Paula Ribeiro Prist

O risco de transmissão da Hantavirose em função do clima e da estrutura da paisagem

Hantavirus transmission risk in function of climate and landscape structure

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Tese apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Doutor em Ecologia, na Área de Ecologia de ecossistemas terrestres.

Orientador(a): Prof. Dr. Jean Paul Metzger

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Prof. Dr. Jean Paul Walter Metzger

Orientador

Dedicatória

Às florestas, aos animais e aos seres humanos.

Epígrafe

"A árvore é a embaixatriz da Paz. Cuidar da saúde do planeta e promover a paz universal são duas faces da mesma moeda".

Wangari Maathai

Ambientalista

Nobel da Paz de 2004

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Resumo Geral

A Síndrome Cardiopulmonar por Hantavirose (HCPS) é uma doença causada por Hantavírus, um conjunto de vírus com RNA negativo pertencentes à família *Bunyaviridae*. Esses vírus são altamente virulentos para os seres humanos, levando cerca de 50% dos infectados a óbito. O principal reservatório de HCPS é constituído por espécies de roedores generalistas, que aumentam em abundância em paisagens agrícolas e fragmentadas, potencialmente elevando o risco de transmissão dessa doença. O clima também pode afetar a dinâmica populacional dos roedores e a sobrevivência do vírus no ambiente, assim como o tempo em que este se mantém virulento, enquanto que fatores sociais podem regular os processos de transmissão dos vírus dos reservatórios para os seres humanos. No entanto, apesar da alta virulência destes vírus e da falta de vacina, não está ainda bem estabelecido como esses diferentes fatores ligados à estrutura da paisagem, ao clima e às condições sociais afetam a dinâmica de transmissão dessa doença. O presente trabalho teve assim como objetivos: 1) identificar quais fatores ecológicos e sociais afetam a transmissão de HCPS, identificando as áreas de maior risco no estado de São Paulo e 2) prever como cenários de mudanças climáticas (RCP4.5 e RCP8.5) e de expansão de cana-de-açúcar influenciam a transmissão de HCPS. Para responder aos nossos objetivos, o sistema de estudo compreendeu os 645 municípios que compõe o estado de São Paulo. Num primeiro capítulo, realizamos uma revisão bibliográfica para entender como as variáveis de paisagem e de clima afetam o risco de HCPS. Num segundo capítulo, utilizamos um modelo Bayesiano para quantificar a associação entre a incidência anual de HCPS no estado de São Paulo, obtida através do número de casos confirmados pelo Ministério da Saúde, entre os anos de 1993 a 2012, e as variáveis de clima (precipitação total anual e temperatura anual média), estrutura da paisagem (porcentagem de vegetação nativa, número de fragmentos e porcentagem de área ocupada com cana-de-açúcar), escolhidas na revisão bibliográfica, além de fatores sociais (número de homens rurais acima de 14 anos - população de risco, e o Índice de Desenvolvimento Humano - IDH). Construimos modelos separados para a Mata Atlântica e o Cerrado. Em ambos os biomas, o risco de HCPS aumentou principalmente com a proporção de terra cultivada com cana-de-açúcar e com o IDH, mas a proporção de habitat nativo, temperatura anual média e população de risco também mostraram relações positivas para Mata Atlântica. O risco médio de HCPS para o estado de São Paulo foi de 1.3%, com 6% dos municípios sendo classificados como de médio a alto risco (≥ 5%). Num terceiro capítulo, utilizamos cenários de expansão de cana-de-açúcar e anomalias de temperatura extraidas dos cenários RCP4.5 e RCP8.5 de 32 modelos de circulação geral (GCMs) do IPCC5 para prever os riscos futuros de HCPS. Com a expansão de cana-de-açúcar, o risco médio de HCPS para o estado aumenta de 1.3 para 1.5%, enquanto que os cenários RCP4.5 e RCP8.5 aumentam o risco para 1.6% e 1.7%, respectivamente. RCP4.5 e RCP8.5 sozinhos são os cenários que mais aumentam o risco máximo de infecção (46.1% para 51.4% e 51.7%), enquanto que a expansão de cana-de-açúcar combinada com os cenários climáticos são os que mais provocam o aumento da expansão do risco no estado de São Paulo, expandindo o número de municípios em alto risco para 7%. Nossas análises fornecem as primeiras evidências sobre a ação de fatores da paisagem, climáticos e sociais na incidência de HCPS nos Neotrópicos. Também, nossos mapas de risco podem ser utilizados para otimizar a correta alocação de recursos, permitindo que ações sejam tomadas para reduzir os impactos da expansão da cana e das mudanças climáticas sobre a propagação da doença.

Abstract

Hantavirus Cardiopulmonary Syndrome (HCPS) is a disease caused by Hantavirus, which are negative-sense RNA viruses in the family *Bunyaviridae*. These viruses are highly virulent to humans, taking about 50% of infected people to death. The main Hantavirus reservoir is constituded by generalist rodents species, which increase in abundance in agricultural and fragmented landscapes, potencially augmenting the transmission risk of the disease. Climate can also affect rodent population dynamics and the virus survival in the environment, as well as the time it remains virulent, while social factors may regulate the processes of transmitting viruses from reservoirs to humans. However, despite the high virulence of these viruses and the lack of vaccine is not yet well established how these different factors linked to landscape structure, climate and social conditions affect the dynamics of transmission of the disease. Thus, this study aimed to: 1) identify which social and ecological factors affect the transmission of HCPS, identifying the areas of greatest risk in the state of São Paulo and 2) predict how climate change (RCP4.5 and RCP8.5) and expansion of sugarcane scenarios influence the transmission of HCPS. To answer these questions the study system corresponded to the 645 municipalities that compose the state of São Paulo. To achieve our goals, in a first chapter, we conducted a literature review to understand how landscape structure and climate variables affect the risk of HCPS. In a second chapter we used a Bayesian model to quantify the association between HCPS annual incidence in the state of São Paulo, obtained by the number of cases confirmed by the Ministry of Health, between the years 1993-2012, and climate variables (total annual precipitation and mean annual temperature), landscape structure (percentage of native vegetation, number of fragments and percentage of area occupied with sugarcane), chosen in the literature review, and social factors (number of rural men over 14 years - risk population, and the Human Development Index - HDI). We build separate models for the Atlantic Forest and the Cerrado. In both biomes, the risk of HCPS increased mainly with the proportion of land cultivated with sugarcane and the HDI, but the proportion of native habitat, mean annual temperatures and risk population also showed positive relationships to Atlantic Forest. The average risk of HCPS for the state of São Paulo was 1.3%, with 6% of the municipalities being classified as medium to high risk ($\geq$ 5%). In a third chapter we used sugarcane expansion and extracted temperature anomalies of RCP4.5 and RCP8.5 scenarios of general circulation models (GCMs) of IPCC5 to predict HCPS risk. With sugarcane expansion, average risk for HCPS increases from 1.3 to 1.5%, while RCP4.5 and RCP8.5 scenarios increased the risk to 1.6% and 1.7%, respectively. RCP4.5 and RCP8.5 scenarios alone are responsible for the largest increase in the maximum risk of infection (46.1% to 51.4% and 51.7%), while the sugarcane expansion combined with climate scenarios are causing the larger expansion in the the number of municipalities at high risk, which goes to 7%. Our analyzes provide the first evidence on the action of landscape, climate and social factors in HCPS incidence in the Neotropics. Moreover, our risk maps can be used to optimize the correct allocation of resources, allowing actions to be taken to reduce the impacts of sugarcane expansion and climate change over this disease propagation.

Chapter 01

General Introduction

General Introduction

Human well-being depends on healthy ecosystems and the provision of services provided by these ecosystems. Ecosystem services are those goods and services that humans obtain from ecosystems, which can be classified as: (1) supporting services such as soil formation, primary productivity, nutrient cycling; (2) regulation, such as climate, flooding and disease control, water purification, etc. (3) provision, as food supply, drinking water, wood and fibers, fuels, etc. (4) and cultural services, which have educational, aesthetic and recreational purposes, for example (Millennium Ecosystem Assessment 2005). However this classification is on constant revision.

Strong evidence suggests that human and ecological factors have played an important role in the increased incidence of some diseases around the world (Patz et al. 2004; Kilpatrick and Randolph 2012), including zoonotic diseases (i.e. diseases transmitted from vertebrate animal hosts to humans; Woolhouse and Gowtage-Sequeria 2005). Since 1976, the World Health Organization has recorded more than 40 emerging and reemerging infectious diseases, many of which are the direct result of land use and land cover changes (WHO 1996), which have profound effects on the distribution and ecology of organisms, including parasites (Dearing and Dizney 2010).

Landscape composition (i.e., the landscape units and their relative abundance) and configuration (i.e., the spatial arrangement of landscape units) affect the incidence of diseases by altering the interactions, abundance and movement patterns of the hosts, vectors and people, although the landscape effects on disease dynamics are understood only for a handful of well-studied diseases (McCallum 2008; Lambin et al. 2010), such as Lyme disease in the United States (Allan et al. 2003; Brownstein et al. 2005), and Hantavirus Cardiopulmonary Syndrome (HCPS) in Panama (Suzan et al. 2008a), both affected by forest fragmentation.

The Hantavirus Cardiopulmonary Syndrome (HCPS) is a zoonotic disease, characterized by severe pulmonary involvement that leads to respiratory failure and cardiogenic shock (Pinto-Junior et al. 2014). It is caused by Hantavirus, which consist of more than 80 genetically related viruses belonging to the Bunyaviridae family (Blasdell et al. 2011). Hantavirus are the only viruses of the Bunyaviridae family that are not arthropod-borne (Clement et al. 2007). They have a lipid envelope, and contain a single-stranded, tri-segmented RNA genome with negative sense (Clement et al. 2007; Blasdell et al. 2011). Due to their lipid covering, they are sensitive to heat, acid pH, detergents, formalin, lipid solvents, and chlorite solutions (Clement et al. 2007).

Rodents in the family Muridae/Cricetidae are the primary hosts of Hantavirus (Mills et al. 1998, 1999; Jonsson et al. 2010; Oliveira et al. 2014), which causes two syndromes in humans: HCPS in the Americas, and Hemorrhagic Fever with Renal Syndrome (HFRS) in Eurasia and Africa (Terajima et al. 2004; Jonsson et al. 2010). HFRS was first identified in the 1970s (Lee et al. 1978) in Asia and later in Europe, while HCPS was first recognized as a clinical entity in May 1993 in the Four Corners region in the USA (United States, CDC 2014), and a few months later, in the city of Juquitiba, in São Paulo, Brazil (Brazilian Ministry of Health 2013).

In Asia and Europe, the Hantaviruses that cause HFRS are responsible for approximately 150.000 to 200.000 cases per year with mortality rates ranging from <1% to 12% (Bi et al. 2008). Instead, HCPS is responsible for approximately 200 cases per year in South America, and is associated with high mortality rates (35% in US; Macneil et al. 2011; 40.75% in Brazil; Brazilian Ministry of Health of Brazil 2013; 38% in Canada; Drebot et al. 2000). To date, the International committee on the taxonomy of viruses (ICTV) recognized 24 Hantavirus species, which have been officially accepted (Fauquet et al. 2005). In Brazil, five strains of Hantavirus have been associated with documented cases of HCPS: Juquitiba virus (JUQV), Araraquara virus (ARAV), Laguna-black virus, Castle of Dreams virus and Anajatuba virus (Silva et al. 1997; Suzuki et al.

2004; Raboni et al. 2005), whose names correspond to the locations where they were first isolated (Ferreira 2003).

Transmission to humans occurs via inhalation of aerosolized virus particles derived from the urine, saliva, and feces of infected rodents (Lee et al. 1981; Doyle et al. 1998). Virus transmission to rodents can occur either by the inhalation of the virus in its aerosolized form, principally during allogrooming (Bernshtein et al. 1999), as through the transfer of infected bodily fluids resulting from aggressive interactions (Root et al. 2005; Calisher et al. 2007; Bagamian et al. 2012). Therefore, the majority of infected rodents are usually males (Childs et al. 1994; Mills et al. 1997; Calisher et al. 1999, 2007, Vadell et al. 2001) in reproductive conditions (Douglass et al. 2007). The virus is maintained in the reservoir populations via horizontal transmission, having a positive correlation with the density of the reservoirs (Mills et al. 1999; Calisher et al. 2007). Once a host is infected it will remain infected and infectious (able to shed infectious virus in the environment) during the rest of his life (Kuenzi et al. 2005), once there is no effect of the virus in the infected rodent (Botten et al. 2000; Calisher et al. 1999; Vadell et al. 2011).

Despite being identified only in the 1970s, the first case of Hantavirus infection dates back to the early 1930s, and occurred when Japanese troops invaded Manchuria (northern China) and 12.600 soldiers were affected by a febrile illness previously unknown, which was called "Songo fever", and later "Epidemic hemorrhagic fever" (HFS). Again in the Korean War (1951-1953), an acute febrile illness led to multiple organ dysfunction (mainly acute renal failure and bleeding) and had a mortality rate of 10 to 15%, affecting more than 3.000 United Nations troops. Despite a massive investigative effort by the American army special committee on hemorrhagic fever, only in 1976 a specific antigen of the virus was discovered in the lungs of a Korean striped field mouse (*Apodemus agrarius*). This led to the isolation and characterization of the disease

responsible agent in 1977 (Lee et al. 1978; Clement et al. 2007), which was called Hantaan-Hantavirus; Hantaan was where most of the cases were reported and infected rodents were collected (Clement et al. 2007).

A priori, each Hantavirus serotype has its main reservoir rodent and its own geographical distribution (Clement et al. 2007), and there is a co-evolutionary relationship between them (Plyusnin and Morzunov 2001). However, recently, other species of rodents and other animals groups have been identified harboring different strains of Hantaviruses (e.g., bats and skunks; Kim et al. 1994; Araujo et al. 2012). Moreover, the presence of the virus in these species, which are not reservoir species, can be considered only the result of accidental and not infectious infection, and does not constitute a part in the virus natural life cycle (Parmenter et al. 1998). Humans are also only accidental hosts for Hantavirus infection, since the increased immune response in humans after the Hantaviral infection often leads to death of the infecting virus (Clement et al. 2007; Dearing and Dizney 2010), and these do not make part in the natural virus ecology.

The risk of acquiring Hantavirus infection is directly related to the density of rodents, as well as the probability of contact of these infected animals with human populations (Childs et al. 1995; Reusken and Heyman 2013). In Brazil at least eight species of rodents were identified as Hantavirus reservoirs, which are considered habitat generalist, being common in altered and agricultural habitats, where they become more abundant (Yahnke et al. 2001; Yates et al. 2002; Umetsu and Pardini 2007; Suzan et al. 2008a; Martin et al. 2012).

