Association of the occurrence of Brazilian spotted fever and Atlantic rain forest fragmentation in the São Paulo metropolitan region, Brazil

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ABSTRACT

Brazilian Spotted Fever (BSF) is a zoonotic disease caused by the bacterium Rickettsia rickettsii. In the São Paulo Metropolitan Region (SPMR) it is transmitted by Amblyomma aureolatum ticks. In this region, annual lethality of the disease can reach 80% and spatial occurrence depends on environmental factors and more particularly on the presence and interaction of domestic and wild carnivores as well as the presence and characteristics of the remnant Atlantic Rain Forest patches. This study analyzed the association between forest fragmentation and its influence on the risk of occurrence of the disease in the human population. Domestic dogs tested for R. rickettsii antibodies in nine different areas under the influence of different patterns of Rain Forest fragmented landscapes and human occupancy. Landscape metrics were obtained by analyzing satellite images and high-resolution orthophotos. Principal component analysis (PCA) was used to determine among the different landscape variables the one that could best explain the data variance, and the results were tested against canine seroprevalence in order to address disease occurrence risk levels. From 270 canine samples, the seroprevalence ranged from 0 to 37%. PCA showed an inverse correlation between functionally connected large forest patches and the canine seroprevalence for R. rickettsii (p = 0.030; Spearman's R = −0.683), while there was a positive correlation between forest border effect and canine seroprevalence (p = 0.037; Spearman's R = − 0.909). The further attributed disease occurrence risk level supported the real spatial prevalence of the disease reported for the last eight years (p = 0.023; Spearman's R = 0.63). The results suggest an important relation of deforestation and fragmentation with the occurrence of BSF in the SPMR.

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1. Introduction

Brazilian Spotted Fever (BSF) is an acute infectious febrile disease caused by the bacterium *Rickettsia rickettsii* transmitted by ticks. In South America the main vector species belong to the genus *Amblyomma* (Labruna, 2009a; Labruna et al., 2009b).

The bacterium *R. rickettsii* belongs to the Alphaproteobacteria group, Rickettsiales order and Rickettsiacea family (Brooks and Carrol, 2012; Tortora et al., 2012). This species is considered the most lethal *Rickettsia* in the world, since untreated cases of BSF can reach a mortality rate of 80% (Labruna and Machado, 2006; Angerami et al., 2012). This bacterium has been detected in Brazil, United States, Canada, Mexico, Panama, Colombia, Costa Rica and Argentina, and its distribution is restricted to the Americas (Labruna, 2009a).

Different tick species have been implicated as vectors of *R. rickettsii* in different regions (Labruna et al., 2011b). In Brazil, transmission of *R. rickettsii* is related to two tick species: *Amblyomma aureolatum* and *Amblyomma sculptum* (Gomes, 1933; Pinter and Labruna, 2006; Horta et al., 2010; Krawczak et al., 2014).

Currently, cases of BSF are often reported in southeastern Brazil, mainly in the states of Rio de Janeiro, Minas Gerais, and São Paulo, with the highest incidence in the State of São Paulo (Szabó et al., 2013), where 555 cases with 224 deaths (case-fatality 40%) were reported between 1985 and 2012 (São Paulo, 2014). In the State of São Paulo cases of BSF are associated with tree tick species. In the countryside, especially in the central region, the cases are related to *A. sculptum* and in coastal areas to *Amblyomma ovale*. In the eastern region within the Atlantic Rain Forest mountain domain, human cases are related to *A. aureolatum* (Barbieri et al., 2015). Human BSF cases associated with *A. aureolatum* occur mainly in places sur-

rounded by forest fragments, when dogs play an important role of carrying adult ticks from inside forest areas into houses. (Pinter et al., 2008; Angerami et al., 2012).

An experimental study carried out by Labruna et al. (2011a) showed that this tick species is able to maintain *R. rickettsii* by transestadial perpetuation and transovarial transmission at a 100% rate, which shows the high vector competence of this species. On the other hand, *R. rickettsii* infection was shown to have a deleterious effect on this tick species, which may explain the low infection rate among the tick populations in nature (1%–10%), as demonstrated by Pinter and Labruna (2006) and Ogrzewalska et al. (2012).

A. aureolatum used to be a very abundant species within the São Paulo Metropolitan Area (SPMR) in the early 20th century, but due to expanding urbanization and consequent deforestation of the native Atlantic Rain Forest, at the present time *A. aureolatum* is restricted to forest remnants in peripheral areas. Forest remnants are found all around the SPMR and vary in patch size, shape, vegetation quality and connection to larger forest areas. The main features required of a forest patch to sustain a population of *A. aureolatum* are: enough vegetation to provide a dense canopy to prevent sunlight reaching the ground, over 90% air relative humidity and annual average temperatures under 23 °C (Pinter et al., 2004). Human communities at risk of being parasitized by this tick species are mainly those located close (up to 300 m) to the edges of these patches (Pinter et al., 2008; Ogrzewalska et al., 2012).

