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Analysis of climate and extrinsic incubation of *Dirofilaria immitis* in southern South America

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Abstract. Dirofilariosis, caused by *Dirofilaria immitis* and *D. repens*, is spreading in several geographic regions. The development of infective larvae in the mosquito vector (extrinsic incubation) needs an accumulated total of 130 degree-days above the 14 °C threshold, normally expressed as heartworm development units (HDUs). Based on this information, temperature-based models have been developed and applied to evaluate the distribution and spread of *Dirofilaria* infections in various countries and continents. Despite the confirmed presence of *D. immitis* in most South American countries, the available information about its epidemiology remains scarce. We analysed the temporal and spatial extrinsic incubation of this parasite in Argentina, Chile and Uruguay, taking into account daily temperatures from 49 meteorological stations during a 30-year period (1982-2012). The theoretically possible number of *D. immitis* generations was calculated based on the number of meteorological stations that reached the 130-HDUs threshold. The resulting information was spatially interpolated using the inverse weighted distance (IWD) model to produce thematic maps. The model shows that 41 of the meteorological stations reach the threshold needed and that *D. immitis* transmission is markedly seasonal with a peak in late spring (December), stable during summer (January to March) and declining in the autumn (April and May). Suitable temperatures exist in Uruguay and most of Argentina, whereas *D. immitis* transmission in Chile is only possible in the north and in the central inlands. The results suggest that the climatic impact on *D. immitis* transmission must have been minimal in the countries investigated since the annual meteorological records did not change much during the 30-year period analysed.

Keywords: *Dirofilaria immitis*, extrinsic incubation period, geographical information system, temperature, South America.

Introduction

Dirofilariosis, caused by *Dirofilaria immitis* and *D. repens*, is spreading in several geographic regions (Genchi et al., 2011; Simón et al., 2012). Dogs are the main reservoirs, while humans are ‘dead-end’ hosts. Still, with about 1,800 cases reported worldwide, human cases are increasing and this has become an important focus of medical interest (Simón et al., 2012).

As in most vector-borne diseases, *Dirofilaria* transmission is dictated by the climate (Otranto et al., 2009; Genchi et al., 2011) since the development of *D. immitis* microfilariae to infective larvae (L_3) in the mosquito vector(s) depends mainly on ambient temperature (Fortin and Slocombe, 1981; Genchi et al., 2011). The total temperature-related energy required for extrinsic incubation to proceed is expressed in degree-days above a proposed 14 °C threshold or heartworm development units (HDUs) (Fortin and Slocombe, 1981; Slocombe et al., 1989). The seasonal dirofilariosis transmission model is based on two assumptions: (i) a requirement of 130 HDUs for the larvae to become infective; and (ii) a 30 days maximum life expectancy for the mosquito vector (Slocombe et al., 1989; Lok and Knight, 1998). Based on this information and with application of a geographical information system (GIS), temperature-based models have been applied to evaluate the distribution and spread of *Dirofilaria* infections (Lok and Knight, 1998; Vezzani and Carbajo, 2006; Medlock et al., 2007; Genchi et al., 2009; Rinaldi et al., 2011).

Despite more than a century since the first description of *D. immitis* in South America (Roncalli, 1998), and its confirmed presence in most of the region’s countries (Labarthe and Guerrero, 2005; Simón et al., 2012), available information about its epidemiology remains scarce (Vezzani et al., 2011a). In Argentina, canine dirofilariosis has been reported in nine provinces, mainly in the temperate and subtropical north-eastern region (Vezzani and Carbajo, 2006;

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Vezzani et al., 2011a; Uhart et al., 2012; Cuervo et al., in press), while Chile is the only surveyed country with no reported cases so far (López et al., 2012; Simón et al., 2012). To our knowledge, studies have not been conducted in Uruguay (Simón et al., 2012). Regarding temperature-based models, a previous assessment of spatial and temporal risk transmission of *D. immitis* only considered Argentina and a 5-year period (1985-1990) (Vezzani and Carbajo, 2006). The aim of the present study was to analyse temporal and spatial extrinsic incubation of *D. immitis* in Argentina, Chile and Uruguay.