Therefore it is often during landscape changes, as *habitat fragmentation* and conversion of natural to anthropogenic habitats that biodiversity drops, and emerging diseases, as HCPS, first appear (Suzan et al. 2009; Wolff et al. 1997). Landscapes are spatially heterogeneous areas, constituted of mosaics of habitat patches (Turner 1989). Landscape structure is usually defined in

terms of composition and configuration (Dunning et al. 1992), which are, respectively, the type and amount of each patch present in the landscape and the spatial relationship between them (Langlois et al. 2001). Habitat fragmentation is different from habitat loss because it reflects configuration aspects (e.g., number of habitat patches, edge density, fragment shape), and not just the total amount of habitat in a landscape (Swift and Hannon 2010). Thus, habitat fragmentation is a landscape configuration change (Langlois et al. 2001; McGarigal and Cushman 2002), usually defined as a process during which a great extension of habitat is transformed into a series of patches of smaller area, isolated from each other by a different habitat of the original, the matrix (Ewers and Didham 2006).

This process results in the creation of low-quality habitats (i.e., the matrix) for a large number of native habitat-dependent species (MMA 2003), with variable implications, because species with different strategies and life stories are differently affected by habitat fragmentation (Gehring and Swihart 2003; Ewers and Didham 2006). Generally, it is expected that smaller and more isolated pieces of habitat in a landscape have relatively small species richness, as well as lower abundances, and an increased risk of population extinction (Bender et al. 2003).

Currently, most of the studies support the hypothesis that habitat loss and fragmentation increases the prevalence of Hantavirus reservoir species within an ecosystem (Susan et al. 2008a, 2008b; Blasdell et al. 2011; Lehmer et al. 2012). This happens because these anthropogenic changes lead to the simplification of mammal communities structure (Pardini 2004; Pardini et al. 2005; Santos-Filho et al. 2012), which tend to be dominated by generalist species (Bentley et al. 2000; Utrera et al. 2000; Yahnke et al. 2001; Laurence et al. 2002; Daily et al. 2003; Pardini et al. 2005), which in turn are recognized as important Hantaviruses hosts (Yahnke et al. 2001; Ruedas et al. 2004; Goodin et al. 2006). These habitat generalist species are favored in disturbed environments (Suzan et al. 2008a, 2008b, 2009), since they can tolerate and adapt to ecological

changes (Hughes et al. 1997), becoming more abundant in these landscapes (Utrera et al. 2000; Yahnke et al. 2001; Daily et al. 2003; Pardini 2004; Susan et al. 2008a).

Climatic factors can also affect Hantavirus risk, because they directly affect the living conditions of animals in terms of temperature and food availability (Klempa 2009), altering rodent population dynamics and abundance. Several studies in U.S. have reported a positive association between rainfall, rodent hosts population sizes and Hantavirus prevalence, through a phenomenon that was called bottom-up trophic control (Abbott et al. 1999; Yates et al. 2002; Luis et al. 2010). Increases in precipitation led to greater vegetation growth, which in turn caused a population explosion of rodents, and an associated increase in infection prevalence, increasing the likelihood of encounters between humans and infected rodents, and consequently the transmission of Hantavirus (Engelthaler et al. 1999; Yates et al. 2002; Luis et al. 2010). However, in Europe, results regarding precipitation were contradictory, with the absence or even the presence of negative effects (Roda-Gracias et al. 2015).

Temperature can also affect reproduction and recruitment rates of rodents, and the time the virus remains infectious in the environment (Dearing and Dizney 2010), affecting Hantavirus transmission risk. In Europe, strong evidence was found for a positive association between temperature and Hantavirus incidence (Roda-Gracias et al. 2015). Moreover, both temperature and precipitation fluctuated between beneficial or detrimental effects on rodent survival and recruitment depending on season (Luis et al. 2010), with these effects varying among habitats and climatic regimes (Loehman et al. 2012).

Important determinants of species biological responses to climate change are the degree of warming itself (IPCC 2007) and the physiological sensitivity of the organism to changes in the temperature of their environment (Calosi et al. 2007). The thermal tolerance of many organisms has been shown to be proportional to the magnitude of temperature variation they experience

(Ghalambor et al. 2006), a characteristic of climate that also increases with latitude. In this sense, tropical species are expected to be highly impacted by increasing temperatures. For Hantavirus infection risk, beyond the effects on rodent reservoir populations, there is also the impact of climate change on virus survival in the environment, and on the transmission path, which is directly linked with climatic conditions.

Climate is typically heterogeneous across landscapes (Hayhoe et al. 2007). Overall, the complex relationships between climate and Hantavirus infections need further exploration to identify specific health risks and initiate appropriate intervention measures, especially in the context of climate change and global warming, which may have serious consequences for human health (Roda-Gracias et al. 2015).

In addition to these factors, *social aspects* may also affect the dynamics of Hantavirus transmission. Most of the HCPS reported cases occur in mans, which perform agricultural activities, living in rural villages, have between 20 and 50 years, and live in poor housing conditions (Purcell 2006; Pereira 2007; Liu et al. 2012; Zhang et al. 2010), being associated with low education levels (Munoz-Zanzi et al. 2015). In recent years, cases are also being reported in the periphery of cities of low and middle income countries, where the unordered growth of human settlements with low sanitation coverage and deforestation practices promoted rodent proliferation and wild rodents invasion of human dwellings (Figueiredo et al. 2001). Additionally, any activity that causes dispersion of aerosols or dust such as cleaning, demolition or removal of grains stored in silos is reported as risk factors for acquiring the disease (Pinto-Junior et al. 2014).

Thus, rural areas are the primary place of transmission. Agricultural crops provide food for rodents, and where traditional agricultural methods are in use, there are numerous opportunities for humans to be exposed to the virus, especially during the harvest season, when

workers sleep next to the fields (Xiao et al. 2013a). Therefore, exposure to a Hantavirus-contaminated environment during work on farms, specific outdoor occupations, and outdoor recreational activities are commonly documented risk factors for infection (Martinez et al. 2010). For HFRS, 93% of the cases include people living in low socio-economic circumstances (Xiao et al. 2013b). For HCPS these association is controversial, with countries showing no relationship with socio-economic level (Elkhoury et al. 2012), while others presents positive associations (Munoz-Zanzi et al. 2015).

In the state of São Paulo two rodent reservoirs species that transmit Hantavirus to humans were recognized: *Necromys lasiurus*, which inhabits the Cerrado and is associated with Araraquara virus; and *Oligoryzomys nigripes*, found in the Atlantic Forest, harboring the Juquitiba virus (Katz et al. 2001; Suzuki et al. 2004; Oliveira et al. 2011; Oliveira et al. 2014). The Cerrado and Atlantic Forest are considered biodiversity hotspots because they have a high concentration of endemic species and high rates of habitat loss (Myers et al. 2000).

The Cerrado is the second largest biome of Brazil's, after Amazonia, occupying 21% of the country's, or 1.5 to 2.0 million km^2 (Borlaug 2002), and extending from the Amazonian forest through the Grand Plateau of Central Brazil, reaching areas in the southern states of São Paulo and Paraná, occupying more than 20° of latitude and an altitudinal range from sea-level to 1800m (Ratter et al. 1997). Currently, 55% of its area has already been cleared or transformed for human uses (Machado et al. 2004), as pasture, cash-crop agriculture, and other uses. The climate is seasonal, with a very strong dry season during the southern winter (April to September), average annual temperatures ranging from 18° to 27° C, and precipitation from 800 to 1500 mm (Dias 1992).

The Atlantic Forest was one of the largest rainforests of the Americas, originally covering around 150 million ha. Extremely heterogeneous in its composition, extends from 3°S to 31°S,

and from 35°W to 60°W, mainly along the Brazilian coast, but also reaching into Paraguay (Cartes and Yanosky 2003) and Argentina (Giraudo 2003), and covering a broad list of climate and vegetation formations, from tropical to subtropical areas (Mantovani 2003).

However, after five decades of human expansion, and harboring 70% of the Brazilian population, the Atlantic Forest is one of the most threatened tropical ecosystems globally because of indiscriminate destruction of its original vegetation (Tabarelli et al. 2010; Joly et al. 2014). Most Atlantic Forest landscapes are located outside protected areas and are formed by archipelagos of small and isolated forest patches, surrounded by open habitat matrixes, such as pastures and sugarcane (Ribeiro et al. 2009), threatening not only its biodiversity but also the services provided by it (Ferraz et al. 2014). Little is known about the impacts of habitat loss and fragmentation, and matrix composition change on the ecosystem services provide by the Atlantic Forest, especially on the regulation of zoonotic diseases.

Originally, the Cerrado and Atlantic Forest occupied 80% and 18.2% of the São Paulo state, respectively (Cavassan 2002). Nowadays, the state has only 14% of its surface still covered with remnants of both biomes (12.4% with Atlantic Forest and 1.6% with Cerrado), with most in small and isolated fragments (Instituto Florestal 2005). The remaining 86% of the state are covered by agriculture, especially a mixture of pasture (Durigan et al. 2007), sugarcane (Nassar et al. 2008), and urban landscapes. Deforestation in the state of São Paulo occurred mainly between 1700 and 1970 with its natural vegetation being replaced by crops, especially sugarcane, which were later replaced by coffee and pasture (Durigan and Ratter 2006). With the creation of the Pro-Alcohol program in 1970, whose goal was to replace a significant percentage of fossil fuels consumption by ethanol produced from sugarcane (Moreira and Goldemberg 1999), Brazil has become a world leader in ethanol production (Martines-Filho et al. 2006) and sugarcane export

(UNICA 2014), with much of that production (~ 74%) coming from the Southeast, where only the state of São Paulo produces 60% of the total (Ferreira Filho and Horridge 2014).

Epidemiological data indicates that the number of cases is increasing each year, as well as the number of viral variants described (Pinto-Junior et al. 2014). Since 1993 more than 2.000 HCPS cases have been reported in the American continent (Jonsson et al. 2010), probably in an underestimated incidence. This occurs mainly because HCPS has a shared clinical feature with a variety of other disease syndromes, including leptospirosis, acute respiratory distress syndrome, and pneumonia (Oliveira et al. 2009), being unnoticed or misdiagnosed. Additionally, studies involving human serosurveys in regions of Brazil with few or no reported HCPS cases have found evidence of Hantavirus infection in a substantial portion of the population (Oliveira et al. 2009), proving that there are still many undiagnosed HCPS cases.

Zoonoses are responsible for over 60% of human pathogens known of emerging and re-emerging infectious diseases (Taylor et al. 2001). Thus, considerable scientific effort has been devoted to understanding the epidemiological relationship between wild animals, which constitute virus reservoirs and/or hosts, and humans (with great attention to wild rodents) in order to develop more effective strategies for preventing these diseases. Therefore, significant progress has been made in recent decades to identify the epidemiology of some diseases, but there is still a lack of knowledge and a great need to examine the interactions between population dynamics of rodents and environmental factors related to these zoonoses (Parmenter et al. 1998), especially in the anthropogenic era, where deforestation, fragmentation, climate change and other human activities are the main threats to environmental preservation.

The Hantavirus Cardiopulmonary Syndrome (HCPS) is an emerging health problem in Brazil (Figueiredo et al. 2009), being recognized as a threat to public health in South America (Donalísio and Peterson 2011), and with an epidemiology mostly unknown (Campos et al. 2003).

Currently, due to the absence of vaccine or specific antiviral therapy, the only way to prevent human infections and reduce mortality from Hantavirus infection is to take preventive measures, and for that it is necessary to better understand the effects of landscape, climate, and social alterations on the prevalence of Hantavirus. Increasing the knowledge and understanding the mechanisms that lead to Hantavirus transmission in Brazil is essential to prevent outbreaks and reduce the incidence of this disease.

This thesis is inserted in the "Projeto Interface", a Thematic Project funded by FAPESP, focused in understanding how habitat loss influences different types of ecosystem services. The general objective of this thesis was to identify which social and ecological factors are related to Hantavirus transmission risk in the Cerrado and Atlantic Forest of São Paulo state. The thesis is divided into five chapters, in which this is the first, providing a brief introduction to the thesis. Chapters 2 to 5 are presented in manuscript format, whose main objectives are presented below.

Chapter 2- Do landscape structure and environmental factors modulate Hantavirus Cardiopulmonary Syndrome outbreaks? Authors: Paula R. Prist; Paulo Sérgio D´Andrea, Jean Paul Metzger

This chapter´s goal was to conduct a literature review in order to explore the state of the art of how landscape structure and climatic factors affect the incidence of HCPS, identifying key knowledge gaps. From this review, we propose a conceptual model showing how these aspects affect HCPS risk, and pointing out other factors that should be addressed in future studies, and their expected relation with the disease. In that way, this review was essential to identify the ecological factors that were used as predictor variables in the other chapters. This manuscript was submitted to the *EcoHealth* journal.

Chapter 3 - Landscape, environmental and social predictors of Hantavirus risk in São Paulo, Brazil. Authors: Paula Ribeiro Prist, Maria Uriarte, Leandro Reverberi Tambosi, Amanda Prado, Renata Pardini, Paulo Sérgio D´Andrea, Jean Paul Metzger

In this chapter we conducted a Bayesian modeling of the relationship between landscape structure variables, climate, and social aspects, with Hantavirus reported cases (HCPS) in the state of São Paulo, to understand which factors are related to the incidence of this disease. By obtaining more knowledge about the factors that lead to a higher incidence of HCPS, and creating a risk map, we were able to identify municipalities and people at high risk for acquiring this disease. Therefore, allocation of resources and the adoption of preventive measures can be optimized and new guidelines facing specific factors acting on disease transmission can be developed, all in order to avoid HCPS propagation. This chapter was published in *Plos ONE* journal.

Chapter 4 - Impacts of climate change and sugarcane expansion on Hantavirus infection risk in the state of São Paulo, Brazil. Authors: Paula Ribeiro Prist, Maria Uriarte, Kátia Fernandes, Jean Paul Metzger

In this chapter we performed predictive analysis on how future scenarios of sugarcane expansion and temperature changes under two climate scenarios will influence the likelihood of HCPS risk in the state of São Paulo in 2050, testing the independent and combined effect of those two factors. By identifying how these scenarios will affect the disease risk, and which municipalities will have the largest increase, we can better support the creation of preventive strategic planning and a proper allocation of resources, reducing the risk of future HCPS outbreaks.

Chapter 5 - General discussion and conclusions

In this chapter we performed a general discussion involving all results obtained in the previous chapters, and make a conclusion of our major results.

