characteristic movement pattern of microfilariae and *D. immitis* is the only filarial species historically recorded in dogs from the study area (Vezzani et al., 2006), as was recently confirmed by acid phosphatase stain technique (Vezzani et al., 2008). In order to characterize infection severity in the dog population under study, a count of total microfilariae in 1 mL was performed over 100 infected blood samples.

Modified Knott technique and serological test were only performed when requested by the veterinary practitioner. Modified Knott was done according to Soulsby (1987) and typical morphology of *D. immitis* microfilariae was observed. For serological test, sera sample was stored at −20 °C until it was performed. In Argentina, three in-clinic heartworm antigen test kits were available during the study period: Speed Diro (Bio Veto Test, France), Witness Dirofilaria (Synbiotics Corporation, USA), and Snap 3dx (Idexx Laboratories, USA). All test kits were used prior to the expiration date and according to manufacturer recommendations.

2.3. Data analysis

2.3.1. Host factors and inter-annual trend

First, the heartworm prevalence for each diagnostic technique was calculated. Then, for the analysis of risk factors and inter-annual trend, an overall prevalence was computed; i.e. a blood sample was considered infected by *D. immitis* if any of the three diagnostic techniques was positive. Microfilaria counts were summarized by median and quartiles because they did not follow a normal distribution (Lilliefors test: $P < 0.01$).

The database consisted of 19,298 blood samples with collection date and source (i.e. geographic location of the veterinarian). Among these samples, the gender, the age, and the breed were known for 17,484, 16,280, and 16,883 respectively. Heartworm prevalence by age was compared between categories <1 year, 1 year, 2 years, 3–5 years, 6–10 years, and older than 10 years. Breed was first classified as mixed-breed and pure-breed, and the latter was then regrouped as short or long hair breeds. In addition, the prevalences of those pure-breeds with sample size greater than 100 were analyzed, and also regrouped by size (large, medium, small). In order to assess temporal trends, monthly and annual prevalences were computed.

All statistics comparisons were performed with WINPEPI software (Abramson, 2004). Prevalences between dichotomous categories (male versus female, pure-breed versus mixed-breed, and short hair breeds versus long hair breeds) were compared using the X^2 test for two independent proportions (Fleiss et al., 2003). The comparisons among three or more prevalences (involving age categories, different pure-breeds, the size, and the six years of study) were made with the X^2 test for multiple independent proportions (Fleiss et al., 2003). In addition, the increasing or decreasing trend in prevalence with age and with date was evaluated using the Mantel's test (Abramson, 2005).

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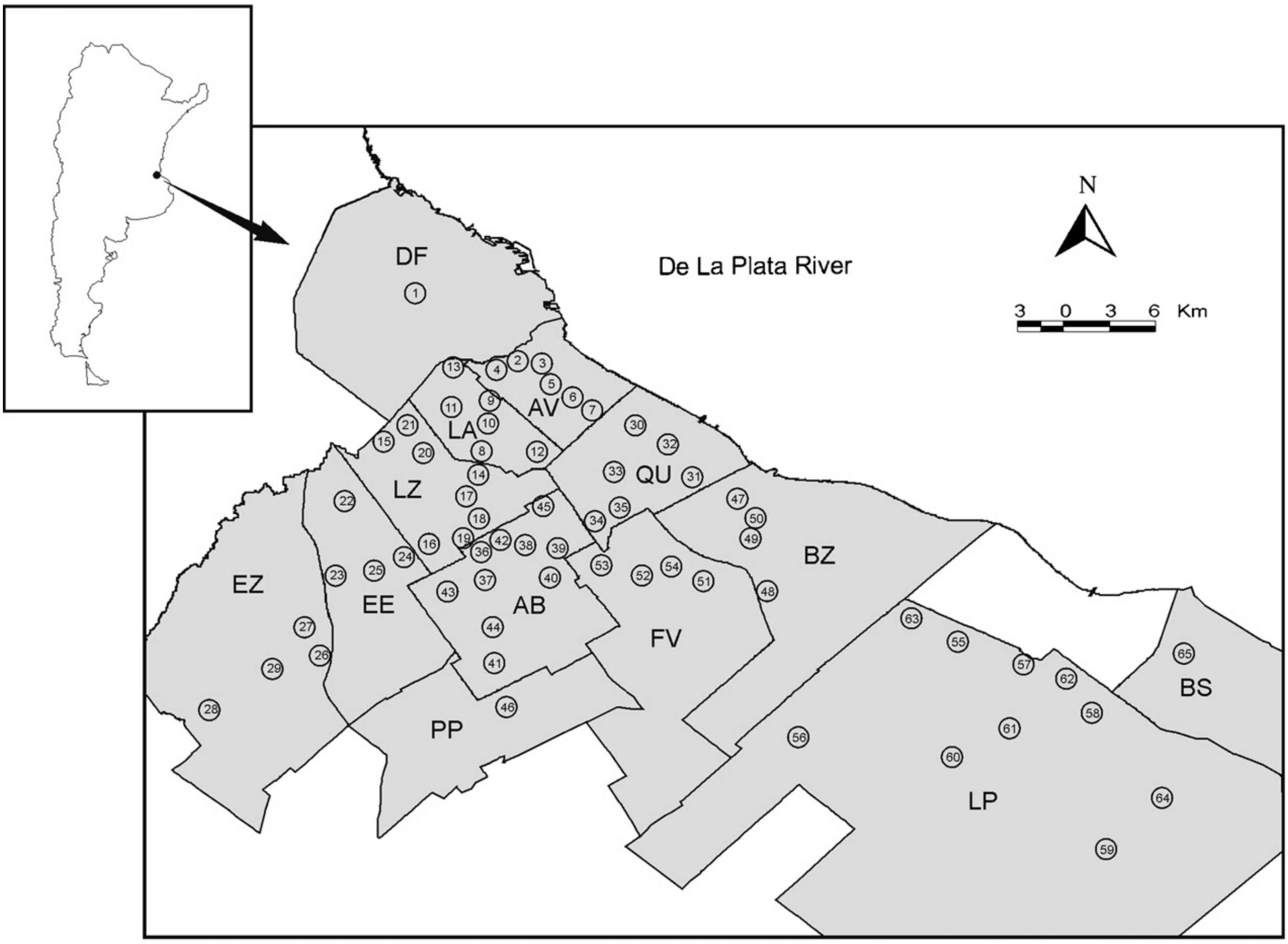

