Ecological study and risk mapping of leishmaniasis in an endemic area of Brazil based on a geographical information systems approach

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Abstract. Visceral leishmaniasis is a vector-borne disease highly influenced by eco-epidemiological factors. Geographical information systems (GIS) have proved to be a suitable approach for the analysis of environmental components that affect the spatial distribution of diseases. Exploiting this methodology, a model was developed for the mapping of the distribution and incidence of canine leishmaniasis in an endemic area of Brazil. Local variations were observed with respect to infection incidence and distribution of serological titers, i.e. high titers were noted close to areas with preserved vegetation, while low titers were more frequent in areas where people kept chickens. Based on these results, we conclude that the environment plays an important role in generating relatively protected areas within larger endemic regions, but that it can also contribute to the creation of hotspots with clusters of comparatively high serological titers indicating a high level of transmission compared with neighbouring areas.

Keywords: visceral leishmaniasis, dogs, geographical information system, hotspots, eco-epidemiology, Brazil.

Introduction

Visceral leishmaniasis (VL) is one of the most severe problems in public health in numerous countries in the Americas. It has been considered a reemerging disease because of several factors including the appearance of transmission cycles in peri-urban areas due to the destruction of primary forests and the establishment of human settlements in modified environments (Asfhord et al., 2000; Werneck et al., 2002; Maia-Elkhoury et al., 2008).

Quite early on, the sand fly *Lutzomyia longipalpis* (Lutz and Neiva, 1912) was shown to be the principal vector of VL in Brazil. However, this mosquito has been now considered a complex of species with a high capacity to colonize different habitats (Lainson and Rangel, 2005). Importantly, the canid element compounds the VL domestic and wild cycles and dogs are

described as the most important reservoirs of *Leishmania chagasi* in the New World (Deane and Deane, 1955; Courtenay et al., 2002). In fact, canid infections precede human cases of the disease as new endemic areas develop (Monteiro et al., 1994). Moreover, the positive correlation between the presence of infected dogs and new human cases in endemic regions was reported long ago (Falqueto et al., 1986).

Control measures in use, including spraying to eliminate adult forms of the vector together with diagnosis and treatment of human patients and elimination of seropositive dogs, have failed in preventing new epidemics (Ministério da Saúde, 2003). Among the several difficulties encountered, the high complexity of eco-epidemiological features involved is probably the most significant barrier. The epidemiological situation is not only greatly influenced by the wide distribution of the disease, but also by the pressure of local environmental factors affecting the populations of human hosts, vectors and reservoirs (Elnaiem et al., 2003).

Geographical information systems (GIS) and remote sensing (RS) are important tools that comprise computational systems, which permit mapping and analysis of environmental factors related to the spatial and temporal distribution of hosts and vectors (Bavia et al., 2005; Margonari et al., 2006). Furthermore, the availability

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of climatic, geological and phytographic digital data, and the accessibility of GIS software, has facilitated the implementation of epidemiological studies in relation to ecological factors and disease prediction (Beck et al., 2000). Considering the scarcity of Brazilian studies of canine VL based on geo-processing techniques, in addition to previous observations of dogs with high serological titers as potentially more infective to the sand fly (Abranches et al., 1991; Courtenay et al., 2002), we utilized this approach to determine the spatial distribution patterns of canine serology in a peri-urban area of Rio de Janeiro, Brazil. The aim was to identify potential risk factors associated with the transmission of *Leishmania infantum chagasi* in this area with the added purpose of exploring the potential utility of an available methodology that could support improved disease control.

Materials and methods

Study area

The work took place in Barra de Guaratiba, a strip of land between the Atlantic Ocean and the mountains of Pedra Branca, which is part of the Serra do Mar massif in Rio de Janeiro state. The total surface of the study site was 1,430,071 m² with a resident population of 4,015 inhabitants (http://www. armazemdedados. rio.rj.gov.br). The climate is tropical with an annual average temperature of 27.5°C and rainfall of 1,400 mm. A secondary Atlantic rain forest covers the mountain slopes but the terrain close to the sea is mainly composed of mangrove vegetation. The latter is, however, gradually yielding to modification by the local population, typically comprising people with low or intermediate income interspersed with tourists. Dogs are common and there are also frequent reports of wild animals such as marsupials, armadillos and primates.