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Chapter 02

DO LANDSCAPE STRUCTURE AND ENVIRONMENTAL FACTORS MODULATE HANTAVIRUS CARDIOPULMONARY SYNDROME OUTBREAKS?

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ABSTRACT

Hantavirus, hosted by rodent species, causes Hantavirus cardiopulmonary syndrome (HCPS) in humans, a highly virulent disease. In order to improve our understanding of how landscape and climate drivers affect HCPS outbreaks, we performed a literature review. We observed that in tropical areas anthropogenic landscape changes as forest loss, fragmentation and agricultural land uses, are related with a boost in Hantavirus reservoir species abundance and Hantaviral prevalence, increasing HCPS risk. Additionally, higher precipitation, especially in arid regions, favors an increase in vegetational biomass, which augment the resources for reservoir rodents, also increasing HCPS risk. Although these relationships were observed, few studies tested it so far, and the ones that did it are concentrated in few places. To guide future research on this issue, we build a conceptual model relating landscape and climate variables with HCPS outbreaks, and identified research opportunities. We point out the need for studies addressing the effects of landscape configuration, temperature and the interaction between climate and landscape variables. Critical landscape thresholds are also highly relevant, once HCPS risk transmission can increase rapidly above a certain degree of landscape degradation. These studies could be relevant to implement preventive measures, creating landscapes that can mitigate disease spread risk.

Keywords: landscape composition and configuration; landscape thresholds; climate; forest cover; fragmentation; disturbance; Hantavirus; HCPS; reservoir species; rodents.

Introduction

Between 1980 and 1990, 40% of the world's tropical forests have been converted to agriculture and other uses (FAO, 1999), increasing the probability of contact between humans and wildlife (Deem et al., 2001), and the transmission of zoonotic diseases (Dearing and Dizney, 2010). Hantavirus Cardiopulmonary Syndrome (HCPS) is one of the most lethal and least studied tropical diseases (Campos et al., 2003), being caused by Hantavirus, which can produce Hantavirus fever in humans with cardiopulmonary and renal involvements (Rasmuson et al., 2011; Clement et al., 2014). HCPS was first recognized in 1993 in United States (CDC, 2014),

and since then has shown low incidence but high mortality rates (US: 35%, Macneil et al., 2011; Brazil: 40.75%, Brazilian Ministry of Health, 2012; Chile: 37%, Ferres et al., 2007). So far there is no treatment options, with no vaccine or drug to prevent or treat HCPS (Custer et al., 2003; Buceta et al., 2004).

Rodents belonging to the family Cricetidae were identified as natural reservoirs of Hantavirus in the new world (Mills et al., 1998; Hjelle and Torres-Pérez, 2010; Oliveira et al., 2014a). Coevolucionary relationships between Hantavirus and their reservoir rodents were observed (Plyusnin and Morzunov, 2000), with reservoir species closely related harboring different species of Hantavirus (Parmenter et al., 1998). Some of these reservoir species are considered habitat generalist, being common in altered habitats, where they become more abundant (Yahnke et al., 2001; Yates et al., 2002; Umetsu and Pardini, 2007; Suzan et al., 2008a; Martin et al., 2012).

The infected rodent shed the virus in the urine, saliva and feces (Botten et al., 2002; Ferres et al., 2007; Godoy et al., 2009), transmitting it to humans through inhalation into their aerosolized form (Schmaljhon and Hjelle, 1997; Peters and Khan, 2002) or bites. Otherwise, there is some evidence of person-to-person transmission in Argentina and Chile (Ferres et al. 2007). Hantavirus infection in rodent hosts is asymptomatic (Hjelle and Glass, 2000) and transmission rodents to rodents is horizontal (Mills et al., 1999). It occurs mostly by intraspecific agonistic encounters among adult males (Mills et al., 1997; Oliveira et al., 2014b; Teixeira et al., 2014), in aggressive interactions associated with reproduction behaviors (Douglass et al., 2001; Bagamian et al., 2012; Teixeira et al., 2014). However, once rodents harboring Hantavirus which cause HFRS can also be infected through the inhalation of the aerosolized virus (Bernshtein et al., 1999), it´s been hypothesized that HCPS rodents can also be infected through these transmission route (Ottenson et al., 1996).

Currently, different causes are associated with the occurrence or increase of HCPS, but this information is scarce and dispersed in the literature, making it difficult to identify patterns. While some studies support the hypothesis that deforestation and land use intensification increase the abundance of reservoir species (Utrera et al., 2000; Yahnke et al., 2001; Pardini 2004; Suzan et al., 2008a,b), and consequently Hantavirus within an ecosystem, others point out that HCPS transmission is affected by climatic factors that affect rodent population dynamics (Calisher et al., 2005a; Andreo et al., 2011).

Due to the need of understanding how climatic factors and landscape structure interact and affect HCPS transmission risk in human populations, we aimed to: (1) summarize the most recent knowledge on how the risk of HCPS epidemics is regulated by climate and landscape structure; (2) provide a conceptual model about the way they influence the spread of HCPS; and (3) identify the knowledge gaps that should be assessed in order to better understand and prevent the spread of HCPS.

Literature review

A literature review was performed considering manuscripts published between 1993 and September 2016 using international scientific databases (Life science database at: http://www.scopus.com/, and Web of Knwoledge at: http://portal.isiknowledge.com/), without restricting the year and using the combination of words: “Hantavirus,” “Landscape Structure”, “America,” “Diseases,” “Zoonoses,” “Rodent,” “Environment,” “Epidemiology,” “HCPS” and “Ecology,” in the title, abstract and keywords. A preliminary scanning of the abstracts was performed and the articles that were unrelated to HCPS or were not conducted in the Americas were discarded. Manuscripts related to the other syndrome (Hemorrhagic Fever with Renal

Syndrome, HFRS) caused by Hantavirus, which is restricted to Eurasia, were not considered. The selected articles were split into four categories concerning the different predictor variables tested: climate (precipitation and temperature), landscape composition (e.g. land use and land cover composition and their relative abundance), landscape configuration (i.e. the spatial arrangement of the landscape units) and landscape structure (i.e. landscape composition and configuration tested together). Studies that related vegetation index (NDVI, EVI) with vegetation productivity were classified as landscape composition studies. Similarly, studies that evaluated human land cover disturbance (i.e. human action that caused the degradation and/or loss of natural vegetation) were also classified as landscape composition studies because those disturbances, e.g. recreational use by all-terrain vehicles (Lehmer et al., 2008; Mackerlprang et al., 2001) or by cattle use (Calisher et al., 2001), created open spaces with no vegetation cover. Some studies tested for more than one predictor variable category at the same time, and were classified in two categories (for example, climate and landscape composition).

We found 327 articles from which only 27 related climatic or landscape factors with HCPS, and thus were considered in this review (Table 1). From these, nine tested only for climatic variables and eighteen for landscape structure variables (composition and/ or configuration) (Figure 1). The majority of them were conducted in the United Stated (74%), followed by Panamá and Paraguay (7.4% each), with few studies from all the other American countries. From these, 22 were conducted in temperate arid regions, and 5 in tropical forest regions. In a revision of the dynamics and drivers of Hantavirus prevalence in rodent populations for the entire world, Khalil et al., (2014) also found a few number of articles (30) that related habitat and HCPS prevalence.

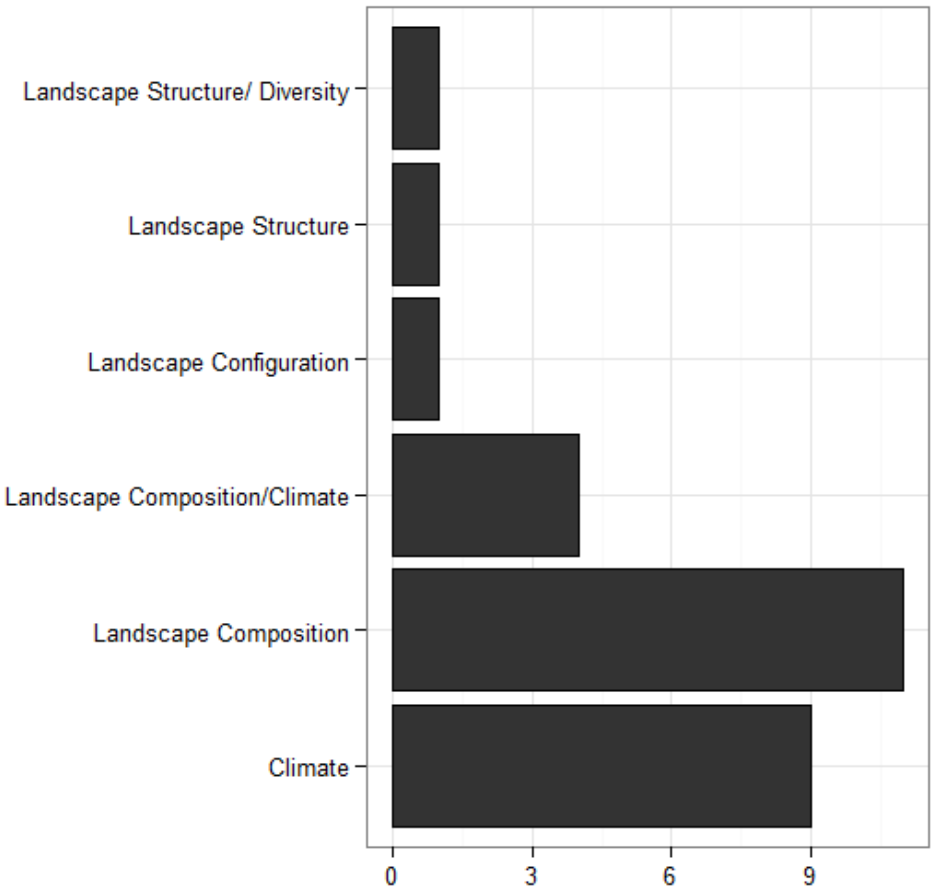

Figure 1. Number of articles relating HCPS with the different environmental variables (see methods section for more details about different categories).

Table 1. Synthesis of the studies that relate environmental predictor variables, including the country and the type of environment where the study was realized, type of predictor variable tested, the category that were classified for this revision (see methods for more information), the response variable, and the effect found on the densities and/or infection prevalence of reservoir rodents and on HCPS outbreaks (response variable).

Local/Environment	Predictor variable	Predictor variable Category	Response variable	Effect found	Author
USA/Temperate-arid	Natural habitat cover and biomass, densities of reservoir rodent	Landscape Composition	Infection prevalence in reservoir rodents	Increases in the rodent reservoir densities lead to a higher infection prevalence in these populations. There was no direct effect of landscape composition	Biggs et al. 2000
USA/Temperate-arid	Normalized difference vegetation index (NDVI)	Landscape Composition	Infection prevalence in reservoir rodents	Sites with a higher prevalence had a greater vegetation productivity then sites with negative prevalence	Boone et al. 1998
USA/Temperate-arid	Normalized difference vegetation index (NDVI)	Landscape Composition	Infection prevalence in reservoir rodents	Sites with a higher chance to have infected rodents had a dense vegetation, while sites with a sparse vegetation had a smaller chance	Boone et al. 2000

USA/Temperate-arid	Land cover type	Landscape Composition	Infection prevalence in reservoir rodents	Infection prevalence was higher in sites with natural vegetation cover than in sites that had the vegetation destroyed by cattle use	Calisher et al. 2001
USA/Temperate-arid	Normalized difference vegetation index (NDVI) and enhanced vegetation index (EVI)	Landscape Composition	Infection prevalence in reservoir rodents	Both vegetation indices had significant correlations with the number of infected reservoir rodents	Cao et al. 2011
USA/Temperate-arid	Land cover type	Landscape Composition	Reservoir rodent densities and infection prevalence	High-risk areas were associated with woody plants, dominated by *Pinus* spp.	Glass et al. 2002
USA/Temperate-arid	Land cover and normalized difference vegetation index (NDVI)	Landscape Composition	Rodent abundance and infection prevalence in reservoir rodents	Sites with higher levels of green vegetation and natural forest cover, were associated with high risk, while areas with cleared ground or shrubland were less often associated with high risk of infected rodents.	Glass et al. 2007

Paraguay/Tropical-forest-shrubland	Land cover type	Landscape Composition	Infection prevalence in reservoir rodents	Infected reservoir rodents where found in intensive and mosaic agricultural landscapes, while none infected rodent where found in natural forest areas	Goodin et al. 2006
Paraguay/Tropical-forest	Land cover type	Landscape Composition	Presence and infection prevalence of reservoir rodents	Reservoir rodent species and infection prevalence were associated with non-forest areas, being higher in areas of herbaceous graminoids	Goodin et al. 2009
USA/Temperate-arid	Land cover type	Landscape Composition	Infection prevalence in reservoir rodents	Infection prevalence on reservoir rodents was higher in sites with the higher levels of natural vegetation cover	Lehmer et al. 2008
USA/Temperate-arid	Land cover type	Landscape Composition	Infection prevalence in reservoir rodents	Infection prevalence on reservoir rodents was higher in sites with no vegetation cover, degraded by human use, that completely denuded the area	Mackelprang et al. 2001
Panama/Tropical-forest	Land cover type, Native habitat fragmentation and native edge habitat areas	Landscape Structure	Densities of reservoir rodents species	Hantavirus reservoirs were more common in pastures, agricultural areas and edge habitats of small fragments than in continuous forest areas	Suzan et al. 2008b
Brazil/Tropical-	Monthly	Landscape	HCPS	Winter precipitation and high photosynthetic mass	Donalisio and

forest	precipitation and enhanced vegetation index (EVI)	Composition and Climatic Variables		(EVI) were closely related to HCPS case distributions	Peterson 2011
USA/Temperate-arid	Monthly precipitation and normalized difference vegetation index (NDVI)	Landscape Composition and Climatic Variables	HCPS	NDVI accounted for a significant part of the variation in the HCPS risk while no effect of precipitation was observed	Glass et al. 2000
USA/Temperate-arid	Monthly temperature, precipitation and vegetation productivity	Landscape Composition and Climatic Variables	Rodent abundance (minimal number alive)	No relationship was found between rodents and weather data or vegetation productivity	Loehman et al. 2012
USA/Temperate-arid	Total seasonal precipitation and land cover type	Landscape Composition and Climatic	Rodent survival	Rodent survival was smaller on sites with no vegetation cover (highly disturbed), when compared to sites with more vegetation cover. The only effect	Previtali et al. 2010