Materials and methods

Data regarding the daily temperatures (minimum and maximum), spanning the 30-year period between 1982 and 2012 for Argentina, Chile and Uruguay were provided by the National Climatic Data Center (Asheville, USA). The study included 49 meteorological stations, of which 27 are in Argentina, 9 in Chile and 13 in Uruguay. The mean daily temperature was calculated and the data was analysed following the temperature-based model by Slocombe et al. (1989) and Lok and Knight (1998). The resulting threshold value of 130 cumulative HDUs was based on a 30-day period moving window: HDUs were calculated on a daily basis and accepted only if the threshold value was reached after 30 consecutive days. The daily data were analysed with the Mann-Whitney test ($P < 0.05$) (Karmeshu, 2012) based on the mean of the full 30-year period, as well as the mean of each of the 10 3-year subsets, to see if presence of significant trends due to climate change could be found. The annual periods used were from July 1 to June 30 following the calendar year.

For each period (30 years and 3 years) and each meteorological station, the following parameters were calculated: (i) the highest HDU score of any 30-day period allowing us to determine meteorological areas and periods where *D. immitis* transmission could take place (HDU scores exceeding the 130-unit threshold); (ii) the first day the extrinsic incubation threshold had been met; (iii) the last day the extrinsic incubation threshold had been met (only considered if no more than three values (≤10%) were missing in the preceding 30 days); (iv) the number of days for permissive periods (this did not necessarily mean that the threshold had to be met on all consecutive days during this period); (v) the total HDU accumulated (only if data were available for 95% of the days in the permissive period); and (vi) the number of *D. immitis* generations supported.

A GIS was constructed using the georeferenced points of all 49 meteorological stations, the South merican country boundaries layer (http://www.gadm.org/), a digital elevation model (DEM) layer at 90 m resolution, the derived hillshade layer and the model output database. All point data layers were spatially interpolated using the inverse weighted distance (IWD) model (Vezzani and Carbajo, 2006) to produce the thematic maps. The GIS databases were developed using ArcGis version 10.1 GIS software (ESRI; Redlands , USA).

Results

Based on the 30-year temperature data, 41 of the 49 meteorological stations reached the 130-HDU threshold (Fig. 1). Fig. 2 shows the temporal variation during the year: three in August, six in September, 20 in October, 34 in November, 40 each in December, January, February and March, 38 in April, 18 in May, four in June and 1 (Easter Island, Chile - not shown in the Fig. 2), where year-round transmission could be supported. The permissive period begins in north-eastern Argentina and Uruguay in October and

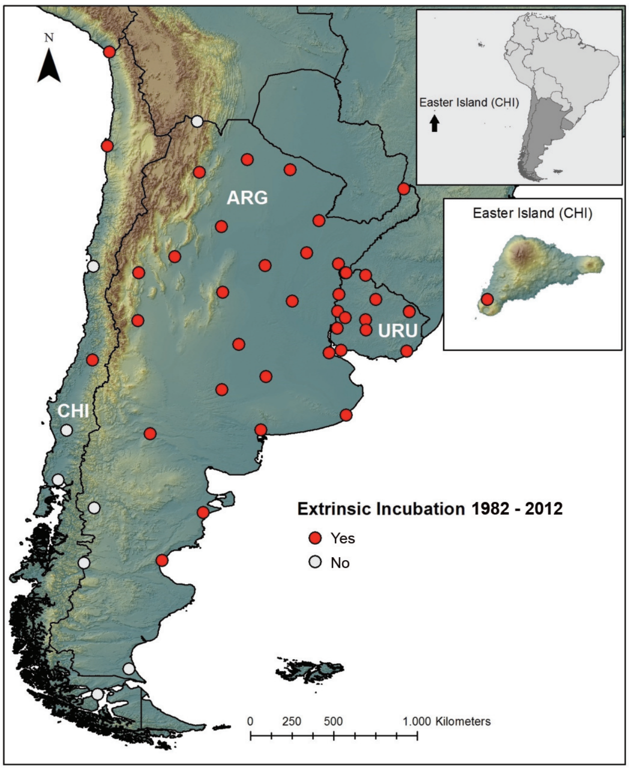

Fig. 1. South American meteorological stations (n = 49) used in the 30-year study. Red dots show stations allowing extrinsic incubation of *D. immitis* at least once; grey dots indicate stations that did not allow extrinsic incubation at any time (ARG, Argentina; CHI, Chile; URU, Uruguay).