The area is endemic for VL, with the parasite lifecycle principally depending on *Lutzomyia* vectors (Table 1). There is a high animal host seroprevalence and some diagnosed human cases have been reported (Cabrera et al., 2003; Silva et al., 2008). However, no cases of cutaneous leishmaniasis (CL) have been reported, which is different from other places in Rio de Janeiro state where both VL and CL exist.

The canine study subjects

At the beginning of the study 294 dogs were monitored through visits to their home places and tested by the immunofluorescence assay (IFA) for the pres-

Table 1. The various sand flies species trapped in the study area (an VL-endemic area in Rio de Janeiro state, Brazil).

Sand flies species	No. of samples	%
Lutzomyia migonei	309	69
Lutzomyia longipalpis	85	19
Brumptomyia cunhai	41	9.2
Lutzomyia intermedia	6	1.3
Lutzomyia edwardsi	5	1.1
Lutzomyia sallesi	2	0.4
Total	448	100

ence of specific antibodies. However, a great number of the animals were lost during follow-up because of various reasons (death by natural causes, traffic accidents, diseases such as cynomosis and gastroenteritis, relocation of owners). Therefore, the final study population consisted of only 100 dogs that remained in the area during the whole study. Out of this population, 40% lived in the mangrove areas, while the reminder was always close to, or lived inside, the Atlantic rain forest. All dogs were clinically followed up every 2 months and tested by the IFA for specific antibodies to *L.* (*i*) *chagasi* during the 2-year study (2005-2006). To be able to know their whereabouts they were georeferenced and traced with a global positioning system (GPS) instrument, using the ArcGIS (ESRI, Redlands, CA, USA) software.

This work was carried out in accordance with the ethical principles of animal experimentation and approved by the ethics committee on the use of animals (CEUA) of Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil.

Entomological survey

CDC traps (Bellamy and Reeves, 1952) were placed in four dog houses and collections were performed one night per month during one year (2005-2006). Buffers represented by circles of 200 m radius based on the mean flight distance of the phlebotomine vectors (Morrison et al., 1993) were centered on sand fly capture points to indicate the areas of vector influence (Fig. 1). Only presence or absence of the vector, not their density, was considered.

Serology

The IFA was performed using the IFI-leishmaniose canina kit (Bio-Manguinhos, FIOCRUZ, Rio de
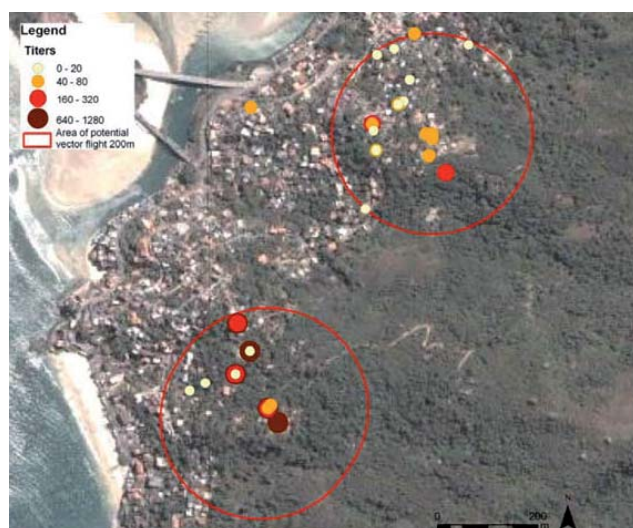
Fig. 1. Visual interpretation of an aerial mosaic of photographs of the study area showing the potential area of vector flight (circles) and the spatial distribution of serological titers in the dogs.