		Variables		found of precipitation was that on sites with no vegetation cover: an increase of precipitation lead to a boost in rodent survival probabilities	
Canada/Temperate-forest	Composition and fragmentation of rodent reservoir preferred habitat (forest, crop fields, clear-cuts and coulees), mean annual temperature and mean total annual precipitation	Landscape Structure and Climatic Variables	Infection prevalence in reservoir rodents	Infection prevalence was (1) lower at sites composed of 40 to 50% preferred habitat (2) lower in unfragmented landscapes (3) lower at warmer sites; there was no effect of precipitation.	Langlois et al. 2001
Panama/Tropical-forest	Native habitat fragmentation, native edge habitat	Landscape Configuration and Diversity Loss	Reservoir rodent abundance and infection prevalence in reservoir	Hantavirus reservoirs species were more commonly found in fragmented and edge habitats than in forest; infection prevalence on reservoir rodents was smaller	Suzan et al. 2008a

	and small mammal diversity loss		rodent abundance	in plots where small mammal diversity were higher	
USA/Temperate-arid	Mean annual and seasonal precipitation	Climatic Variables	Desert rodent densities	Increases in precipitation caused declines in rodent populations	Brown and Ernest 2002
USA/Temperate-arid	Climate (daily temperature and precipitation)	Climatic Variables	Rodent abundance	High rainfall amount during a cold period, and hot, dry conditions during the spring and summer were associated with declines in rodent abundance	Calisher et al. 2005a
USA/Temperate	Temperature and precipitation (means daily)	Climatic variables	HCPS	HCPS had a negative relationship with precipitation and a positive effect with temperature at regional scales	Carver et al. 2015
USA/Temperate-arid	1998 Precipitation anomaly relative to previous 20 years average	Climatic variables	HCPS	HCPS were more likely to occur in areas where precipitation was in the upper 75th percentile of deviations from annual precipitation.	Hjelle and Glass 2000
USA/Temperate-arid	Climate (monthly precipitation sums and monthly average	Climatic Variables	HCPS	HCPS were related to above average precipitation during winter and spring of 1992 and 1993 and the month of HCPS exposure had higher and smaller	Engelthaler et al. 1999

	of daily temperature)			temperature ranges	
USA/Temperate-arid	Climate (mean temperature and total precipitation both monthly and five months previously experiment)	Climatic Variables	Reservoir rodent survival and recruitment	Rodent survival was determined by precipitation and temperature in the current month and 5 months previously; rodent recruitment was determined by sum of precipitation over the last 4 months and the sum of temperature over the last 4 months	Luis et al. 2010
Chile/Temperate-arid	Total precipitation and mean, maximum and minimum temperature monthly	Climatic Variables	HCPS	Higher total precipitation leads to increases in HCPS cases. Temperature showed no effect.	Nsoesie et al. 2014
USA/Temperate-arid	Climate (mean annual precipitation and temperature)	Climatic Variables	Reservoir rodent abundance	Increases in precipitation leads to increases in reservoir rodent abundance	Parmenter et al. 1993

USA/Temperate-arid	Total seasonal precipitation	Climatic Variables	HCPS	Increases in precipitation leads to HCPS outbreaks	Yates et al. 2002

Landscape composition and configuration can modulate the risk of diseases transmission between humans and wildlife (Langlois et al., 2001). Landscape alterations, such as deforestation, fragmentation and land conversion from natural to agricultural uses, lead to changes in small mammal diversity through the loss of habitat specialist species, that are not able to persist in fragmented and disturbed habitat, and the increase in population densities of generalist species (including Hantavirus reservoir species), that benefit from edge forests, agricultural and disturbed habitats (Utrera et al., 2000; Yahnke et al., 2001; Daily et al., 2003; Pardini, 2004; Suzan et al., 2008a).

Biodiversity may be fundamentally important in the risk of exposure to certain zoonotic diseases (Ostfeld and Keesing, 2000), with biodiversity loss affecting the transfer of pathogens among species and influencing disease transmission. The dillution effect (e.g., species diversity reduces disease risk; Keesing et al., 2006) was experimentally proven for Hantavirus prevalence (Suzan et al., 2009), with increases in both abundance and Hanta-viral prevalence among competent hosts after the removal of non-reservoir Hantavirus species. This supports the hypothesis that a large number of species can decrease the risk of Hantavirus infection among reservoir rodents, and that higher the density of reservoir rodents, higher will be the intra-specific encounter rates and the potential delivery rate of pathogens from rodents to rodents and into air, and consequently the higher the risk to humans (Ostfeld and Holt, 2004).

Landscape configuration can also affects Hantavirus prevalence, depending on the response of reservoir rodents to landscape fragmentation (Langlois et al., 2001) and matrix habitat. If individuals of those species are less likely to move across more fragmented landscapes, such landscapes may form a partial barrier to Hantavirus transmission, and rodent reservoir populations would show a lower Hantaviral prevalence. Alternatively, if reservoir rodents move farther in fragmented habitat, they may have a wider range of contacts, increasing the

transmission probability (Langlois et al., 2001; Bender and Fahrig, 2005). In this second case, forest fragmentation increases the connectivity of the landscape for some species, frequently generalist ones, besides boosting their densities. Preliminary studies performed with some Hantavirus reservoir rodent species showed that these animals have greater interpatch movement through different matrix types (Bender and Fahrig, 2005), may use matrix as habitat (Pires et al., 2002; Puttker et al., 2006; Rizkalla and Swihart, 2007), and may move two times further in more fragmented habitats (~ 293 mean squared distance/year), when compared to less fragmented (~140 mean squared distance/year; Diffendorfer et al., 1995; Root et al., 1999). These show that these species can be connected even in fragmented landscapes, which increases the likelihood of intraspecific contact and competition between reservoir species population, and consequently virus transmission between both rodents and humans (Diffendorfer et al., 1995; Langlois et al., 2001).

The majority of the studies performed in arid regions (US studies) showed that there is a positive effect of natural vegetation cover on infected reservoir rodents (but see Loehman et al., 2012). Therefore, areas with higher risk of HCPS were the ones with more natural vegetation cover and higher NDVI and EVI values (Boone et al., 1998, 2000; Glass et al., 2000; 2002; 2007; Calisher et al., 2001; Cao et al., 2001; Lehmer et al., 2008; Previtali et al., 2010). On the other hand, the lowest incidence of Hanta-viral prevalence in rodent reservoir species was found in areas with no vegetation cover, that were degraded by different human uses, as recreational use and all-terrain vehicles, causing the full loss of natural vegetation (Mackelprang et al., 2001).

For tropical regions, all studies found that fragmentation of natural habitats, and agricultural land uses presented the higher densities and Hanta-viral prevalence of reservoir rodents (Goodin et al., 2006, 2009; Suzan et al., 2008a,b), showing that landscapes composed by a mosaic of small fragments and crop fields presented the higher risk for Hantavirus transmission

when compared to intact natural forest, that were associated with non-infected reservoir rodents (Goodin et al., 2006), and no HCPS risk.

Precipitation is the most studied climatic predictor of HCPS outbreaks, with several authors pointing out a positive correlation with rodent densities and prevalence of Hantavirus (e.g., Engelthaler et al., 1999; Glass et al., 2000; Hjelle and Glass, 2000; Yates et al., 2002; Luis et al., 2010; Donalisio and Peterson, 2011). The hypothesis to explain those relationships was a bottom-up trophic control, used to explain 1993 and 1998 HCPS outbreaks in United States (Yates et al., 2002). In these events, augments in precipitation associated with an El Nino Southern Oscillation caused an increase in primary productivity and a higher availability of rodent resources, resulting in a boost in reservoir rodent densities. Increased population densities resulted in increased intraspecific contact and higher rates of virus transmission, both for rodents and humans, augmenting HCPS risk. In subsequent studies, HCPS risk was also associated with high levels of precipitation in the dry season (Donalisio and Peterson, 2011), and with high levels and longer duration of green vegetation (Glass et al., 2000, 2007; Cao et al., 2011), confirming this positive association between precipitation and HCPS. However, this positive relation may apply only within arid ecosystems, where water is a limiting factor in plant growth (Loehman et al., 2012), and majority of studies were performed, lacking information for tropical regions (with exception of Donalisio and Peterson, 2011).

Alternatively, studies performed in U.S. considering regional scales found a strong and negative relationship between HCPS cases and precipitation (Carver et al., 2015). It has been suggested that precipitation may impose also indirect impacts on small rodents because once moderate precipitation may benefit plant growth and then rodents abundance, whereas heavy precipitation may kill rodents by flooding their burrows and nests (Brown and Ernest, 2002), causing catastrophic decline in rodent populations. Therefore besides the mean amount of

rainfall, the magnitude and timing of individual rainfall are also important (Brown and Ernest, 2002).

Some studies have shown that the prevalence of Hantavirus is also affected by temperature (Luis et al., 2010; Carver et al., 2015), but compared with precipitation, effects of temperature are relatively less investigated, especially in Hantavirus reservoir species. Four studies have tested it together with precipitation, showing no effect (Nsoesie et al., 2014), or negative (Calisher et al., 2005a) and positive relationships (Luis et al., 2010; Carver et al. 2015) with rodent densities.

Integrated conceptual model

According to this review, it is possible to identify the main climate and landscape drivers that may lead to HCPS outbreaks and point out some knowledge gaps that should be addressed in future studies. We propose a conceptual model in order to organize this knowledge (Figure 2), considering the main studied drivers (items 1 and 2 in Figure 2), suggesting new drivers (items 3 and 4), and identifying knowledge gaps (items 5 and 6).

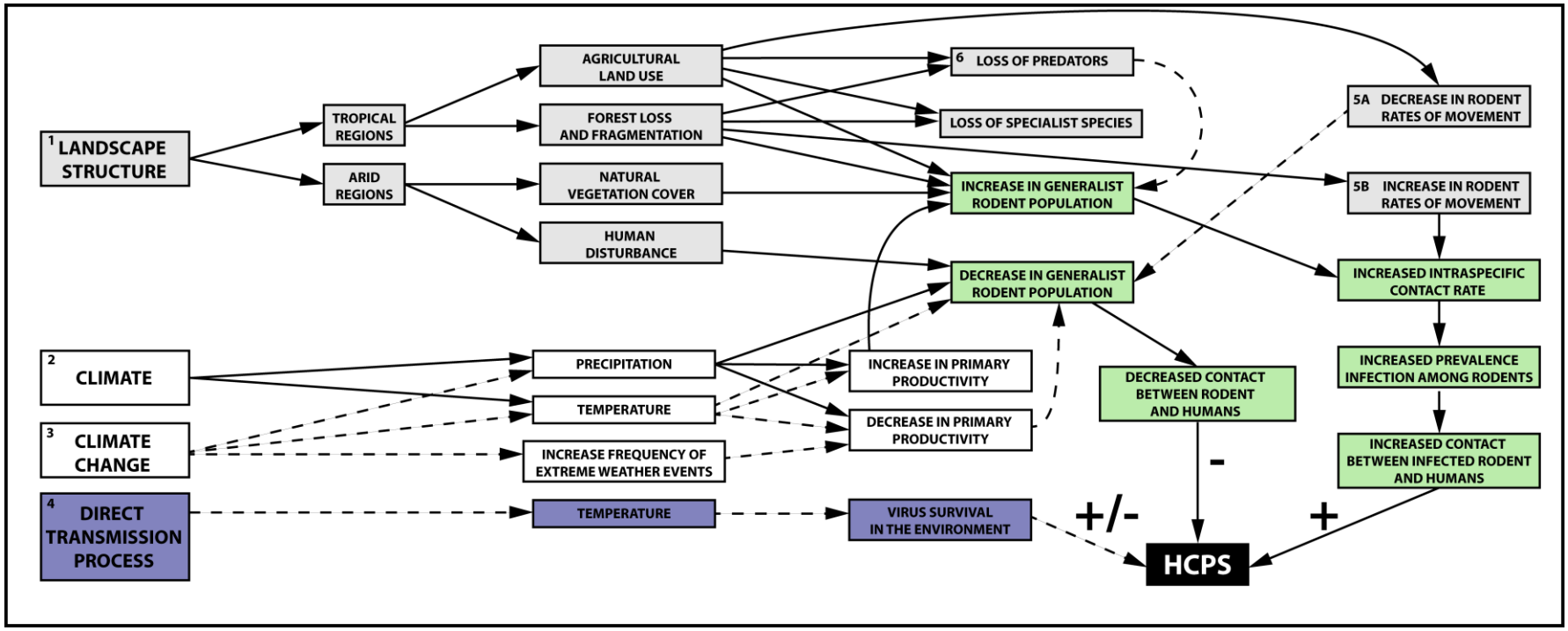

Figure 2. Conceptual model showing the main relationships and parameters that regulate HCPS outbreaks. All mechanisms that are triggered by landscape structure variables are represented in light grey; the ones triggered by climatic factors, and that act through reservoir rodent population, are represented in white; and the ones that act directly in the virus survival, are represented in purple; the mechanisms that are affected by more than one driver are represented in green. Continous arrows represent relationships with a more strong support from the literature review. Dashed arrows indicate hypothetical relationships that deserve to be better investigated.

In temperate arid regions, the presence of *natural vegetation cover* is connected with increases in reservoir rodents' population, which are common in vegetations ranging from grasslands to woodlands (Mills et al., 1997). This positive association is probably a result of the requirements for food and shelter of the reservoir rodents, which do not find this needs in degraded open areas. Higher densities are also connected with increased intraspecific contact rates and Hanta-viral prevalence, enhancing the probability of encounters between humans and infected rodents. On the other hand, human disturbance, that leads to the loss of natural vegetation, is related with decreases in rodents populations, diminishing HCPS risk, once these animals cannot survive in environments with no vegetation cover.

In tropical regions, *forest fragmentation* and *conversion of forest to agricultural land uses* leads to changes in mammal community that normally lose their habitat specialist species, which are not able to persist in altered landscapes. At the same time, habitat generalist, including some Hantavirus reservoir rodent species, are benefited by these altered environments and thus become more abundant. Also, in fragmented landscapes, there is an increase in movement rates for these species. As a consequence of higher densities, increased movement rates, and matrix use, there is an increase in intraspecific contact rates and thus in Hanta-viral prevalence. Therefore, the chance of contact between humans and an infected rodent are enhanced in these landscapes, increasing the risk of HCPS.

Precipitation has a strong literature support when compared to other predictors and shows, in majority of studies, a positive association with HCPS, especially for arid regions. Increases in precipitation affects positively vegetation growth, what means more food for rodents. With higher primary productivity, rodents abundance increases (including some Hantavirus reservoir species), and consequently, intraspecific contact rates and Hantavirus prevalence are enhanced, increasing the probability of encounters between humans and infected rodents. The

transmission of Hantavirus is thus amplifyied and results, finally, in HCPS outbreaks. At the same time that precipitation can affect positively HCPS, it can also have none or negative effects, depending on its magnitude and region. Strong rains can flood rodent refuges and destroy vegetation, reducing rodent population densities, especially for exclusively terrestrial species, and decreasing the chance of contact between humans and rodents and consequently HCPS risk. However this effect would be limited by the habits of the reservoir rodent, and may not have an effect on semi-aquatic and scansorial species. Moreover, in regions where water is not a limiting resource, as in Tropical areas, precipitation may have no effect in rodent abundances (Carver et al., 2015).