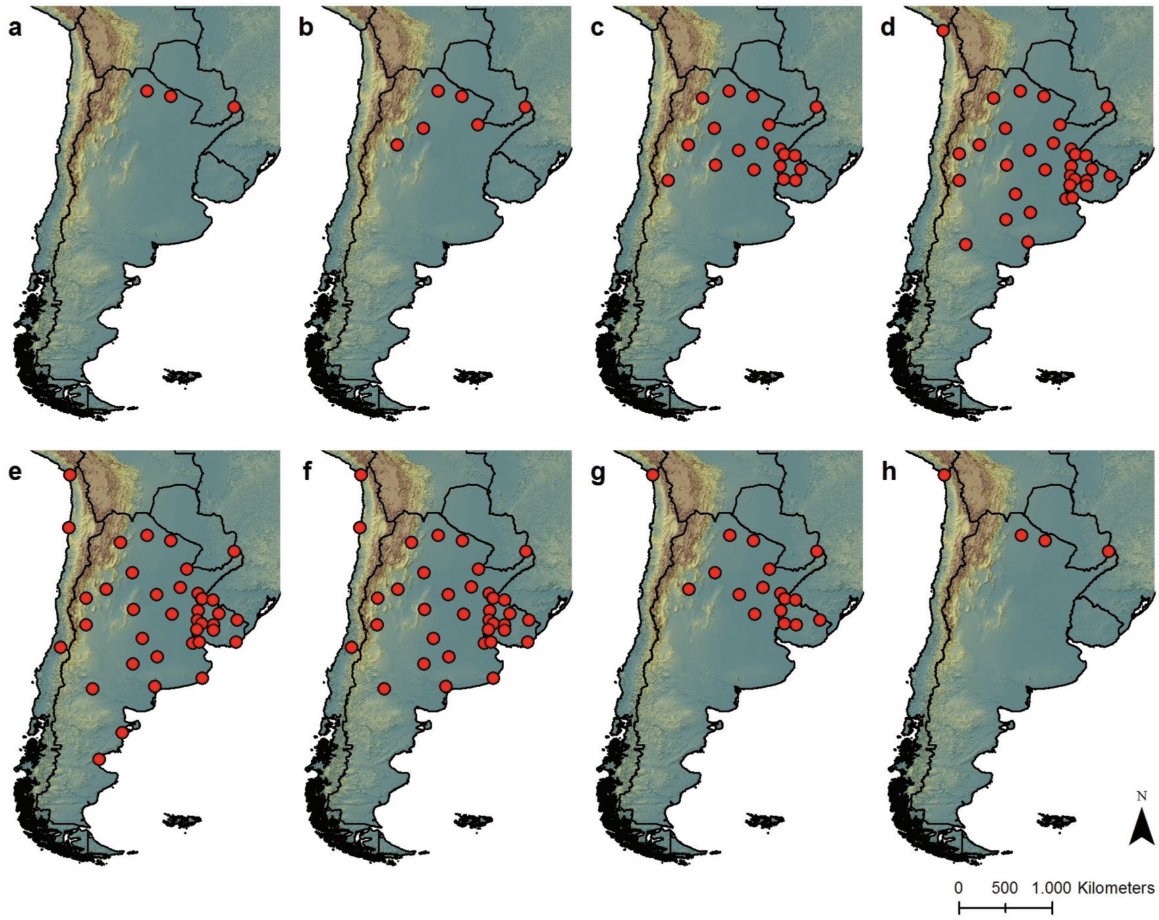

Fig. 2. Meteorological stations reaching the 130 HDUs required for *D. immitis* extrinsic incubation at least once in the 30-year period studied (Easter Island not shown). (a) August; (b) September; (c) October; (d) November; (e) December; (f) April; (g) May; and (h) June.