Janeiro, Brazil). The antigen utilized was obtained from *L. (i) chagasi* promastigotes (strain L-579) harvested at the exponential growth phase from an axenic culture in the NNN + LIT medium. All procedures were performed according to the manufacturer's instructions.

Serum dilutions ranging from 1/10 to 1/2,560 were tested with the cut-off dilution set at 1/40, meaning that a change from the titer level of 1/10-1/20 (negative) to ≥1/40 (positive) was required to consider dogs to have undergone seroconversion. The results were sorted into five titer groups: very low, i.e negative = 0-1/20; low = 1/40-1/80; medium = 1/160-1/320; high = 1/320-1/640 and very high = 1/640-1/1280.

Diagnostic techniques

Parasite presence was investigated by imprints and skin smears, fixed with methanol and stained with May Grünwald-Giemsa (SIGMA-ALDRICH®) followed by microscopy. Observation of amastigotes in the tissues under the optical microscope and a positive polymerase chain reaction (PCR), carried out according to Passos et al. (1996), confirmed the diagnosis. In some specific cases, Western blot analysis, as used by Aisa et al. (1998), was also employed.

Geospatial techniques

GIS and spatial clustering techniques were applied to evaluate the presence of high-risk areas of *L. infantum chagasi* infection. Locations of sand fly capture areas, presence of dogs (seropositive and seronegative),

chicken houses and other features of interest in the area were mapped using GPS receivers to obtain latitude, longitude and altitude. For analysis, the ArcGIS software was used to merge the location database with the study group data. The database included a 1:2,000 scale digital map of Barra de Guaratiba and vegetation maps made from the visual interpretation of a mosaic of aerial photographs available at the website of the Pereira Passos Institute (http://www.armazemdedados.rio.rj.gov.br/), Municipality of Rio de Janeiro, using the ArcGIS software and the points chosen for data collection in the field.

Maps were created using kernel density estimator with Gaussian function and smooth kernel of 100 m radius (ArcGIS version 9.1, ESRI; http://www.esri.com/software/arcgis/index.html). The kernel density estimator is an interpolation and smoothing technique for generalizing point location to an entire area. It consists of a bi-dimensional function of the events forming a surface, whose value is proportional to the intensity of samples for the area. Kernel density estimators belong to a class of estimators called non-parametric density estimators. In comparison to parametric estimators, where the estimator has a fixed functional form (structure) and the parameters of this function are the only pieces of information needed to store, non-parametric estimators have no fixed structure and depend upon all the data points to reach an estimate.

Statistical analysis

The Spearman correlation test was applied to evaluate the parameters studied.

Results

Land characterization

The following distribution of geographical characteristics in the study area was observed: low vegetation = 8.5%, dense vegetation = 46.4%, rocky soil = 2.3%, exposed soil = 0.3%, urban areas = 23.5% and water (lakes, rivers, etc.) = 19%.

Entomological survey

L. migonei was the most common species (69%), while *L. longipalpis* composed 19% of the total sand fly population. Four other species, making up the remaining 12% of the population, were also found at the capture points (Table 1).

CL cases

The 40 animals from the mangrove areas were consistently negative throughout the study period, while 50% of the other 60 dogs were seropositive in the beginning of the study. After 1 year, the percentage of positive dogs was 55% and by the end of the study, 2 years later, 63% had positive serological titers ranging from 1/40 to 1/1,280. All positive cases where found in habitats with an altitude range of 63-111 m.

A cluster analysis based on georeferenced dog houses with dogs with positive serology demonstrated a non-random distribution of the seropositive dogs (Figs. 1-2). Two focal areas with dogs with particularly high titers, indicating a comparatively higher risk of *L. (i) chagasi* transmission (hotspots), were found: Caminho da Bica (place A) and Chico Buarque de Holanda (place B) (Fig. 2). Both places had almost the same number of the seropositive dogs (Table 2). In

place A, only 18.7% of the positive dogs presented serologic titers ≥1/160, while 78.6% did so in place B. The seroconversion rates observed were 12.5% and 87.5%, respectively, and all the dogs that showed serum conversion were traced to spend most of their time inside or very close of these hotspots (Fig. 2). All dogs that presented symptoms, tissue parasitism and tested positive by PCR, were also traced in the same places (data not shown).