Research opportunities

Based on the current set of empirical evidences, we raised some hypotheses about relevant but still underexplored processes and mechanisms that can influence HCPS risk, and should be addressed in future studies (Figure 2, items 3, 4, 5 and 6).

First, despite studies showing a clear *relation between HCPS risk and landscape configuration* (e.g., landscape fragmentation), these studies are scarce and restricted to few reservoir species. From the 26 selected studies, only three tested for these effects, and were restricted to two countries (Panama and Canada) with different forest vegetation (tropical and temperate forest). Therefore, more studies concerning this topic are needed to confirm the results found in these three articles, particularly considering other Hantavirus reservoir species (Figure 2, item 1).

Some studies have shown that reservoir rodent species can move further in fragmented landscapes, or use matrix habitats (Figure 2, item 5 b) (Diffendorfer et al., 1995; Pires et al.,

2002; Puttker et al., 2006), but more studies addressing this issue are necessary, once this issue was ignored in most population studies, especially with reservoir species. The dispersion capacity of reservoir species can determine the magnitude of Hantavirus transmission and prevalence among these populations, influencing HCPS risk, because movements of greater distances relative to areas where movements are short, should increase the chance of encounters with conspecifics (Root et al., 1999). Therefore studies with different reservoir species, in different matrix types, in different regions are necessary to fully understand how and which matrix types are considered as a barrier for these species, and if there is a maximum distance where rodents are not able to move further in the landscape (Figure 2 item 5 a).

Second, landscape composition and configuration affect the structure and diversity of medium sized and large bodied mammal communities, with fragmented and degraded landscapes leading to the loss of large mammals and mesopredators. Therefore, some rodent species, including generalist reservoirs, that are able to persist and benefit from anthropic landscapes, also became free form the effects of predation (Terborgh et al., 2001; Nie and Liu, 2005). It may seem intuitive that predators control populations of rodents and thus are important for controlling the transmission of pathogens, but this has been difficult to prove empirically (Ostfeld and Holt, 2004). Consequently, we suppose that *Hantavirus transmission increases with the loss of predators* (Figure 2, item 6), and that these loss is modulated by the structure of the landscape. Therefore, fragmented and agricultural landscapes, also present higher HCPS risk due to the loss of mesopredators. Studies that take into account these trophic interactions can be useful to understand if predators can control rodent reservoir population and thus HCPS risk. At the same time, studies considering this topic in different landscapes structures can define if and how landscapes can modulate HCPS transmission through this top-down control.

Third, evidences of *non-linearity in small mammal response to landscape changes* have already been showed. For example, Suzan et al., (2008a) indicate a minimum size of 5 ha for forest fragments to maintain a more diverse small mammal community and keep HCPS risk low. Abrupt changes in small mammal species composition were also observed in the Atlantic forest, with a shift from a community dominated by specialist and endemic species to a community composed essentially by generalists when forest cover is reduced below 30% of the landscape (Pardini et al., 2010). In landscapes below this forest cover threshold, communities become dominated by generalist species (Banks-Leite et al., 2014), as some Hantavirus reservoir species, that can move further in fragmented habitats (Diffendorfer et al., 1995; Pires et al., 2002), and use resources that are sparsely dispersed (D´Eon et al., 2002), increasing HCPS transmission risk. We can thus hypothesize that a critical threshold should exist for HCPS transmission risk in tropical areas (Figure 3), which should coincide with those changes in small mammal community composition. Studies testing the existence of critical thresholds would be important to understand at which point landscape structure can modulate HCPS transmission risk. Such thresholds are essential for understanding rodent population dynamics, Hantaviral transmission and HCPS risk for humans, and would be important to future landscape management programs.

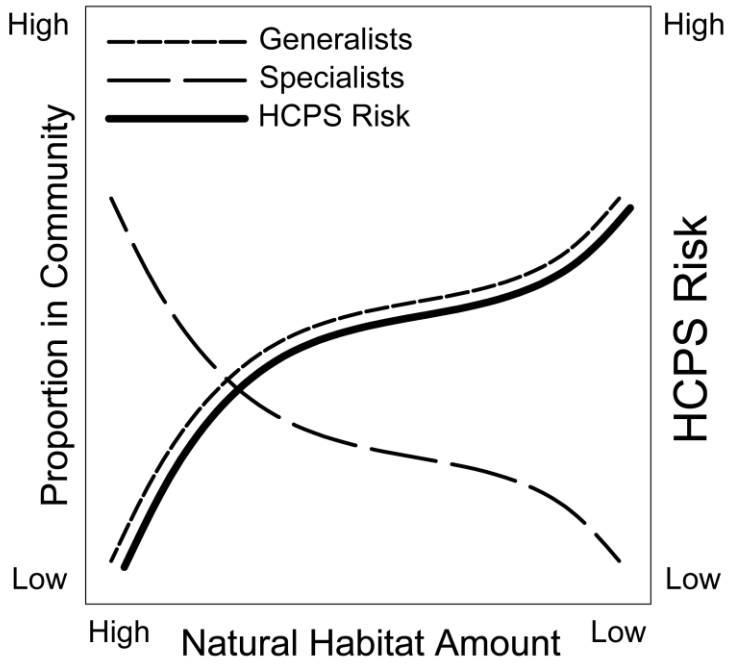

Figure 3. Expected relationship between habitat amount, HCPS transmission risk and proportion in the community of habitat generalist and specialist rodent species, based on landscape structure mechanisms raised through the literature review for tropical regions. As forest habitat is lost, specialist species are lost while generalist species become more abundant (Banks-Leite et al. 2014), as some Hantavirus reservoir species, increasing Hantaviral prevalence and HCPS transmission risk. In a specific threshold, where a disproportionate loss of specialist species takes place, there is an abrupt increase in generalist species and consequently in HCPS tranmsission risk. In landscapes above this threshold the HCPS risk should be low, once small mammal community is more diverse and Hantavirus reservoir populations are "controlled", decreasing infection rates.

Fourth, *temperature may be an important driver of HCPS transmission* (Figure 2, item 2). Similarly to precipitation, temperature can also affect positively vegetation growth (Luis et al.,

2010; Wang et al., 2011). Experimental studies have shown that small mammal populations are frequently food-limited (Prevedello et al., 2013), and therefore increases in vegetation growth may augment population densities (Luis et al., 2010), increasing HCPS risk. Temperature also affects reproduction and survival of small rodents (Zeier et al., 2005; Luis et al., 2010), and can have a positive or negative effect depending on its magnitude (Calisher et al., 2005b; Luis et al., 2010; Carver et al., 2015). For HFRS reservoirs rodents, mild temperatures (10-25° C) are most favorable for breeding (Liu et al., 2012), therefore we can expect the same pattern for HCPS reservoirs, once they are all rodent species. In that way, increases in temperature may have a positive effect on reservoir rodent abundance until reaching a certain threshold, from where it will exert a negative effect, once will become detrimental to those species.

Additionally, temperature can also affect the direct process of HCPS transmission (Figure 2, item 4), once humans (and probably also rodents) became infected through inhalation of the virus in their aerosolized form (Hjelle and Glass, 2000). Reservoir rodents normally have a peak of infection during warmer months (Oliveira et al., 2014b; Teixeira et al., 2014), probably because high temperature leads to greater aerosolization of the virus and higher rates of inhalation by both humans and rodents (Langlois et al., 2001). Yet, climatic conditions can enhance virus survival in the environment (Sobsey and Meschke, 2003; DHSS 2014), with exposure to sunlight and high temperatures (37°C) decreasing and even losing virus viability, while milder temperatures (23°C; 73°F) increases the time the virus remains infectious (Sobsey and Meschke, 2003; Kallio et al., 2006; DHSS, 2014). Therefore, temperature should affect positively virus aerosolization, increasing HCPS risk, again until reaching a certain threshold, from which the effect would be negative. Studies addressing this point are important to understand the role of temperature on Hantavirus transmission risk, especially considering future climate change (Figure 2, item 3).

Finally, studies addressing the *interaction between climate and landscape variables* are almost non-existent and inconclusive. In one study, the effects of landscape structure (composition and configuration) were stronger on virus incidence than climate variables (Langlois et al., 2001), showing that landscape played a major role in HCPS transmission than climatic variables. In another study, alternatively, precipitation and land cover interact to affect rodent survival probabilities (Previtali et al., 2010). In periods of reduced precipitation, rodent survival probabilities were extremely low in areas with no vegetation cover, however, with higher precipitation, rodent survival probabilities were also higher. In this last case, precipitation alone did not have a significant effect, but when combined with land cover had significant effects, changing the influence of land cover (Previtali et al., 2010) and showing that climatic variables can diminish the negative effects of landscape changes. If we are able to understand if one variable can attenuate or accentuate the effect of another one, we would be able to implement management and design programs that can diminish HCPS risk, even in the worst future scenario of deforestation and/or climate change.

Conclusion

It is noteworthy that HCPS is a disease that causes 50% of infected patients to death, and that their outbreaks are linked to deforestation, fragmentation, agricultural land use, loss of biodiversity and increases in rates of rainfall. We found that there is a large gap of studies concerning the relationship between HCPS and landscape configuration, temperature and the interaction between climate and landscape variables. Overall, despite many remaining questions, current evidence indicates that maintaining the landscape above a certain structural threshold,

which allows preserving the integrity of small mammal community, may reduce the prevalence and risk of HCPS.

To identify high risk areas and be able to monitor and effectively prevent HCPS outbreaks, we need to address questions such as: What is the best landscape structure to maintain pathogenic host rodent species population below critical sizes, even considering future climate changes? Is there a forest cover threshold that can maintain these populations under control, reducing Hantavirus transmission low? Forest restoration and landscape management programs designed to diminish landscape HCPS risk should be based on strong scientific evidences of how landscape and climate affect HCPS, however those relationships still need to be better established or consolidated.

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Chapter 03

LANDSCAPE, ENVIRONMENTAL AND SOCIAL PREDICTORS OF HANTAVIRUS RISK IN SÃO PAULO, BRAZIL

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Abstract

Hantavirus Cardiopulmonary Syndrome (HCPS) is a disease caused by Hantavirus, which are negative-sense RNA viruses in the family *Bunyaviridae* that are highly virulent to humans. Numerous factors modify risk of Hantavirus transmission and consequent HCPS risk. Human-driven landscape change can foster transmission risk by increasing numbers of habitat generalist rodent species that serve as the principal reservoir host. Climate can also affect rodent population dynamics and Hantavirus survival, and a number of social factors can influence probability of HCPS transmission to humans. Evaluating contributions of these factors to HCPS risk may enable predictions of future outbreaks, and is critical to development of effective public health strategies. Here we rely on a Bayesian model to quantify associations between annual HCPS incidence across the state of São Paulo, Brazil (1993-2012) and climate variables (annual precipitation, annual mean temperature), landscape structure metrics (proportion of native habitat cover, number of forest fragments, proportion of area planted with sugarcane), and social factors (number of men older than 14 years and Human Development Index). We built separate models for the main two biomes of the state (cerrado and Atlantic forest). In both biomes Hantavirus risk increased with proportion of land cultivated for sugarcane and HDI, but proportion of forest cover, annual mean temperature, and population at risk also showed positive relationships in the Atlantic forest. Our analysis provides the first evidence that social, landscape, and climate factors are associated with HCPS incidence in the Neotropics. Our risk map can be used to support the adoption of preventive measures and optimize the allocation of resources to avoid disease propagation, especially in municipalities that show medium to high HCPS risk (> 5% of risk), and aimed at sugarcane workers, minimizing the risk of future HCPS outbreaks.

Keywords: Landscape structure, Hantavirus, social variables, HDI, climate, landscape ecology, fragmentation, environmental factors, sugarcane, temperature, disease ecology, risk map

Introduction

Multiple lines of evidence suggest that ecological and anthropogenic factors play important roles in elevating incidence of diseases around the world (Kilpatrick and Randolph 2012). Landscape composition (e.g., relative abundance of landscape units) and configuration (e.g., spatial arrangement of landscape units) may affect disease incidence by altering interactions, abundance, and movements of hosts, vectors, and people, although the effects of these landscape variables on disease dynamics are understood only for a handful of well-studied

cases (Lambin et al. 2010). For instance, in the Amazon Basin and East Africa, deforestation increases standing water and sunlight, and enhances breeding success of some mosquito species, which can increase risk of malaria transmission (Yasuoka and Levins 2007). Habitat fragmentation and decreasing habitat patch size increase the risk of Lyme disease transmission in North America (Allan et al. 2003; Brownstein et al. 2005) and Hantavirus Cardiopulmonary Syndrome (HCPS) transmission risk in Panamá (Suzan et al. 2008a).

HCPS ranks among the major emerging diseases of the last century, and is expected to remain a public health threat into the future (Pereira et al. 2007). It was first recognized in May 1993 in the Four Corners region of the US (CDC 2014), and a few months later, in the city of Juquitiba, in the state of São Paulo, Brazil (Brazilian Ministry of Health 2013). Rodents in the family Cricetidae are the primary hosts of HCPS in Brazil (Mills et al. 1999; Jonsson et al. 2010), a virus (family *Bunyaviridae*) that causes two syndromes in humans: HCPS in the Americas, and hemorrhagic fever with renal syndrome (HFRS) in Eurasia and Africa (Jonsson et al. 2010). Transmission to humans occurs via inhalation of aerosolized virus particles derived from the urine, saliva, and feces of infected rodents (Lee et al. 1981; Vapalahti et al. 2010). HCPS is associated with high lethality rates (35% in the US; 41% in Brazil; 38% in Canada) (Macneil et al. 2011; Brazilian Ministry of Health 2013; Drebot et al. 2000).

Currently, most studies support the hypothesis that forest loss, forest fragmentation, and anthropogenic landscape change, as consequences of natural habitats conversion to agricultural areas increases prevalence of Hantavirus in reservoir species (De Souza et al. 2008; Suzan et al. 2008a; Suzan et al. 2008b; Lehmer et al. 2012). This effect occurs because these species are generally habitat generalists (Yahnke et al. 2001; Goodin et al. 2006) that can tolerate and adapt to ecological changes (Hughes et al. 1997), being favored in disturbed environments (Suzan et al. 2008a,b) and becoming abundant in altered landscapes (Yahnke et

al. 2001; Suzan et al. 2008a; Pardini et al. 2010; Puttker et al. 2015). In addition, greater population densities of these reservoir species increase intraspecific encounters and consequent Hantavirus transmission (Goodin et al. 2006; De Souza et al. 2008).