DF: DISTRITO FEDERAL
1. Buenos Aires City

AV: AVELLANEDA
2. Avellaneda
3. Crucecita
4. Piñeiro
5. Sarandi
6. Villa Dominico
7. Wilde

LA: LANUS
8. Escalada
9. Gerli
10. Lanus Este
11. Lanus Oeste
12. Monte Chingolo
13. Valentin Alsina

LZ: LOMAS DE ZAMORA
14. Banfield
15. Ingeniero Budge
16. Llavallol
17. Lomas de Zamora
18. Temperley
19. Turdera
20. Villa centenario
21. Villa Fiorito

EE: ESTEBAN ECHEVERRIA
22. 9 de abril
23. El Jaguel
24. Luis Guillon
25. Monte Grande

EZ: EZEIZA
26. Canning
27. Ezeiza
28. Spegazzini
29. Tristan Suarez

QU: QUILMES
30. Bernal
31. Ezpeleta
32. Quilmes Este
33. Quilmes Oeste
34. San Francisco Solano
35. Villa La Florida

AB: ALMIRANTE BROWN
36. Adrogue
37. Burzaco
38. Calzada
39. Claypole
40. Don Orione
41. Glew
42. Jose Marmol
43. Loma Verde
44. Longchamps
45. San Jose

PP: PRESIDENTE PERON
46. Guernica

BZ: BERAZATEGUI
47. Berazategui
48. Gutierrez
49. Ranelagh
50. Villa España

FV: FLORENCIO VARELA
51. Bosques
52. Florencio Varela
53. Gobernador Costa
54. Zeballos

LP: LA PLATA
55. City bell
56. El Peligro
57. Gonnet
58. La Plata
59. Los Hornos
60. Melchor Romero
61. San Carlos
62. Tolosa
63. Villa Elisa
64. Villa Elvira

BS: BERISSO
65. Berisso

Fig. 1. Geographic location of the localities of Buenos Aires Province included in the study; municipalities names and two letters codes in capital letters.

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2.3.2. Spatial analysis

The assessment of the spatial distribution of the prevalence was performed under the assumption that dogs inhabit the same locality of the veterinary clinic that submitted the samples. A preliminary analysis of the prevalence at municipality and locality levels was done for descriptive purposes. With this aim, X^2 tests for multiple independent proportions (Fleiss et al., 2003) were applied to assess differences of prevalence among municipalities, and multiple pairwise comparisons by Tukey procedure to identify groups of similar prevalences (Zar, 1999). Multiple independent proportions tests were also done among localities of the same municipality.

Finally, the spatial distribution of heartworm prevalence was studied in relation to environmental variables. Each of 65 localities was represented by a point in a Geographic Information System (GIS) that included the main cartographic features of the study area (IGM, 2005). The study spatial resolution of all the analyses was a square cell of approximately 1 km side. In order to represent environmental variables, Thiessen polygons were built around each locality. Series of environmental variables were obtained from the GIS to be used as explanatory variables as follows: mean distance to De La Plata River (MDR), lagoons total surface (LTS), proportion of the surface with lagoons (PSL), and human population density (HPD). The vegetation continuous fields from MODIS images (Hansen et al., 2003) were also used as explanatory variables. The polygon around each locality was used to calculate mean cover by trees (MTC), by grass (MGC), by bare soil (MBSC) and their standard deviations (SDTC, SDGC and SDBSC, respectively). All these explanatory variables represent environmental characteristics that might determine the presence and/or abundance of potential mosquito vectors of the parasite, and also the density and/or behavior of the dogs. HPD, MTC, MGC, MBSC, SDTC, SDGC, and SDBSC are highly associated with the urbanization degree, whereas MDR, LTS, and PSL with the presence of medium and large water bodies.