All animals with serologic titers ≥1/160 emanated from inside, or in the boundary of, the forest (≤15 m). On the other hand, the dogs with lower positive serological titers (1/40-1/80) were predominantly traced to be in degraded areas outside the forest, although they were sometimes observed in the forest as well (Figs. 1-3). Interestingly, some animals remained negative during the whole study, in spite of living together at the same places with others with higher titers.

Serological titers in relation to the presence of chicken houses

It was observed that 75% of all chicken houses were situated in place A. In contrast to place B, the chicken houses were scattered around the area with a distance up to 100 m between them (Fig. 3). Analysing the statistical correlation between distance from the closest chicken house and the serological titer, a significant inverse correlation was demonstrated, i.e. the closer to the chicken houses, the lower the frequency of dogs with high titers.
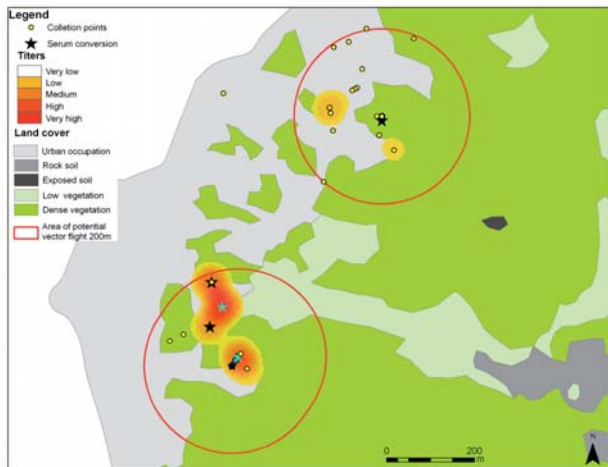

Fig. 2. Geographic distribution of dogs in the study area. Kernel density estimator map (Gaussian function and smooth kernel of 100 m radius) showing the correlation between the land cover and the location of the collection points and the corresponding canine serological IFA titers: very low = negative (0-1/20); low (1/40-1/80); medium (1/160-1/320); high (1/320-1/640) and very high (1/640-1/1,280). Symbols explained in the figure legend.

Table 2. Serological data of a dog population in the study area.

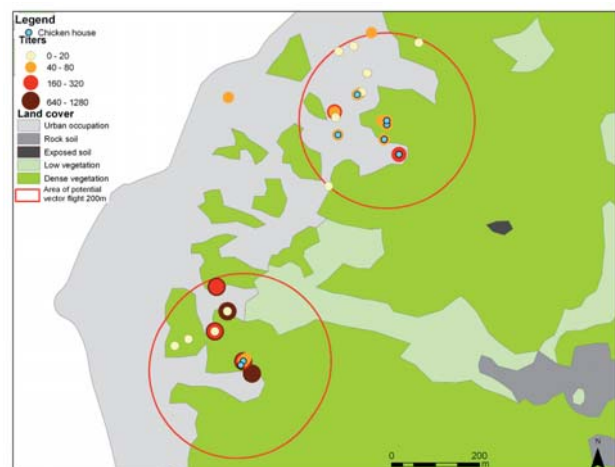|	Sero-negative dogs	Sero-positive dogs		Serum conversion
		1/40-1/80	≥1/16	
Place A	13		0	1
		14	3	
Place B	9			7
		4	17	
Total	228	18	20	8

Fig. 3. Digital map of the study area. The geographic distribution of the canine serological titers and chicken houses in relation to the potential area of vector flight and land cover features.