November. Finally, in Chile, *D. immitis* extrinsic incubation is possible in the most northern part of the country between November and June, with two other stations (one located in the north and the other inland central, presenting suitable temperature conditions between December and April (Fig. 2).

In the beginning of the season (August), *D. immitis* extrinsic incubation is only possible in northern and north-eastern Argentina (Fig. 2a). Then it spreads to the northwest of the country during September but no cases have been reported there so far. However, the number of stations reaching the HDU threshold greatly increases from October (Fig. 2c) onwards, peaking in December with 41 of the meteorological stations reaching the threshold level (Fig. 2e). In December, the limit for areas that could support *D. immitis* extrinsic incubation reached latitude 45º 46' 58.8'' S in Argentina (Comodoro Rivadavia) and 34º 58' 1.20'' S in Chile (Curicó) (Fig. 2e), permitting between three and six *D. immitis* generations (Fig. 3b). The number of stations recording permissive temperatures remained stable until April but decreased dramatically after that in the May and June period (Fig. 2g-h).

No differences were observed between the number of meteorological stations recording permissive temperatures in the first three-year period (July 1982 - June 1985) and the last one (July 2009 - June 2012), and no station changed the status in this respect during the whole 30-year period. When comparing the 10 3-year periods, a non-significant (Mann-Whitney test, P >0.05) trend was observed in most of the meteorological stations (Fig. 4). Inland meteorological stations and those situated along the Atlantic coast presented a positive trend, i.e. increases of the total permissive period for *D. immitis* extrinsic incubation, while stations located in the Patagonia region in southern Argentina, as well as in the Andes and Pacific coast, demonstrated a negative trend, i.e. shorter permissive periods (Fig. 4a). With some exceptions (a few coastal and inland stations), a similar behaviour was observed for the number of *D. immitis* generations that could theoretically be completed (Fig. 4b).