Generalized linear models (GLMs) (McCullagh and Nelder, 1989) were used to study the association between heartworm prevalence and the environmental variables. These GLMs permit a wider range of models than linear regression, which assumes normal error distributions and constant variance, and are preferable to the log transformation of the response variable when the distribution is very skewed (Wilson and Grenfell, 1997). Because the model uses maximum-likelihood estimators, the fit is measured by the reduction in deviance instead of variance (typical of least-squares estimation). The response variable was the number of positive samples per locality. A quasi-Poisson error distribution was used, as the variance-mean ratio was higher than one (overdispersion). So, the model estimates a dispersion parameter for the variance-mean relation instead of supposing it to be 1. The explanatory variables ($x1, x2$) are related to the response variable through a linear predictor (LP); $\text{LP} = a + bx1 + cx2$, where a, b, c are parameters to be estimated. To force the predicted values to be positive the LP was linked to the number of positive samples (ps) as $\text{LP} = \ln(\text{ps})$. As the number of samples in each site differed, an offset was applied in the model, adding the log(number of samples) to the LP. This procedure makes a weighted regression equivalent to model the prevalence (ps/number of samples) with a binomial error, but resulted in a better model fit.

A manual upward stepwise multiple regression procedure was used to find the best models. First, explanatory variables were fitted individually. Significance was evaluated for each term addition with an *F*-test on the change in deviance (Zuur et al., 2007). The statistic was D2-D1/phi(d.f.1 − d.f.2), with D1 the deviance of the model in consideration, D2 the deviance of the model with the additional term, d.f. the corresponding degrees of freedom and phi the estimated dispersion parameter. Due to the high number of variables tested, the used significance alpha for retention was 0.01. The significant variables that explained the higher deviance were used in turn as start up (three branches were started). Subsequent variables were added with the same restrictions provided they had not a correlation coefficient module higher than 0.5 with any variable already included. The quadratic terms and interactions were also tested. The final model parameters were bootstraped to discard the effect of very influential observations and further compensate for the different number of samples in each locality. If the 95% confidence interval of a parameter contained zero the term was deleted from the model. Residuals plots were used to verify the model validity. Semivariograms (Bailey and Gatrell, 1995) were used to detect any spatial correlation in the residuals. The explanatory power of the model was estimated with D^2, the ratio of the residual to null deviance (equivalent to R^2 in least-square models). To evaluate the final models accuracy, the localities were classified into positive or negative for heartworm. A confidence interval for ps was built as ($\text{ps} \pm t_{95}$ Standard Error_{ps}), with $t_{95} = 2$. If the interval included zero then it was classified as negative, while if it did not it was coded as positive. The sensitivity, specificity and correct classification ratio were calculated from comparing the predicted and observed localities with or without heartworm. The predicted ps were used to calculate a prevalence for each locality (ps/analyzed samples). A map was built with the predicted heartworm prevalence assigned to each polygon when the ps differed significantly from zero. S-plus 6.1, S+ SpatialStats, Arcview GIS add-on (Insightful Corp., 2002) and Arcview Gis 3.2 (ESRI, 2000) were used for modeling and mapping.

3. Results

Considering the entire study period and area, the heartworm prevalence was 1.63% (312/19,136) for microhematocrit tube, 3.65% (26/713) for Knott and 14.41% (17/118) for serological commercial kits; all these values differed significantly at $P < 0.001$. Microfilaremic dogs showed a median of 1933 microfilariae per millilitre ($\text{min} = 10$, $q1 = 375$, $q3 = 5625$, $\text{max} = 37{,}500$, $n = 100$).

3.1. Host factors

All host factors considered (i.e. gender, age and breed) showed some importance in the determination of the heartworm prevalence. The males (166/7621: 2.18%)

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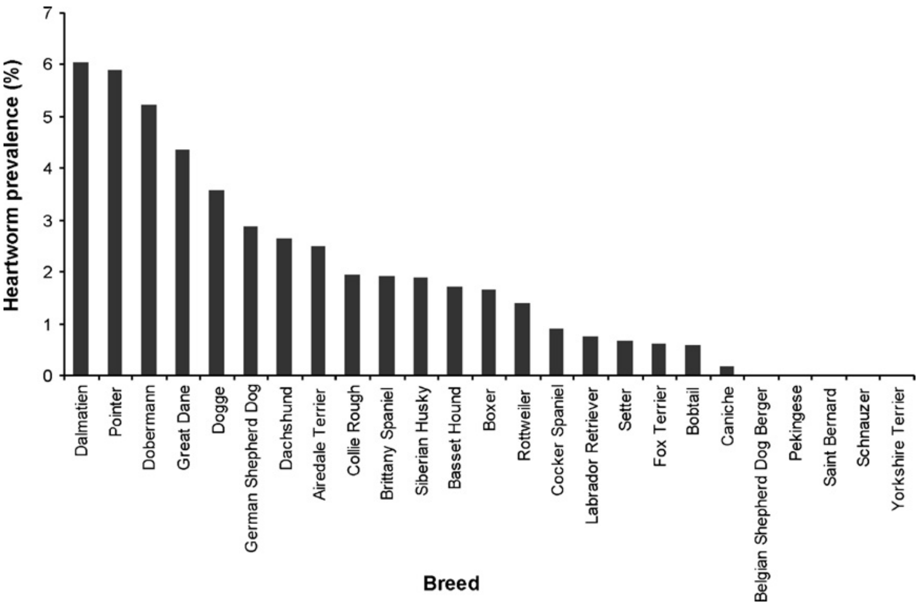

Fig. 2. Heartworm prevalence by breed in Buenos Aires (Argentina) during the period 2001–2006.

recorded higher prevalence than the females (134/9863: 1.36%) ($X^2 = 17.124$, $P < 0.001$).