Serological titers in relation to vector presence

At two capture points, one in each place, the presence of *L. longipalpis* was detected. The superimposition of the vector mean flight distance with the waypoints from the dog houses, demonstrated that only one seropositive animal was outside the potential area of vector influence (Figs. 1-3). All the other species observed were collected at one or more of the four capture points.

Discussion

The VL vector *L. longipalpis* is a highly adaptable sand fly species. In Brazil, it has been reported from the Amazonian and Atlantic rainforests as well as from the caatinga (xeric shrub land in northeastern Brazil) and cerrado (tropical Brazilian savanna, common in the states of Goiás and Minas Gerais) vegetation types, which all represent very different environments. The species has been captured in remote primary forests far from human habitation, but it has also been found in sparsely forested areas or places suffering from forest degradation (Lainson and Rangel, 2005). The ability to colonize a wide spectrum of different habitats and interact with a great variety of potential sylvatic and domestic hosts contributes to the high complexity of VL epidemiology. The currently adopted measures, based mainly on the elimination of the adult forms of the vector plus diagnosis and treatment of human patients together with elimination of seropositive dogs, represent a simplistic approach that may not be enough to solve the problem. Indeed, after 2 years of follow-up, despite implementation of control measures, we identified a high prevalence (63%) of infected dogs as well as a considerable rate of seroconversion (13%), emphasising the need for an improved control strategy.

The GIS methodology employed, allowing the inclusion and evaluation of environmental factors related to the spatial and temporal distribution of hosts and vectors, provides evidence that its use is indispensable in the elaboration of control plans. Here, we present the first study of VL based on GIS methodology in an endemic Atlantic rain forest area in Rio de Janeiro state, Brazil. In a study carried out in the state of Bahia in northeastern Brazil, Nieto et al., (2006), using a broader approach to analyse macro-habitats through models based on genetic algorithm for rules-set prediction (GARP) and growing degree day-water budget analysis, predicted a similar distribution and abundance patterns for the *Lutzomyia longipalpis*-

Leishmania chagasi system. They also predicted a high to moderate risk in the caatinga and a low risk in the cerrado. A low risk was also predicted for the interior forest as well as the coastal forest. Our study, in contrast, observed a high risk of *L. (i) chagasi* infection in an endemic area in the Atlantic rain forest. We think the difference could be related to the fact of *L. longipalpis* is a species complex and consequently could present different behaviour in different regions. It is important to point out that, besides the occurrence of *L. longipalpis* in semi-arid regions, the observation of this species in the Amazon region of Brazil (Lainson and Rangel, 2005) indicates that it is primordially a sylvatic species and that it can still be captured in remote primary forest far from human habitation (Lainson and Rangel, 2005).

In another study based on serial space temporal scan statistics, also performed in Bahia, this time in a semi-arid area, the authors showed that VL can also appear as endemic clusters in the cities (Carneiro et al., 2007). Those observations, in addition to our results that showed a similar pattern in an endemic area situated in the Atlantic rain forest, demonstrate that the *L. (i) chagasi* infections can assume the same trend in different habitats. It underlines how complex the VL epidemiology is since it depends on the biological characteristics of various hosts and vectors as well as their interplay with the habitat. In relation to the vector, we could mention the need for suitable conditions related to humidity and temperature that gives rise to ideal breeding sites as well as the influence of sugar and blood meal sources located inside its mean flight distance.

Cluster analysis of the serological titers in dogs in the study area showed a non-random distribution, demonstrating that the patterns of transmission of canine VL can undergo local alterations, producing hotspots where the risk of infection is very high compared to neighbouring areas. The presence of the eight dogs that serum converted addition to those that presented tissue parasitism confirmed by microscopy of imprints and PCR inside or very close to the hotspots, confirms the higher transmission risk in certain places.

The fact that one part of the study area presented an elevated concentration of dogs with high serological titers, while the other was mostly inhabited by animals with low titers, emphasises the dynamic nature of VL transmission. Transmission patterns may thus be highly influenced by environmental, focal factors capable of producing micro-epidemiological differences resulting in different transmission risks.