Climate can also influence host rodent population abundance and Hantavirus transmission dynamics. Several studies in North America have uncovered positive associations between precipitation, population size of rodent hosts, and Hantavirus prevalence (Meserve et al. 1995; Mills et al. 1999; Luis et al. 2010). High precipitation increases vegetation growth, boosting rodent densities and enhancing probability of human-rodent encounters and consequent Hantavirus transmission (Engelthaler et al. 1999; Yates et al. 2002). Temperature may also affect reproduction and survival rates of small rodents, as well as the time that the virus remains infectious in the environment (DHSS 2014); these effects influence transmission risk, although their direction is not entirely clear (Jiang 2011).

Additionally, several socio-economic variables can also influence disease transmission. HCPS epidemiology is complex, involves many factors, and the distribution and abundance of the reservoir species does not necessarily imply transmission of the disease. Agricultural practices (such as mechanization, use of personal protective equipment and adequate infrastructure to handle and stock production), public sanitation, types of preventive measures used, education, behavior, and economic conditions also influence HCPS transmission (Pereira 2007).

The relationship between landscape, climate, and social factors associated with Hantavirus transmission remains unexamined, particularly in Latin America. Evaluating the relative contributions of these factors to Hantavirus transmission can enable predictions of future outbreaks, and can be critical to design effective surveillance, control, and mitigation programs. Here, we rely on a Bayesian model to fill this research gap for the state of São Paulo, Brazil: we quantify associations between HCPS incidence and the size of risk

populations (e.g., number of rural men older than 14 years) and potential drivers including landscape structure (e.g., percentage of landscape units and fragmentation of native habitats), climate (e.g. temperature, precipitation), and social factors (Human Development Index, HDI dismembered, poverty and Gini Index). We make the following predictions:

(a) HCPS incidence will be greater in municipalities with a lower proportion of native habitat cover, a large proportion of agriculture and habitat edge areas, and with a large number of fragments, because HCPS reservoir species are habitat generalist which increase in abundance in edge habitats and in agricultural landscapes;

(b) HCPS incidence will be greater at higher precipitation, once it affects rodent population dynamics, increasing their abundance;

(c) HCPS incidence will be greater in municipalities with lower human development index (HDI), and with a large number of population at risk, since economic and social conditions can also affect HCPS transmission, and the bigger the number of people in contact with infected rodents the greater is the chance of HCPS transmission. We then use the results to identify high-risk areas for HCPS incidence across the state of São Paulo.

Materials and Methods

Study Area

We focused analyses in São Paulo, Brazil, including both the cerrado and Atlantic forest biomes (Fig 1), in southeastern Brazil, with an area of ~248,210 km^2, and a population of ~42 million (21.5% of Brazil's population); (IIBGE 2014). At present, only 13% of the state of São Paulo is still covered by remnants of its original biomes (cerrado and Atlantic forest), with the remaining area being covered by agriculture, especially a mixture of sugarcane plantations (Nassar et al. 2008), pasture (Durigan et al. 2007), and urban landscapes. Cerrado covered originally 33% of the state (MMA 2015) but now almost 81% of its area is converted

to anthropic uses (MMA 2015). It comprises a mosaic of vegetation types ranging from savanna with sparse shrubs and small trees to almost-closed woodland (Coutinho 1978). The region sees rainy summers and dry winters, with annual precipitation of 1390 mm (Coutinho 1978). The Atlantic forest originally covered ~69% of the state (Fundação SOS Mata Atlânica/INPE 2015). Only 13.9% of the original vegetation remains, and is now highly fragmented (Fundação SOS Mata Atlânica/INPE 2015). These areas are characterized by hot and rainy summer, without a defined dry season (Peel et al. 2007); annual precipitation ranges 1000-2200 mm Alvares et al. 2014).

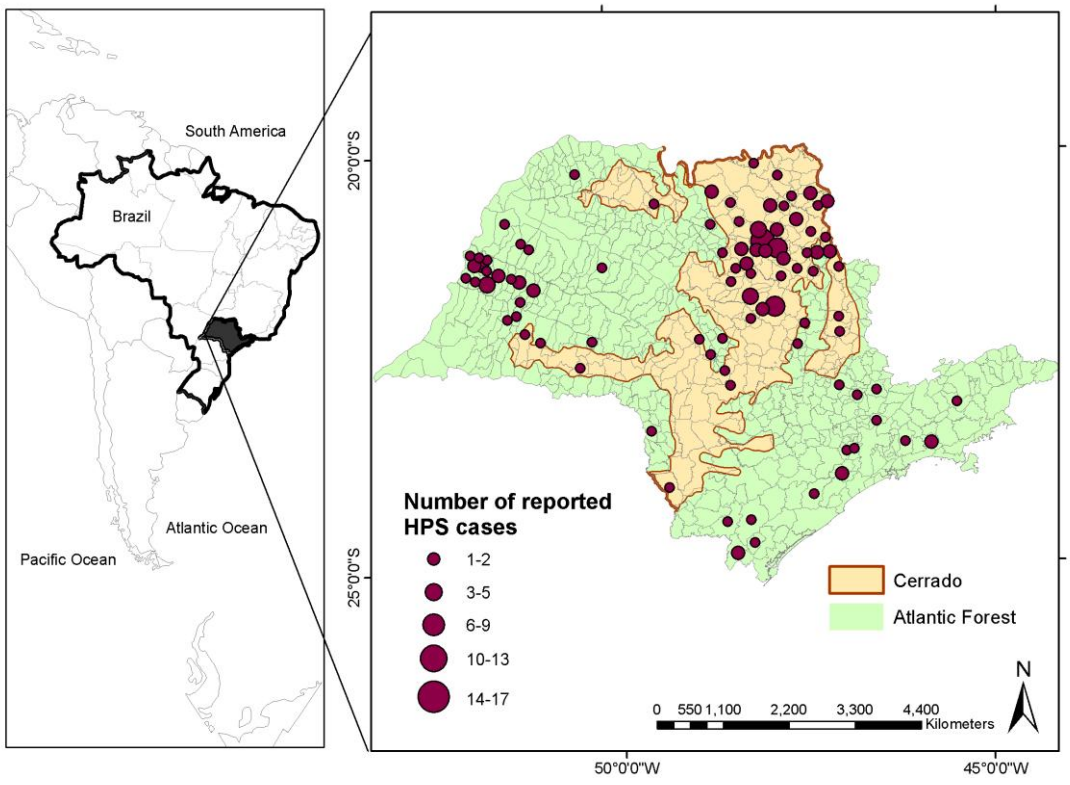

Fig 1. Hantavirus incidence between 1993 and 2012 across the 645 municipalities of the state of Sao Paulo, and cerrado (in orange) and Atlantic forest (in green) delimitation in the state.

Disease and social data

HCPS incidence data are collected at the municipality level, so we treated the 645 municipalities within São Paulo state as our sampling units. The number of reported HCPS cases in each municipality per year from 1993 to 2012 was extracted from the website of the

Center for Epidemiological Surveillance of the State of São Paulo (CVE-SP) (1993-2012), and the Health Portal SUS (http://portal.saude.gov.br/portal/saude/profissional/area.cfm?id_area=1558). The CVE-SP compiles this information, once the cases of Hantavirus infection are of mandatory notification to the local health authorities in Brazil. The data is provided by every hospital in the state, once the patients' living address is a confidential information. Therefore CVE considers all HCPS cases confirmed by laboratory analysis (antibody positive) that were recorded in any hospital presented in each municipality of São Paulo state. Since the majority of the municipalities had 0 (98.39%) or 1 case per year (1.61%), with the maximum of 4 cases per municipality per year, we transformed the data to binary, reflecting presence *versus* absence of HCPS. Then, we modeled the probability of incidence of HCPS in each municipality based on the reported cases from 1993 to 2012 and covariates reflecting social, climatic and landscape condition described on the next paragraphs. In that way, the probability of HCPS risk was defined as the probability of an HCPS infection to occur in a municipality.

Epidemiologic data indicate that more than 70% of HCPS-infected people was working or living in agricultural areas, and ~93% were men over the age of 20 (Figueiredo et al. 40,41; Elkhoury et al. 2012). Because the available data are relatively coarse with respect to age distribution, we used the number of rural men older than 14 years in each municipality as the population at risk for HCPS. This information was extracted from the National Institute of Geography and Statistics (IBGE) website (www.ibge.gov.br), and was available only for 1996 and 2006. Since we wanted to model the incidence of HCPS from 1993 to 2012, we thus used the 1996 data as covariates to predict disease incidence for 1993-2001, and 2006 data to predict incidence for 2002-2012.

Since socio-economic development can influence HCPS transmission, Human Development Index (HDI), HDI elements including life expectancy, income, and education, Gini index and poverty were tested for association with HCPS incidence across São Paulo. The Human Development Index (HDI) is a summary measure of average achievement in key dimensions of human development and socioeconomic status of the populations, and includes elements of life expectancy, income (GDP/capita), and education. HDI is thus a measure of human development and poverty, and can be used as a proxy of the socio-economic factors (education, poverty and health) that influence HCPS risk (de Oliveira et al. 2015). HDI data at the municipality level were extracted from IBGE (www.ibge.gov.br) website, with data available for 1991, 2000, and 2010. We used HDI data from 1991 as covariates to predict incidence for 1993-1998, data from 2000 to predict incidence for 1999-2005, and data from 2010 to predict incidence for 2006-2012. HDI elements (e.g., life expectancy, income, and education) were extracted from United Nations Development Programme (UNDP) (http://www.pnud.org.br/arquivos/ranking-idhm-2010.pdf), with data available only for 2010. Gini index is used to measure inequality, and together with poverty, was extracted from IBGE (www.ibge.gov.br) being available only for 2003. Therefore, we used HDI elements data from 2010 and Gini and poverty data from 2003 as covariates to predict incidence for the entire period (1993-2012).

Landscape composition and configuration metrics

We used the São Paulo state forest inventory map (http://www.iflorestal.sp.gov.br) for the years 2000 and 2010 to calculate landscape composition and configuration metrics for each municipality. This native vegetation inventory covers two dates in our study period (2000 and 2010), and was generated at a 1:50.000 scale, with a minimum mapped area of 2.5 ha, being able to identify small fragments, which are very common in the São Paulo state. Additionally, using the IF inventory made possible to use information with same spatial resolution and

mapping method for both cerrado and Atlantic Forest biome in the state. Native vegetation cover aggregated both Atlantic Forest and cerrado remnants, and we considered each municipality as individual landscapes for analysis. Landscape composition was measured considering the relative abundance of each landscape unit (percentage of native vegetation cover - forest and cerrado; and sugarcane, pasture and corn), while landscape configuration refers to the degree of fragmentation of native vegetation cover types (forest and cerrado), measured by the number of habitat fragments (e.g., number of forest or cerrado patches in a landscape) and density of habitat edge (e.g., total length of habitat/non-habitat edge per area of landscape). A municipality was considered as part of the Atlantic Forest or cerrado biome depending on the percentage of its area that overlapped the distribution of these biomes (see Statistical analyses).

All landscape analyses were done in ArcGis 10.0 and Fragstats 4.2. We used metrics extracted from the 2000 map as covariates to model incidence for 1993-2001, and metrics extracted from the 2010 map as covariates for period 2002-2012.

The main agricultural land uses in São Paulo - sugarcane (Gheler-Costa et al. 2012), pasture (Martin et al. 2012), and corn (de Souza et al. 2008) - result in habitats favorable to generalist rodent species achieving high abundances (Olifiers et al. 2005). Some of these land uses have a relatively high temporal heterogeneity (e.g., massive biomass production from planting to harvest in a few months or years, providing considerable amounts of high-energy food) (Gheler-Costa et al. 2012, 2013; Olifiers et al. 2005; Parshad et al. 1986]. Small rodents take advantage of this tremendous food supply, increasing their abundances (Gheler-Costa et al. 2013), which may influence Hantavirus incidence (Olsson et al. 2009). Therefore, we also used data for these agricultural land uses, to test for associations with Hantavirus incidence. We obtained annual data from the Agricultural Census of the Institute of Agricultural

Economics (www.iea.sp.gov.br) to use the proportion of sugarcane, pasture, and corn in each municipality as covariates to model annual disease incidence for 1993-2012.

Climatic variables

Meteorological data used were obtained from the International Research Institute for Climate and Society (IRI) Data Library (http://iridl.ldeo.columbia.edu/index.html). Gridded land surface temperature data were obtained from National Centers for Environmental Prediction (NOAA NCEP) from combined GHCN and CAMS station data at 0.5° spatial resolution, and extracted for each municipality (as average value across the municipality). The monthly data (Fan et al. 2008) were used to calculate annual mean, minimum, and maximum, and seasonal mean, minimum, and maximum temperature values, for each municipality over 1993-2012.

Precipitation data were obtained from the University of California Santa Barbara from the Climate Hazards Group Infrared Precipitation with Stations (CHIRPS) data set, with a spatial resolution of 0.05°, and extracted for each municipality (as average value across the municipality). The 10-day averages data (Funk et al. 2014); were used annual mean, minimum, maximum, and total, and seasonal mean, minimum, and maximum, precipitation for each municipality for 1993-2012.

Statistical analysis

Hantavirus shows high host specificity. Therefore, as expected, for each Brazilian region there are different reservoir species hosting distinct virus strains (Pereira 2007). Although some geographic overlap occurs (Figueiredo et al. 2009), Araraquara virus (ARAV) is the dominant pathogenic Hantavirus in cerrado, and is commonly associated with HCPS cases there (Figueiredo et al. 2009; Suzuki et al. 2004), whereas Juquitiba (JUQV) is the dominant pathogenic Hantavirus in Atlantic forest (Figueiredo et al. 2009). Given the geographic distribution of the two viruses, and the assumption that *Oligoryzomys nigripes* is the chief reservoir for human HCPS cases in Atlantic forest (D´Andrea et al. 2007) and *Necromys*

lasiurus is the reservoir in cerrado, Hantavirus transmission risk was modeled separately in the two biomes. Municipalities were considered as cerrado or Atlantic forest if >50% of their surface area fell inside one or the other biome. Biome distribution was obtained from IBGE (www.ibge.gov.br).