Regarding the age of the dogs, the heartworm prevalence differed significantly among categories ($X^2_{(5)} = 50.38$, $P < 0.001$) and only dogs younger than 12 months were not found infected. The prevalence increased with the age up to 2.41% in dogs of 6–10 years and then suffered a slight decrease in older dogs. This increasing trend was significant according to Mantel test ($X^2 = 28.44$, $P < 0.001$).

There was not significant differences ($X^2 = 0.052$, $P = 0.819$) between dogs of pure-breed (173/9794 = 1.77%) and mixed-breed (122/7094 = 1.72%). Among the pure breeds, those of short hair (92/3284 = 2.80%) registered prevalence significantly higher ($X^2 = 30.5$, $P < 0.001$) than those of long hair (81/6510 = 1.24%). When the most represented breeds (sample size > 100) were compared, there was significant difference among them ($X^2_{(24)} = 134.97$, $P < 0.001$) (Fig. 2). Those breeds with prevalence higher than 3% belong to short hair breeds and those never found infected belong mainly to long hair breeds. The prevalence also increased significantly as the dog size category ($X^2_{(2)} = 40.6$, $P < 0.001$; small: 0.27%, medium: 1.59%, large: 2.61%).

3.2. Inter-annual trend

Although values of heartworm prevalence varied among months without a clear seasonal pattern, the annual prevalence showed a marked decreasing trend (Fig. 3). This

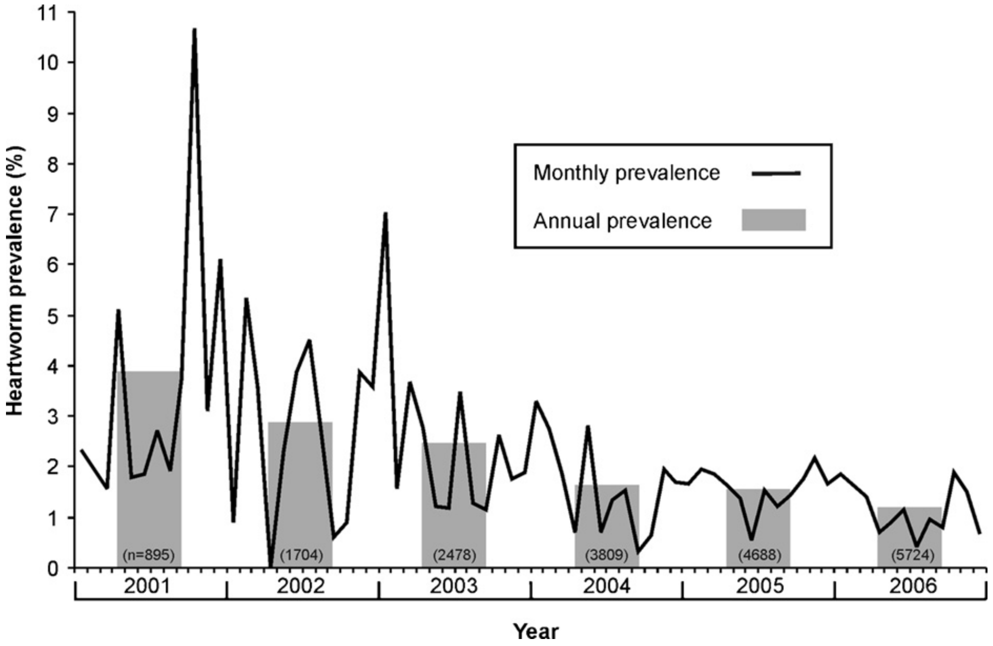

Fig. 3. Monthly and annual values of heartworm prevalence in Buenos Aires (Argentina) during the period 2001–2006; annual sample size within brackets.

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Table 1
Canine heartworm prevalence by municipality in Buenos Aires, period 2001–2006.