The vertebrate host component, besides constitut-

ing a blood meal source for the vector, may represent two opposite possibilities. The first, mainly represented by the dogs, play a key role in maintaining the VL domestic cycle, in which case the spatial distribution of serological titers may indicate the occurrence of a hotspot. This would correspond to areas with clusters of infectious dogs, which favor sand flies infections and consequently contribute to increased transmission rates. However, it could well be that the presence of chicken houses may reduce the mammal risk of infection by attracting the sand flies and thus keeping them away from other hosts.

Several studies suggest that dogs with high serological titers are immunologically unable to control the infection (Cabral et al., 1992; Pinelli et al., 1994). Consequently, they may be much more infective to the phlebotomine vector than dogs with lower titers (Abranches at el., 1991; Courtenay et al., 2002; Palatnik de Souza at al., 2001). Our observations are in agreement with respect to higher infectivity of dogs with high titers since 100% of the cases of serum conversion occurred inside the hotspots that were also the places where there was a concentration of animals with high serological titers.

The animals which remained negative until the final part of the 2-year study were probably either less exposed to the vector (i.e. located far away from the hotspots) or had some degree of resistance. The latter possibility is supported by the fact that five dogs did not become infected, which was confirmed with Western blot and PCR tests, in spite of being kept during the whole study at places together with other dogs presenting very high serological titres (1/1,280). The lower titers, reported in dogs having controlled the infection through cellular immune responses or, conversely, due to a longer incubation period or coming from dogs in the initial phase of the infection (Pinelli et al., 1994), may have a bearing on this result.

Considering the pattern formed by the distribution of serological titers and hotspots observed, it is interesting to note that place A produced just one serum conversion, while seven serum conversions were observed in place B, which also showed a higher frequency of high titers. It is likely that two factors could be at play here: (i) the higher number of chicken houses in place A, thanks to the odor, temperature and CO_2 emitted by chickens, competed successfully with the dogs in attracting blood-seeking sand fly females (Alexander et al., 2002; Caldas et al., 2002; Lainson and Rangel, 2005); and (ii) the forest proximity to place B which increases the risk of transmission due to the reported higher vector density close to the forest

(Deane and Deane, 1955; França-Silva et al., 2005).

The role of the elimination of seropositive dogs as one of the measures in VL control has been fully discussed in relation to its effectiveness by several authors (Dietze et al., 1997; Moreira Jr. et al., 2004). However, culling animals that do not show signs of disease often results in owners hiding their animals, preventing them even to be examined and tested and consequently leading to the persistence of many parasitised dogs. In previous studies, we have suggested that in case of the adoption of control measures that include the removal of seropositive dogs, only animals with titers of ≥1/160 should be eliminated (Silva et al., 2009), because animals that show high serological titers and tissue parasitism must be considered more infective for sand flies (Abranches et al., 1991; Courtenay et al., 2002). The high number of seroconversions in places with a higher number of animals with high titers, observed in the present study, supports that hypothesis. In addition, there is the risk that the infection would persist in spite of culling since the owner would most probably replace the first dog with another and there is no guarantee that the new animal would not be susceptible to *Leishmania* and thus potentially become a new potential source of human infection.

The presence of hotspots in an endemic area of *L.* (*i*) *chagasi* transmission, suggests that environmental factors are important and have to be considered when planning control strategies. Identification of incidence is more important than that of prevalence, because it indicates the areas where transmission can occur in principle.

The work presented here shows that it is possible to predict the specific places of high-risk VL transmission within an endemic area through the mapping of canine serological titers. The risk maps produced from this study should be useful for VL control since we have shown that the areas of the VL incidence coincide with places characterized by clusters of high serological titers.

The identification of circumstances that favor the spread and maintenance of VL based on eco-epidemiological indicators, could eventually be used to set priorities for implementing disease control measures, thus reducing operational costs and increasing effectiveness.

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