To reduce numbers of predictor variables we fitted and compared generalized linear mixed models (see further detail on methods and results of exploratory analyses in S1 to S3 Tables). We then fitted a Bayesian model containing only 7 predictor variables as fixed covariates: proportion of sugarcane, proportion of native vegetation cover, number of native vegetation patches, HDI, mean annual temperature (°C), total annual precipitation (mm), and rural male population >14 years old (S4 Table). All variables included in the model had correlations <0.4 relative to other variables, and are available in S1 Data. Non-linear correlations between variables were assessed visually; when necessary, a quadratic form was fit to covariates and compared with linear relationships using the Deviation Information Criterion (Bayesian method for model comparison; Spiegelhalter et al. 2002). In every case, the linear form provided a better fit to the data. Percent of native vegetation cover and annual precipitation were log-transformed prior to analysis.

Municipality was included as a random effect to account for differences among administrative units not captured in the fixed covariates. To facilitate interpretation, all estimated parameters were standardized, centered on their means, and divided by two standard deviations (Gelman and Hill 2007).

All priors were assigned as uninformative distributions. We used the *rjags* package in R, and examined model convergence and performance via Gelman-Rubin diagnostics. Parameters were considered significant if the 95% quantiles of their distribution did not overlap 0. We also calculated Bayesian *p*-values to examine discrepancies between means of simulated and real data (e.g., values close to 0.5 represent a good model; (Gelman et al.

1996), and R^2 to examine the square of the correlation between true and predicted outcomes (Gelman and Parode 2006). As HCPS can be considered as a rare event in the state of São Paulo (1% of success) we did not validate our model by test-training procedure (e.g., removing a random part of data points to fit the model to the remaining data), as it was necessary to have all data available for calibrating the model. Therefore, we are using Bayesian *p*-values and R^2 as measures of validation.

We tested HCPS incidence and model residuals for both models, constructed for Atlantic forest and cerrado biomes, for spatial autocorrelation, by calculating Moran's *I*. For this analysis we used the spatial contiguity matrix based on the Queen´s case neighborhood relation and treat each year, from 1993 to 2012, separately. This test is commonly used and accepted as a fair evaluation of spatial autocorrelation and dependence (Fortin et al. 2002), especially in disease studies (Fang et al. 2006; Khamis et al. 2007; Tsai 2012). For both models and for HCPS incidence, Moran´s *I* results showed no spatial autocorrelation (see S5 and S6 Tables) for the majority of years, justifying our use of a non-spatial model.

Mapping is a primary goal in spatial epidemiology (Stevens and Pfeiffer 2011), as it allows immediate visualization of the extent and magnitude of public health threats (Hay et al. 2009). We used model results to generate a map of Hantavirus risk areas for the state of São Paulo. Risk was defined as the probability of an HCPS infection to occur in a municipality. The mean and coefficient of variation of simulated results among years were summarized for each municipality, and imported into ArcGIS 10.0 for visualization.

A t-test or an one-way analysis of variance (ANOVA), followed by Tukey's Multiple Comparison Test, were performed to check whether significant differences existed in the final predictor variables between available years.

Results

During 1993-2012, 207 HCPS cases were reported for the state, with increasing numbers in the last 10 years (Fig 2). Of the total, 57 cases were reported from the cerrado region (161 municipalities; 0.35 cases per municipality) whereas 150 (484 municipalities; 0.30 cases per municipality) in the Atlantic forest region. The largest number of HCPS cases are concentrated in the northeastern region, followed by the western region, although there are cases statewide (Fig 1). Increases in HCPS incidence were particularly marked in the 2000s, raising was 250% if compared to the number of cases in the 1990s. Only in 2005 we can observe a decrease in the number of reported cases especially for cerrado region, but after this period it augment further, reaching a peak of 28 cases only in 2010 (Fig 2).

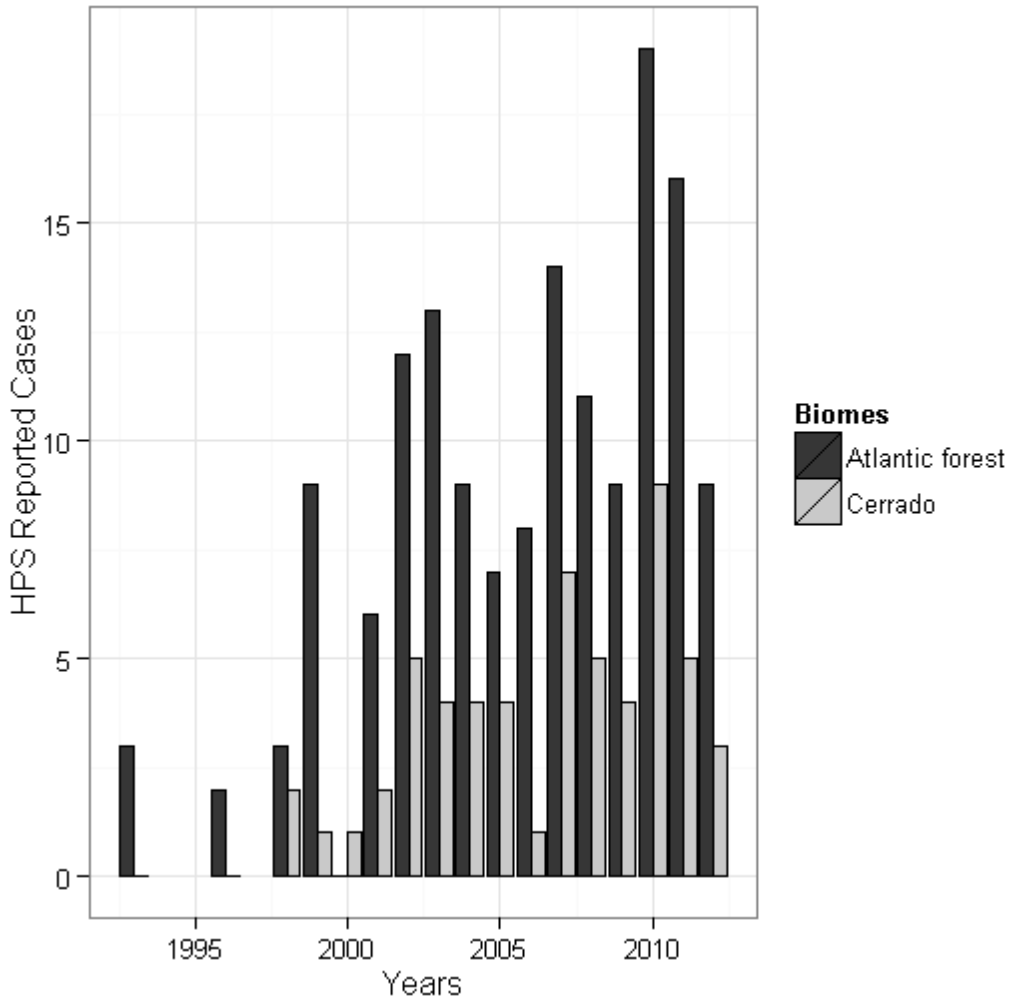

Fig 2. Reported HCPS cases between 1993 and 2012 in the cerrado and Atlantic forest regions of the state of São Paulo.

The majority of the factors considered in our model exhibited significant trends over the study period. Proportion of native vegetation cover increased between 2000 and 2010 for both cerrado (8.10 to 11.38%) and Atlantic forest (12.16 to 16.20%) municipalities, despite concomittant increases in proportion of sugarcane cultivated in these regions over the same period (Table 1). The average number of native vegetation patches also increased in both cerrado (208 to 557) and Atlantic forest (149 to 413) regions (Table 1). HDI and the size of the population at risk (e.g., males > 14 yrs old) also increased over the years for which data

were available in both regions (Table 2); annual mean temperature and annual precipitation did not show any significant trend (S7 Table).

Table 1. Average and range values of percent of sugarcane cultivated, percent of native vegetation cover, and number of patches for the municipalities of cerrado and Atlantic forest regions, for the years 2000 and 2010. *** represents significant difference among years.

Year	Native vegetation cover	Sugarcane	Number of patches
	Cerrado		
2000	8.10 (0.65-79.24)***	15.44 (0-86.39)***	208.39 (11-1869)***
2010	11.38 (1.9-82.34)	27.60 (0-81.72)	557.12 (31-3624)
	Atlantic forest		
2000	12.16 (0.17-89.3)***	11.57 (0-91.6)***	149.84 (2-925)***
2010	16.20 (0.06-91.8)	22.95 (0-97.85)	413.36 (1-2611)

Table 2. Average and range values of HDI (Human Development Index) and Population at risk (rural men older than fourteen years) for the municipalities of cerrado and Atlantic forest regions, for the years that the data is available. *** represents significant difference among years.

	Human Development Index			Population at Risk	
Year	Cerrado	Atlantic forest	Year	Cerrado	Atlantic forest
1991	0.50 (0.31-0.64)***	0.49 (0.23-0.69)***	1996	1209 (4-4610)	993 (2-7473)
2000	0.65 (0.5-0.74)***	0.64 (0.46-0.82)***	2006	1327 (10-7085)	1052 (1-7549)

2010	0.74 (0.65-0.82)	0.73 (0.63-0.86)

Overall, our statistical models fit well to the data (cerrado: R^2 = 0.19; Bayesian *p*-value = 0.49; Atlantic forest: R^2 = 0.23; Bayesian *p*-value = 0.50), and showed significant effects of landscape, climate, and social variables on Hantavirus infection risk, as is described below in detail.

In cerrado, probability of Hantavirus infection risk was significant and positively related to HDI and to proportion of municipality occupied by sugarcane plantation (Fig 3). Number of patches and annual mean temperature also showed a positive relationships, but they were not significant, with greater risk of HCPS infection in more fragmented habitats and in years with higher annual mean temperatures (Fig 3).

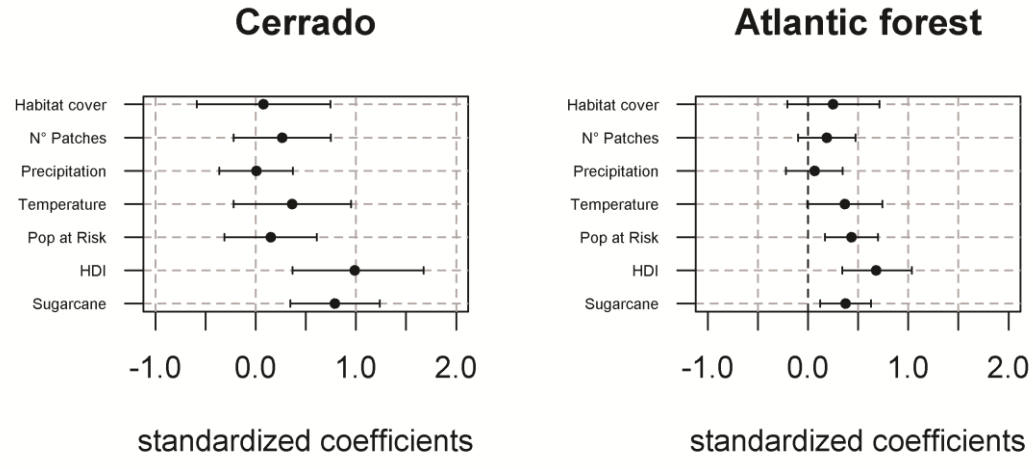

Fig 3. Parameter estimates with mean (black dot) and credible intervals (2.5 - 97.5%) of predictors of HCPS risk for cerrado and Atlantic forest region. Habitat cover = percentage of native vegetation cover; N° Patches = number of native vegetation fragments; Precipitation= total annual precipitation; Temperature = mean annual temperature; Pop at Risk= population at risk, i.e. rural men aged over 14 years; HDI= Human Development Index; Sugarcane= percentage of municipality occupied by sugar cane plantations.

For Atlantic forest, population at risk, HDI, and proportion of sugarcane were all significantly positively associated with Hantavirus infection risk (Fig 3). Annual mean temperature and number of patches had marginally significant positive relationships to Hantavirus infection, with a higher chance of Hantavirus infection in municipalities with higher temperatures and more fragmented forests.

Using results from the statistical models, we mapped HCPS risk across the state. Overall, 6% of the state was classified as medium (5-10%) or high (> 10%) risk category for HCPS infection, and 94% was indicated as low risk (<5%) category (Fig 4A). All municipalities with a medium to high risk of Hantavirus infection are shown with black outlines in the risk map and represent municipalities where preventive measures should be allocated.

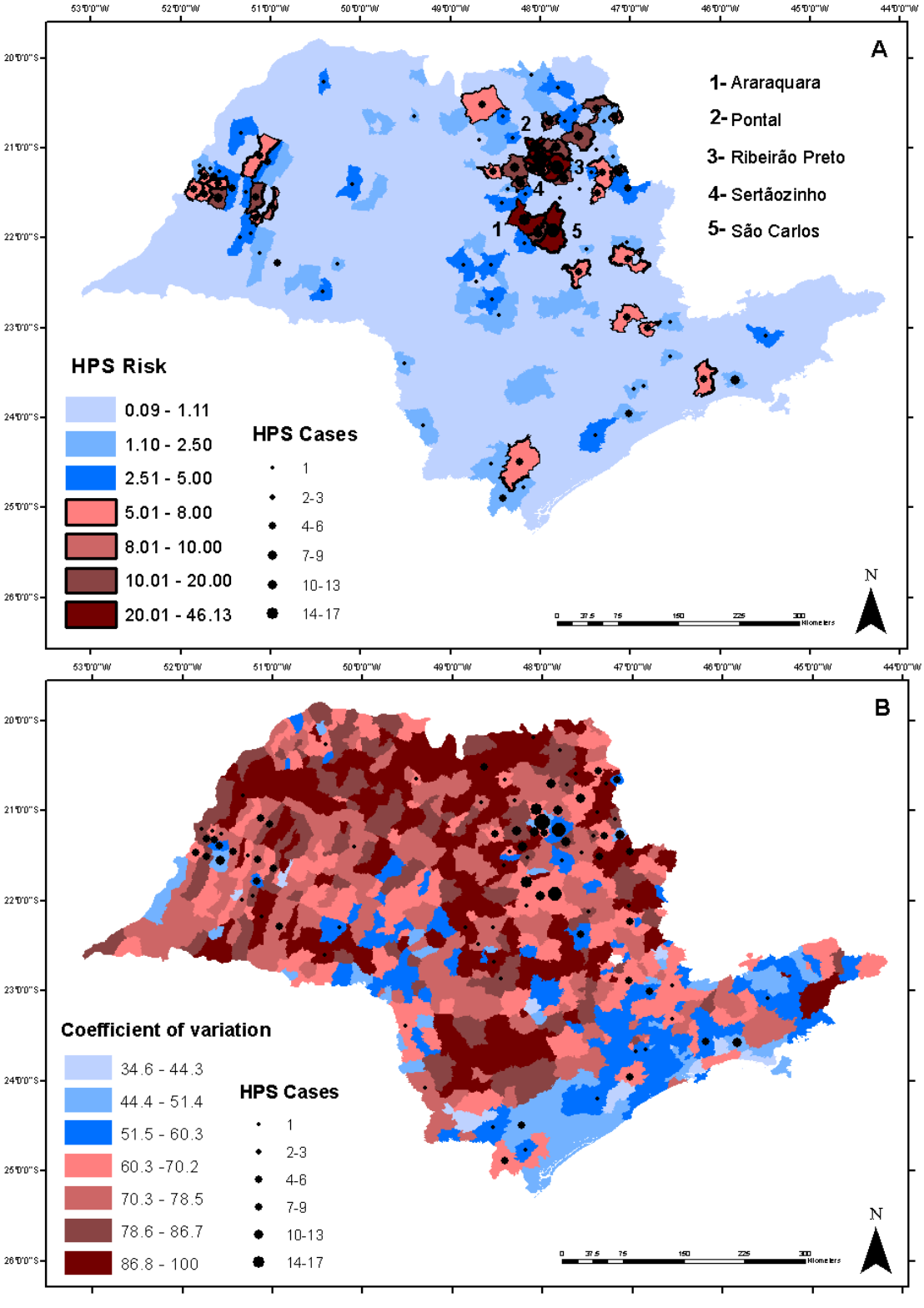

Fig 4. Map of Hantavirus infection probability (%) (A) mean (B) and coefficient of variation (B) among years across the state of São Paulo. Black dots depict number of reported cases between 1993 and 2012. Municipalities with no symbol means no reported cases of HCPS. Black outlines indicate municipalities with medium to high risk (> 5%) of Hantavirus infection and where preventive effort should be allocated.