Municipality (code)	Sample size	Prevalence (%)
Avellaneda (AV)	4180	2.44 a
Lomas de Zamora (LZ)	4366	2.20 a
Almirante Brown (AB)	4473	2.17 a
Quilmes (QU)	1886	1.64 a b
Florencio Varela (FV)	474	1.05 a b c
Berazategui (BZ)	201	1.00 a b c
Lanus (LA)	1935	0.67 b c
Esteban Echeverria (EE)	402	0.25 b c
Ezeiza (EZ)	460	0.22 b c
La Plata (LP)	800	0.00 c
Distrito Federal (DF)	44	0.00 c
Berisso (BS)	42	0.00 c
Presidente Peron (PP)	35	0.00 c

Same letters indicate no significant differences ($P > 0.05$) between municipalities according to the multiple pairwise comparisons (Tukey procedure).

trend was significant according to Mantel test ($X^2 = 49.82$, $P < 0.001$). The annual prevalence differed significantly ($X^2_{(5)} = 54.52$, $P < 0.001$) among the years involved in the study, being the highest value 3.91% in 2001 and the lowest 1.17% in 2006.

3.3. Spatial patterns

The prevalence differed significantly among municipalities ($X^2_{(12)} = 62.24$, $P < 0.001$), with the highest values around 2.17–2.44% (AV, LZ and AB) and four municipalities without infected dogs (DF, PP, LP, and BS) (Table 1). Within each municipality, the prevalence was internally heterogeneous for AB ($X^2_{(9)} = 65.20$, $P < 0.001$), LA ($X^2_{(5)} = 15.40$, $P < 0.01$), LZ ($X^2_{(7)} = 16.69$, $P < 0.05$) and QU ($X^2_{(5)} = 16.83$, $P < 0.01$). At locality level, the presence of *D. immitis*-infected dogs was registered in 32 out of 65 localities assessed, with a prevalence range of 0.2–6.7% (Fig. 4).

The univariate GLMs significant variables were MBSC, MGC, HPD, and SDBSC (Table 2). In all cases the quadratic relations explained more deviance than the linear ones. Most significant explanatory variables were correlated as follows: (a) MBSC positively with HPD and negatively with MGC, MTC, SDTC and MDR; (b) MGC positively with MDR and negatively with HPD and SDMBSC; and (c) HPD negatively with MTC, LTS and SDTC. No multivariate model resulted significant. The models based on HPD and SDBSC showed very poor explanatory power (less than 19%). The models based on MBSC and MGC explained the variability of canine heartworm cases better. The residuals of these models showed no spatial correlation. Between these two models we chose MBSC to proceed with the analysis due to its highest explanatory power and correct classification (Table 3). The spatial distribution of the prevalence predicted by this model showed two clusters, one south of Buenos Aires City and another around La Plata City (Fig. 5). The maximum heartworm prevalence was predicted around middle values of bare soil cover, and its lowers at high and low covers (Fig. 6).

4. Discussion

Considering the comprehensive database analyzed, canine heartworm infection in urban temperate Argentina could be described as endemic, spatially heterogeneous and relatively low. A revision of the surveys of canine heartworm prevalences in different continents suggests that

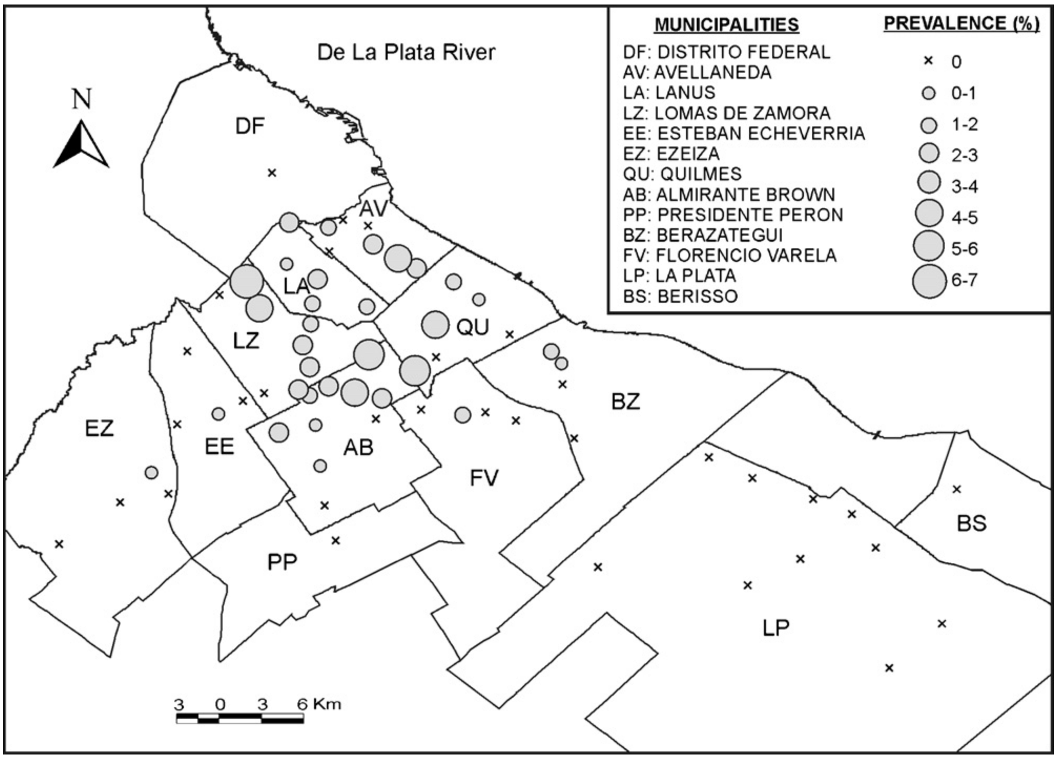

Fig. 4. Spatial distribution pattern of heartworm prevalence at locality level in 13 municipalities of Buenos Aires (Argentina) during the period 2001–2006.