The immature stages of *A. aureolatum* mainly use passerine birds as primary hosts, while the adult stage uses wild carnivores as natural primary hosts. However, in areas with high anthropogenic changes, the adult stage successfully adapts to domestic dogs. Humans are only parasitized by the adult stage of this tick species, usually by a single tick specimen, most likely when an adult tick is transferred from a dog to a person during physical contact (Pinter and Labruna, 2006; Labruna et al., 2011b; Ogrzewalska et al., 2012). Saraiva et al. (2014) demonstrated that the adult stage of *A. aureolatum*, when transferred from one host to another, after starting the bloodmeal intake, is able in only ten minutes after skin attachment to transmit enough burden of the bacterium *R. rickettsii* to cause the disease.

Dogs are also susceptible to *R. rickettsii* infection and develop a mild disease with rare death outcome (Piranda et al., 2008). Furthermore, dogs develop an immune response against the bacterium, which can be detected by serological tests. Studies carried out by Pinter et al. (2008) and Ogrzewalska et al. (2012) showed that the serological monitoring of dogs is an important epidemiological tool to determine whether the bacterium *R. rickettsii* is present among the tick population in areas where *A. aureolatum* is established.

Therefore, since dogs are considered sentinel animals for risk of human cases of BSF, seroprevalence studies of dog cohorts to detect de circulation of *R. rickettsii* can be useful in determining risk areas for the disease (Pinter et al., 2008; Ogrzewalska et al., 2012)

Based on these data, this study aimed to test the hypothesis that the circulation of *R. rickettsii* in *A. aureolatum* ticks is correlated with the fragmentation process of the Atlantic Forest in the SPMR, using the domestic dog as a sentinel for the presence of the bacterium in different locations and further considering the number of human case reports.

2. Material and methods

2.1. Studied areas

The present study investigated localities within the SPMR with known occurrence of *A. aureolatum* ticks, according to ecological data from previous studies and epidemiological data from human BSF case investigations. All localities were composed of human settlements in direct contact with remaining Atlantic Rain Forest patches.

Nine areas were selected with different levels of forest fragmentation in order to have significant variation of forest and human occupation profiles. Areas were identified from one to nine, with sites 1, 2 and 3 being within well preserved forest areas, sites 4, 5 and 6 within intermediate to severe deforestation areas, and sites 7, 8 and 9 within areas with very advanced stages of deforestation (Fig. 1).

2.2. Seroprevalence assay

In order to evaluate whether a spotted fever group rickettsia was circulating among the *A. aureolatum* tick population, a cohort of 30 dogs was sampled from each of the nine sites. All samples were obtained in 2014. The geographical coordinates of each dog owner were obtained with a GPS device (Garmin, 60CSx model, Datum WGS 84).

For each studied location, 30 dogs (270 dogs total) were selected that satisfied the criteria of being healthy, maintained unleashed, having unrestricted access to forest patches, having been raised in the area, not having traveled to other places, and being at least one year old (Pinter et al., 2008). Approximately 2 ml of blood was drawn per animal, using disposable syringes and needles. The collected blood was transferred to tubes that were further centrifuged in the laboratory and separated into serum aliquots, which were stored at −20 °C until use in the serological tests. Veterinary doctors performed all the process.

The experiment was approved by the animal ethics committee of the Faculty of Medicine, University of São Paulo (Research Protocol # 103/14).

2.3. Indirect immunofluorescence assay

Each of the 270 dog sera was tested by indirect immunofluorescence assay (IIFA) following previously described protocols (Horta et al., 2004) using as crude antigens, vero cells infected with the bacterium *R. rickettsii* strain Taiaçu (Pinter and Labruna, 2006) or *R. parkeri* strain At24 (Silveira et al., 2007). The antigens slide production and IFA tests were carried out in the Department of Specialized Laboratories of the Superintendência de Controle de Endemias, São Paulo, SP, Brazil.

The sera were initially analyzed at 1:64 dilution. Titers ≥ 1:64 were considered positive. Positive sera were titrated to the endpoint titers by dilution in 2-fold increments.

2.4. Landscape data analyses

In each of the nine selected sites, the forested patches and dwellings were identified by visual analysis of high-resolution high satellite images (CNES/Astrium, DigitalGlobe and Terrametrics compositions, with passage date until October 2012), provided in Google Earth Pro 7.1.5.1557 (Google, Inc. Mountain View, CA, USA).

Polygons of the forest areas and the points representing the residences and other buildings were scanned on screen, and the linear and area calculations were performed in the same software. To assist in solving any sort of classification doubt, the polygons of forest patches were superimposed on panchromatic orthophotos with 1 m spatial resolution, years 2010–2011 (EMPLASA) in the Idrisi Selva program.