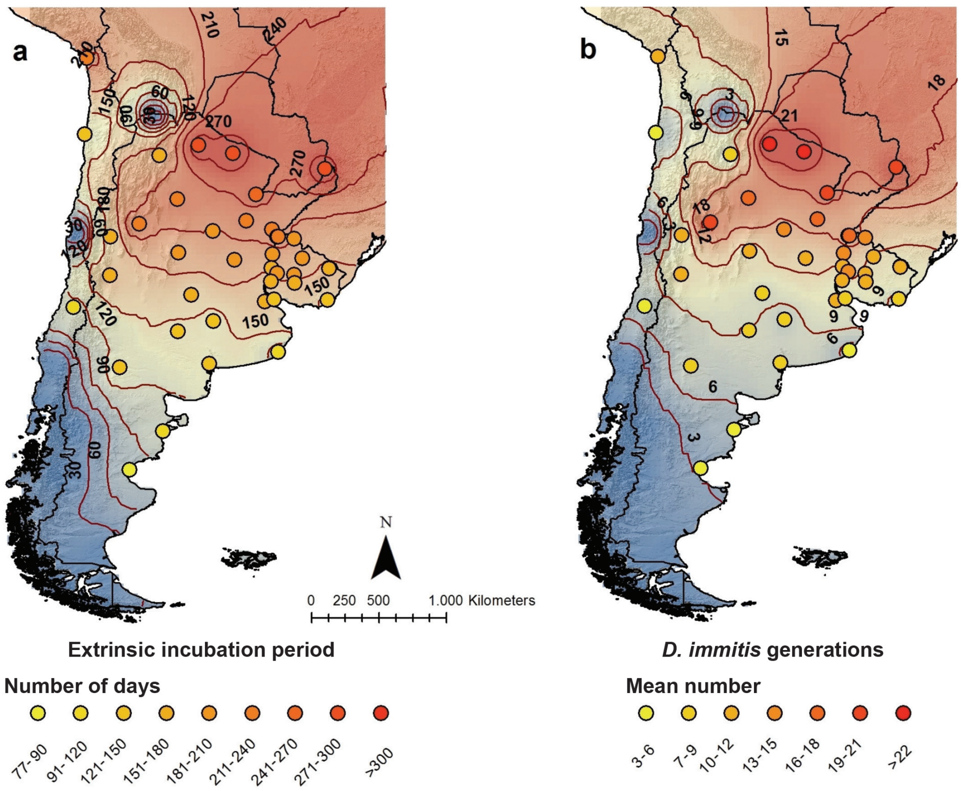

Fig. 3. Length of the permissive period for *D. immitis* extrinsic incubation in days (a) and the mean number of predicted *D. immitis* generations obtained by inverse weighted distance interpolation (b).

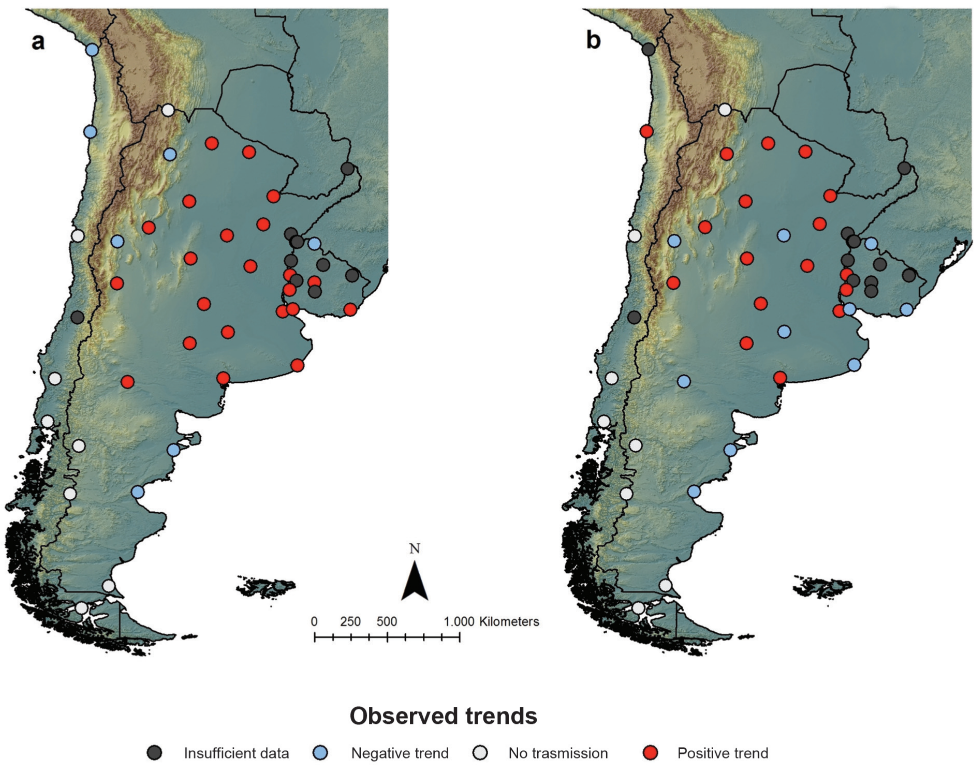

Fig. 4. Meteorological stations showing the different trends observed based on prolonged length of the permissive period for *D. immitis* extrinsic incubation (a) and the number of *D. immitis* generations possible (b).

Discussion

The study showed that *D. immitis* extrinsic incubation is markedly seasonal in southern South America with the climate-based model demonstrating that suitable temperatures in southern South America are mainly found in Uruguay and in most of Argentina.