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Table 2
Univariate GLM significance for the number of heartworm positive samples in Buenos Aires, Argentina.

Variable code	Description (units)	Linear model P (e.d.)	Quadratic model P (e.d.)
MBSC	Mean bare soil cover (%)	0.205 (4.8)	0.000 (86.9)***
MGC	Mean grass cover (%)	0.236 (4.1)	0.000 (77.2)***
HPD	Human population density (inhabitants/ha)	0.002 (25.5)**	0.001 (38.3)**
SDBSC	Standard deviation of bare soil cover (%)	0.390 (2.1)	0.009 (27.2)**
SDGC	Standard deviation of grass cover (%)	0.011 (18.9)	0.026 (21.4)
MDR	Mean distance to De La Plata River (km)	0.096 (8.5)	0.024 (21.0)
MTC	Mean tree cover (%)	0.293 (3.3)	0.036 (20.4)
PSL	Proportion of the surface with lagoons	0.700 (0.4)	0.042 (18.6)
LTS	Lagoons total surface (ha)	0.097 (8.2)	0.050 (17.5)
SDTC	Standard deviation of tree cover (%)	0.061 (10.6)	0.153 (11.8)

The model was a quasi-Poisson with log link and log(number of samples) offset. Significance (P) and explained deviance (e.d.) for the linear (x) and quadratic ($x + x^2$) models of each variable are shown; P values correspond to an F-test on the change of deviance (see text for details). The null model deviance was 207.14.

** $P < 0.01$.

*** $P < 0.001$.

Table 3
Parameters and explanatory power of the two selected GLMs for the number of heartworm positive samples in Buenos Aires (Argentina).

	Mean bare soil cover (MBSC)			Mean grass cover (MGC)		
	Parameter	S.E.	d.f.	Parameter	S.E.	d.f.
Intercept	−7.6736	1.2565		−52.0548	23.8882	
Variable	0.3182	0.1012	1	1.3788	0.6675	1
Square of variable	−0.0063	0.0021	1	−0.0098	0.0047	1
Explained deviance	86.9		2	77.2		2
Null deviance	207.1		64	207.1		64
Sensitivity	87.5			90.6		
Specificity	60.6			51.5		
Correct classification (%)	73.8			70.8		
Explained deviance (%)	42			37		

Parameters and S.E. were obtained by bootstrap resampling.

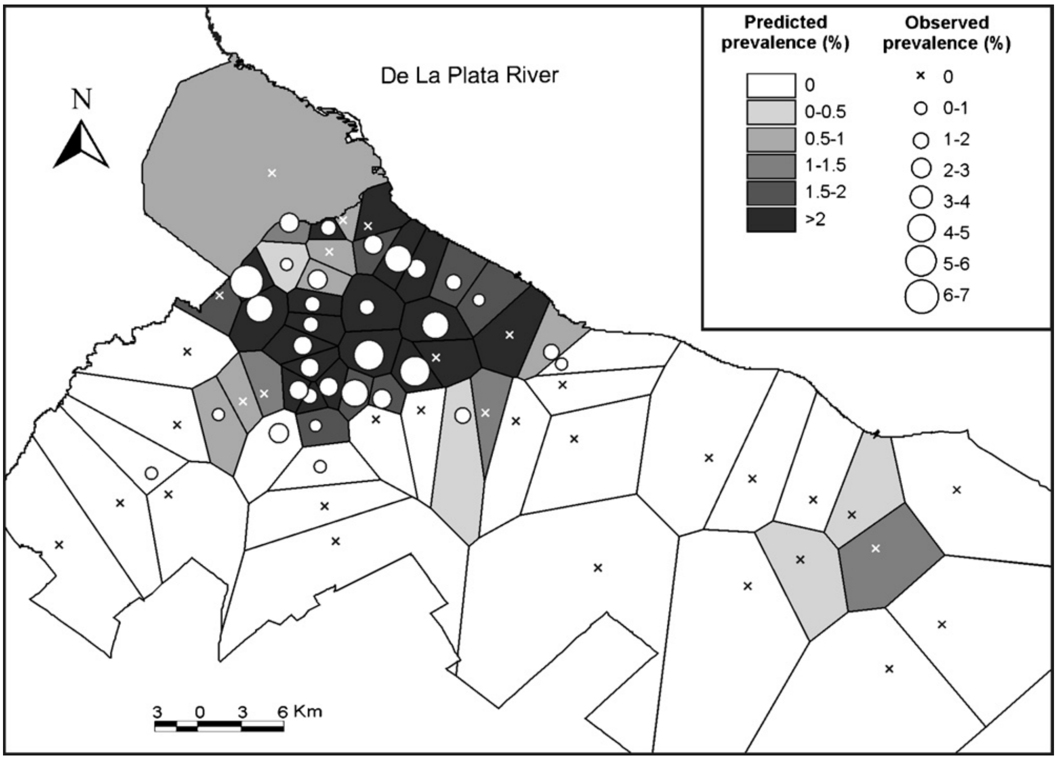

Fig. 5. Predicted spatial distribution pattern of heartworm prevalence in Buenos Aires (Argentina) according to a GLM function of bare soil cover (see text for model details). Observed values of heartworm prevalence at locality level were also included.

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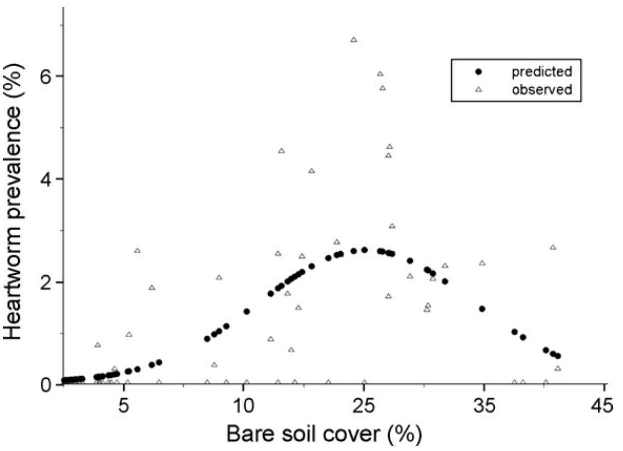

Fig. 6. Predicted and observed heartworm prevalences in Buenos Aires (Argentina) as a function of bare soil cover. Predicted values correspond to a GLM function of bare soil cover (see text for model details).

practically any value up to 84.4% has been reported (Genchi et al., 2007a; McCall et al., 2008). This variability of results may reflect actual differences related to epidemiological factors but, in some cases, just could be due to different testing methodologies, the dog population included (e.g. stray or owned) or also the sample sizes used.

Microhematocrit tube and modified Knott techniques showed a marked difference in the prevalence estimated; 1.63% and 3.65%, respectively. Both concentration methods showed lower values than antigen test kits (14.41%), as expected according to the suggested sensibilities of different diagnostic methods (Genchi et al., 2007b). However, it is noteworthy to mention that antigen testing is not requested by veterinary professionals as a routine diagnostic in our study area, and in many cases this request presumes a heartworm infection. On the other hand, a previous survey in the same study area suggested that about 20% of the microfilaremic dogs are antigen negative due to low worm burden (Vezzani et al., 2008). Therefore, it is hard to determine if prevalence estimated by antigen commercial kits was over- or subestimated in our database.

Regarding host factors associated with heartworm infection, the associations detected suggest that adult males of short hair and large size breeds are at higher infection risk. There is a general consensus that heartworm prevalence increases with age because there are more opportunities for dogs to become infected considering a longer exposure to mosquito bites (e.g. Glickman et al., 1984; Araujo et al., 2003; Montoya et al., 2006; Yildirim et al., 2007; Furtado et al., 2009). We observed a slight decrease in the prevalence for the older category analyzed (>10 years). Among possible reasons of this pattern could be that infected dogs die, worms die, or dogs that live to older age have better medical care including heartworm chemoprophylaxis (Glickman et al., 1984). Another explanation could be that these dogs spend more time indoor.

On the contrary, the gender seems to be a somewhat controversial issue and many researches stressed that there is no difference between sexes (e.g. Glickman et al., 1984; Araujo et al., 2003; Montoya et al., 2006; Furtado et al., 2009). Others studies suggested that males are more prevalent due to the time expended outdoors for guardian or similar tasks (e.g. Selby et al., 1980; Yildirim et al., 2007). However, when only dogs maintained outdoors were compared, Theis et al. (2001) found no difference between genders and Bolio-Gonzalez et al. (2007) reported a higher prevalence for females in a survey of stray dogs. In our survey, there is no available information about the time that dogs spend outdoors or other behaviors that potentially affect to males and females in different ways.

In regard to the physical characteristics of the breeds, our results match with several previous studies (e.g. Selby et al., 1980; Montoya et al., 2006; Yildirim et al., 2007). Larger breeds might be more bitted by mosquitoes due to their use as guardian and hunting pets, and the skin of short hair breeds could be more accessible to mosquito proboscis. Not surprisingly, other investigations found no significant association between these characteristics and the infection with heartworm (e.g. Glickman et al., 1984; Araujo et al., 2003). But, the fact that breeds of long hair or small size have never been found more prevalent than the others, clearly suggests that these host factors might have a major effect on the parasite transmission, in agreement with our findings.

A spatially heterogeneous pattern of heartworm infection among dog populations of the same region was observed, for both municipality and locality scales. The Federal District and La Plata city with its suburban surroundings were negative whereas almost all the municipalities of the Southern Greater Buenos Aires were positive. Within this heartworm focus, we identified a well-defined core area with relatively high prevalences up to 6.7% surrounded by localities with marginal (e.g. 0.2%) or null values. In general, our positive/negative records agree with the available information summarized in Vezzani et al. (2006). The exceptions are La Plata and Berisso, where the presence of *D. immitis*-infected dogs has been well documented (Arias et al., 1994; Notarnicola and Navone, 2007) but were negative localities in our survey.