The situation of dirofilariosis in Uruguay is unclear. Several factors indicate that disease due to *D. immitis* should be present: (i) the temperature-based model shows that extrinsic incubation is possible in most of the territory during 8 months (October-May) (Fig. 2); (ii) eight out of the 11 mosquito species previously found infected in Argentina and Brazil (Vezzani and Carbajo, 2006; Vezzani et al., 2011b) are present (Rossi and Martínez, 2003; WRBU, 2013); and (iii) large areas are in close proximity to the endemic regions in Argentina and southern Brazil (Labarthe et al., 2003; Vezzani et al., 2006). On the other hand, the situation in Chile is completely different as it is the only South American country where *D. immitis* has been extensively searched but never been found (López et al., 2012; Simón et al., 2012). The climate-based model indicates that *D. immitis* extrinsic incubation is not possible in the southern half of the country (below latitude 34º 58' S), where several surveys have been conducted or in low-lying (usually coastal) sites below 150 m above the mean sea level in the central region (Fig. 1). Information regarding presence and distribution of suitable mosquito species is scarce and only three of the potential vector species have been described there (Vezzani and Carbajo, 2006; Vezzani et al., 2011b): mosquitoes of the *Culex pipiens-quinquefasciatus* complex and *Aedes [Ochlerotatus] albifasciatus*, which may be present in most of the country, and *Aedes [Stegomya] aegypti* restricted to the northern region (Heinemann and Belkin, 1979; Angulo and Olivares, 1993; WRBU, 2013). Consequently, extrinsic incubation of *D. immitis* in Chile seems to be restricted to inland sites in central Chile and, mainly, the northern region. A species similar to *D. repens* has been described in the region (López et al., 2012) and a child with an unidentified *Dirofilaria* spp. subcutaneous infection, where *D. immitis* cannot be ruled out, has been reported in central Chile (Pérez and Arce, 2007).

The model indicates that favourable temperatures reach as far as latitude 45º 47' S in the Atlantic coast of southern Argentina (Patagonia), far beyond the observed southern fringe for positive cases (Vezzani et al., 2011a). Only three (*C. pipiens*, *A. [Ochlerotatus] albifasciatus* and *A. [Ochlerotatus] scapularis*) of the proposed vector mosquito species (Vezzani and Carbajo, 2006; Vezzani et al., 2011b) have been reported from Patagonia (Rossi and Vezzani, 2011). Despite suitable temperature conditions and presence of potential vectors, effective transmission in southern Argentina seems unlikely. According to Vezzani and Carbajo (2006), *D. immitis* transmission could only occur in areas with 5 months in which extrinsic incubation would be possible, a condition not met in southern Argentina (Fig. 3).

The results suggest that the impact of climate change on extrinsic incubation of *D. immitis* is minimal in southern South America. The meteorological stations reporting temperatures supporting extrinsic incubation did not change, nor was there significant HDU variations during the 30-year period analysed. The main climatic changes reported are reflected in a decreasing percentage of cold nights and an increasing percentage of warm nights, most pronounced during summer and autumn (Vincent et al., 2006). However, an increasing number of cold days and a decreasing number of warm days, mostly in the summer, were also found in certain parts of Argentina (mainly in Patagonia) (Rusticucci and Barrucand, 2004). Meanwhile, a different situation has been described in central and northern Chile (between latitudes 17º S and 37º S), where there is a strong contrast between the relatively colder temperature observations along the coast and the central valley and western Andes only 100-200 km inland at 500-2,500 m higher altitude where is now warmer (Falvey and Garreaud, 2009). This will, without a doubt, influence the non-significant (positive or negative) trends observed either with regard to the length of the permissive period for extrinsic incubation or the number of *D. immitis* generations.

Although climate change certainly will influence dirofilariosis transmission in southern South America, it is important not to overlook the effects of other changes, e.g. movement of untreated dogs, introduction of competent vector species and increasing urbanization. Given that climate change over *D. immitis* extrinsic incubation in southern South America are minimal, the apparent expansion of *D. immitis* in mid-western and central Argentina (Uhart et al., 2012; Cuervo et al., in press) could be the consequence of some of these other changes. Despite the light trends detected on the extrinsic incubation of *D. immitis*, its major impact may be on the distribution and life span of mosquito species acting as vectors. For a more accurate measure of these impacts, further research on the ecology and the role in disease transmission of mosquito species present in southern South America is required. In conclusion, the factors that influence the transmission and spread of dirofilariosis are multiple, and this study only represents one step forward on this complex path.

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