Similar spatial patterns of canine heartworm infection were previously described at different spatial scales. Yildirim et al. (2007) found a focus of high prevalence in the Kaiseri Centrum (Turkey) surrounded by negative or very low prevalent districts, concluding that there were some factors affecting prevalence at local scale. In a national survey in USA, positive samples tended to occur in clusters of endemic foci, surrounded by areas of relatively low prevalence (Bowman et al., 2009). Song et al. (2003) found a higher prevalence in shoreline areas than in urban and mountain areas in South Korea, despite the fact that all the study sites have similar temperature and humidity ranges. One contrasting example is that of Tenerife Island (Spain), where heartworm prevalence seemed to be homogeneous through different climatic zones (Montoya et al., 2006).

Independently of the pattern observed, all these studies described prevalence variations through the space (regions, states, cities, districts) but unfortunately they did not contain spatial explanatory variables in the analysis. There is a scarcity of studies that include an ecological analysis of the spatial distribution regarding canine filariosis (Rinaldi et al., 2007). Our analysis included only broad scale variables because we did not have the exact dog locations. A further study with detailed spatial data surely could

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increase the explanatory power of our models. The final predictive model differed from our field data mainly in the positive cluster predicted for La Plata city, despite the fact that around 400 dogs were tested. The explanatory variables related with the prevalence in our models (soil cover and human density) are basically descriptors of the urbanization degree. Our final model suggests that medium urbanization degrees are the most favorable settings for heartworm transmission in urban areas of its southern distribution limit. The distance to De La Plata River and other variables related with water bodies were originally included because riverside areas are usually mentioned as more prevalent due to higher mosquito abundance (e.g. Rosa et al., 2002; Song et al., 2003). However, our field data and models do not support this hypothesis. Moreover, the only mosquito species found harboring *D. immitis* in temperate Argentina were *Aedes aegypti* and *Culex pipiens* (Vezzani et al., 2006); both are artificial container-breeding mosquitoes particularly abundant within premises and gardens.

Inter-annual variations of heartworm infection are also presumably dependent on multiple factors affecting parasite transmission. According to Labarthe and Guerrero (2005), the downward trend observed in Brazil was probably due to effective chemoprophylaxis, the abusive use of injectable ivermectin and the extensive use of tetracyclines. Similarly, Montoya et al. (1998) associated the downward trend of heartworm infection observed in Canary Islands (Spain) with the education of pet owners and the increased use of chemoprophylaxis. The strong downward trend observed through the six-year period in our study area is almost certainly associated to similar reasons. During the last decade, the local use of injectable and oral ivermectin or related drugs for the prevention and/or treatment of filarial and intestinal parasites, ticks, scabies and demodicosis has been markedly increased (D.F. Eiras, personal observation).

In summary, climatic and environmental factors could have major effects on vector, parasite, and host at different spatial scales. For example, temperature acts at regional scale influencing seasonal abundance of mosquitoes and the extrinsic incubation period of *D. immitis*. At a more detailed scale, urban and rural settings from the same region surely differ on mosquito species composition, and therefore, on which species acts as main vector. Among or within cities, different urbanization degrees could be associated with the density of dogs and the time expended outdoors, or different socioeconomic situations could determine the access to veterinary care and also its quality. The arrangement of climatic and environmental features in each setting probably determines the complexity of the epidemiological situation, and thus, the directions of locally significant host factors and the spatio-temporal pattern of the infection.

Present and future geographic distribution of any vector-borne disease is currently discussed in the context of climate change. The dirofilariasis is not the exception, and in Europe, climate change enhanced by globalization has been pointed out as one of the main drivers of *Dirofilaria* infection spreading (Genchi et al., 2009; Otranto et al., 2009). In South America, there are still no signs of dirofilariasis spreading to previously free infected areas, at least towards the south. Historical records (Vezzani et al., 2006) and current findings from temperate Argentina suggest that the southern fringe for *D. immitis* transmission has remained the same at least since 1980.

The exposed results also serve to stress that canine heartworm prevalence could be described through contrasting sets of values depending on the geographic extension of the area considered and the period involved. So, it is evident that characterizing heartworm infection through a single value of prevalence might be of doubtful validity, and comparisons among dissimilar study designs should be performed with caution. The main bias of the present research was the dog population under study (i.e. owned dogs from urban areas). A further survey including samples from rural areas and stray dogs is essential to complete the current approach of canine dirofilariasis in its southern distribution limit. Several others *Dirofilaria* issues still need to be addressed in Argentina; e.g. feline dirofilariasis, identification of mosquito vectors, current national prevalence, effectiveness of locally available treatments and identification of wild *Dirofilaria* species. Finally, the zoonotic potential of *D. immitis* is an important neglected issue, with only four human infections documented in the country (Vezzani et al., 2006). A serological survey is needed to evaluate the actual epidemiological situation of human dirofilariosis in this endemic area.

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