Not surprisingly, municipalities with highest mean risk were those that already had many HCPS cases (Fig 4A). Municipalities in the northeastern region have particularly high mean risk (up to 46%), followed by some municipalities of the east, close to the Serra do Mar (in Atlantic forest region) and in the west of the state, with a mean risk up to 21%. The municipalities of São Carlos, Ribeirão Preto, Pontal, Sertãozinho and Araraquara, located in the northeast of the state (Fig 4A), are the ones that present a very large mean risk for Hantavirus infection (> 20%). The coefficient of variation of Hantavirus infection risk across years (Fig 4B) is smaller in the eastern part of the state, where habitat cover is relatively high (S1A Fig). Based on our model, a large number of municipalities that have not registered HCPS infection have HCPS risk of up to 2.5% (Fig 4A). At the same time, some municipalities that had HCPS cases have a small risk of Hantavirus infection (8% of the state). Maps with the minimum and maximum risk are shown in S2 Fig.

Discussion

This study is the first to link landscape structure, climate, and social variables to Hantavirus infection risk in the Neotropics. Our model identified >6% of the state of São Paulo as presenting medium-to-high risk of Hantavirus transmission (39 municipalities). This disease that has so far killed at least 99 people (47.8% lethality rate) in the state, and 637 people in Brazil, since 1993 (Brazilian Ministry of Health 2013). A key finding of our study is that the extent of sugarcane plantations was the most important predictor of Hantavirus infection, and this relationship held in both the cerrado and the Atlantic forest. Increases in HCPS incidence were noted in the 2000s, when high oil prices led to substantial expansion of sugarcane cultivation in the state (Ferreira-Filho and Horridge 2014).

Sugarcane plantations support greater abundances of rodents than other ecosystems, whether natural or agricultural (De Souza et al. 2008; Gheler-Costa et al. 2012; Verdade et al.

2012). Brazil is the largest producer (~ 490 x 10^6 tons/year in 2011-2012) and exporter of sugarcane in the world (Rudorff et al. 2010), with most of this production (~76%) in São Paulo (Borrero et al. 2003). Yields in these plantations can reach 120 tons/year/ha (Norman et al. 1995), serving as a readily available source of food and cover for rodents (Parshad et al. 1986). Moreover, in recent years sugarcane cultivation has become increasingly mechanized, as a result of legislation limiting burning prior to harvest (Law n°. 11.241, 19 September, 2002). These shifts in predominant harvest mode may have reduced rodent mortality (large numbers previously were killed by burning), increasing population sizes, and consequently augmenting Hantavirus infection risk (Elkhoury et al. 2012; Verdade et al. 2012; but see Gheler-Costa et al. 2013). Sugarcane cultivation is projected to increase still further in coming decades (Smeets et al. 2008), with implications for HCPS risk across São Paulo and neighboring states in southern and central Brazil.

Surprisingly, high HDI was associated with increased HCPS incidences in both regions. This result was unexpected because a number of studies have found no relationship between Hantavirus infection and socio-economic status (Figueiredo et al. 2001; Elkhoury et al. 2012); others studies have found poor sanitary and living conditions to be positively associated with incidence (Vapalahti et al. 2010; Linard et al. 2007). For São Paulo, however, the positive association between HDI and HCPS risk may reflect better socioeconomic conditions in municipalities where sugarcane is dominant economically: sugarcane municipalities have, on average, stronger social welfare indicators (Camargo et al. 2008; Martinelli et al. 2010), with this sector contributing to the concentration of income (Camargo et al. 2008), even outperforming the greater São Paulo Metropolitan Region (Camargo et al. 2008).

HCPS studies elsewhere have found that most individuals affected by HCPS participate in agricultural or forestry activities (Pereira 2007; Figueiredo et al. 2001; Elkhoury

et al. 2012; de Oliveira et al. 2015; Martinelli et al. 2010). This connection exists because HCPS transmission requires contact between humans and aerosolized excreta of infected rodents, which is most likely in this demographic group. This pattern was clear for the Atlantic forest, which showed a positive relationship between population at risk (number of males older than 14 years old) and HCPS incidence; however, this relationship did not hold for the cerrado. Another study in the cerrado (Watson et al. 2014) also failed to uncover an association between Hantavirus infection and the size of rural populations. One possible reason for absence of such association may be that cerrado municipalities have a longer history of sugarcane cultivation and more land proportionally cultivated for sugarcane than Atlantic forest municipalities (S1B Fig); this crop also brings large numbers of temporary workers from other states who do not end up in the official population statistics (Campos et al. 2003), which may have reduced the strength of any association between the size of the population at risk and HCPS incidence. Another possibility is that some Atlantic forest municipalities have taken longer to achieve large-scale mechanization (e.g., in 2007, regions such as Pindamonhangaba and Guaratinguetá still had 0% mechanization; Ficarelli and Ribeiro 2010), leading to greater transmission probabilities for workers in this biome. Workers in unmechanized plantations may have greater probability of contact with rodent excreta, as unmechanized plantations use workers in all steps of the production process, whereas in mechanized systems they work only in some steps of the process (Ficarelli and Ribeiro 2010).

In Atlantic forest, our model showed marginal positive associations of fragmentation with Hantavirus incidence, supporting studies elsewhere (Suzan et al. 2008a,b; Fredo et al. 2014), whereas in cerrado this association was not significant. The same result was found for proportion of habitat cover. In tropical regions HCPS risk is expected to be higher in areas with a small proportion of habitat cover (Suzan et al. 2008a,b), which contrasts with our

results for Atlantic forest. For cerrado, the proportion of habitat cover had no association with HCPS risk. We hypothesize that this may reflect the fact that most of municipalities within Atlantic forest have a small amount of habitat cover (~8.6%), and are composed of second-growth forests in early to medium stages of succession (Rubio et al. 2014). Both Hantavirus reservoir rodent species in São Paulo (*N. lasiurus* and *O. nigripes*) are habitat generalists (Gheler-Costa et al. 2012; Metzger et al. 2009). While *O. nigripes* is known to survive in small and isolated forest remnants (Puttker et al. 2015; Umetsu and Pardini 2007), and prefers early successional stages inside the forest (Metzger et al. 2009; Umetsu and Pardini 2007; Puttker et al. 2008), becoming more abundant in these landscapes, *N. lasiurus* seems to prefer less dense and open areas (Gheler-Costa et al. 2012; Dalmagro and Vieira 2005), occurring in disturbed and open habitats (Metzger et al. 2009).

High annual mean temperature was marginally associated with greater HCPS risk in the Atlantic forest. For cerrado, this association was not significant. This result supports findings elsewhere (Engelthaler et al. 1999; Vieira et al. 2005; Tersago et al. 2009; Li et al. 2009). Temperature can affect vegetation growth (Jiang et al. 2011) and the survival rate of rodents (Carver et al. 2015), with mild temperatures (10-25°C) being most favorable for rodent breeding (Zeier et al. 2005). Additionally, reservoir rodents normally exhibit a peak in Hantavirus infection during warmer months (Liu et al. 2012; Vadell et al. 2011; Oliveira et al. 2014; Teixeira et al. 2014), probably because high temperature leads to greater aerosolization of the virus and higher rates of inhalation by both humans and rodents (Lee et al. 1981; Vapalahti et al. 2010). At present there are no data available on the effects of temperature on HCPS virus survival, but laboratory experiments found that Puumala viruses (aetiological agent of Hantavirus infection in Western Europe) can remain infectious for longer (i.e.12 to 15 days) at room temperature (23°C; 73°F), losing their viability if kept at 37°C (Kallio et al. 2006). The average temperature for São Paulo municipalities, from 1993 to 2012 was 22.9° C,

with maximum temperature of 27° C, while the highest HCPS infection risks were found between 22.5°C and 25°C, intermediate conditions for São Paulo state. This temperature range would be ideal for virus survival in nature, if JUQV and ARAV virus conditions are the same as for the Puumala virus. Studies conducted over shorter spatial scales than the work presented here would be necessary to link climatic factors to HCPS incidence.

There was no effect of precipitation on Hantavirus risk, even in the cerrado, which has a marked dry season (Oliveira et al. 2005). The association between precipitation and Hantavirus infection is still controversial, with no association in some studies (Tersago et al. 2009; Murua et al. 2003), negative effects in others (Carver et al. 2015), while in others increased rainfall in fall to spring have resulted in higher HCPS transmission (Engelthaler et al. 1999; Yates et al. 2002), and in rodent outbreaks (Meserve et al. 1995). These studies, however, were performed in arid and semi-arid regions (85 - 100 mm precipitation per year) (Meserve et al. 1995; Brown and Ernest 2002), which show an increase in both rodent richness and abundance in response to high precipitation (Meserve et al. 1995; Brown and Ernest 2002). Since our study was performed in a tropical region where annual mean precipitation is considerably high, the effects of this climatic variable on rodent resources and population dynamics may not be important.

According to our risk map, a large number of municipalities that had no reported cases nevertheless have some HCPS risk (0.9-4.9%). Although this risk may seem small, the combination of lack of effective prevention and treatment options (with high lethality makes HCPS a serious public health risk. Additionally, as rare events (~1% of events) and undiagnosed asymptomatic infections that are typically not reported to official statistics (Linard et al. 2007), these numbers may be underestimated. This situation is of special concern in view of the high variation of infection risk in several municipalities that had no HCPS infections reported. Due to its high lethality, an infection risk up to 5% should be

considered as a medium risk, and municipalities with risk higher than this value or with very high coefficient of variation should be included in preventive measures programs. Northeastern municipalities showed the highest risk across the state (up to 46%), probably because sugarcane cultivation is intense in that region: it is essential to implement awareness campaigns and reinforce diagnostic protocols to detect HCPS infections in these municipalities, especially in sugarcane properties. Simple, low cost measures such as use of personal protective equipment in any environment where wild rodent excreta is frequently present, rodent control and proper clean-up of their excrement in human dwellings, seal homes against the entry of rodents, and apply other rodent-proofing techniques, may go a long way toward reducing HCPS incidence and human mortality.

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Supporting information

Exploratory analysis

We fitted generalized linear mixed models to reduce the number of predictor variables among landscape, climate and social factors to be included in the final Bayesian model. We split the data into cerrado and Atlantic Forest biomes and used "lme4" package in R program version 3.03, with a Binomial family error distribution. Precipitation and temperature variables were selected through the comparison of 21 simple models, for each biome, each containing one of the 21 climatic variables as fixed factor, municipality and year as random effects, and the presence of HCPS cases as the response variable. Only the variables included in significant models were selected to enter in the model selection with landscape, social and agricultural variables (e.g., annual mean temperature, mean temperature of winter, annual total precipitation and annual maximum precipitation).

After that we performed a maximum likelihood model selection procedure, considering the second-order Akaike´s information criterion (AIC) (Burnham and Anderson 2002) to compare a set of 216 candidate models, for each biome, combining climate, social, landscape and agricultural variables, including only variables with correlations lower than 0.4. As we wanted only to reduce the number of predictor variables to test the hypothesis that social, climate and landscape factors have an effect on Hantavirus incidence on the Bayesian model, we always set up combinations of models containing climate, landscape and social variables (e.g., climate variables selected above; native vegetation cover, number of native vegetation fragments, native vegetation edge density; amount of corn, pasture and sugarcane; HDI, HDI elements; Gini index and poverty). Again, we used generalized linear mixed models, considering municipality and year as random effects, and the presence of HCPS cases as the response variable. All estimated parameters were standardized, centered on their means and divided by two standard deviations. The model with the lower AIC were used in the

Bayesian analysis (Table S1 shows the results of the best models of exploratory analysis for both regions).

Table S1. Exploratory analysis results made with generalized linear mixed model to reduce the number of predictor variables. Only the models that had an AIC value close to 2 are shown (PopRisk = population at risk; HDI = Human Development Index; PLAND = percent of habitat cover; NP = number of fragments; ED= habitat edge density; Cane = amount of sugarcane; Pasture = amount of pasture; TotalP= annual total precipitation; MaxP= annual maximum precipitation; MeanT = annual mean temperature; MeanTWint = annual mean temperature of winter).

Cerrado

Model name	Predictors Variables	AIC	Df	logLik	Deviance
m7	PopRisk; HDI; PLAND;NP; Cane; TotalP; MeanT	384.79	9	-183.39	366.79
m11	PopRisk; HDI; NP; Cane; MaxP; MeanTWint	385.04	9	-183.52	367.04
m5	PopRisk; HDI; ED; Cane; MaxP; MeanT	385.6	9	-183.8	367.6
m9	PopRisk; HDI; ED; Cane; MaxP; MeanTWint	385.73	9	-183.87	367.73
m6	PopRisk; HDI; PLAND; Cane; MaxP; MeanT	385.91	9	-183.95	367.91
m10	PopRisk; HDI; PLAND; Cane; MaxP; MeanTWint	386.2	9	-184.1	368.2
m31	PopRisk; HDI; NP; Pasture; MaxP; MeanT	386.73	9	-184.36	368.73
m4	PopRisk; HDI; PLAND; NP; Cane; MaxP; MeanT	386.73	10	-183.37	366.73
m8	PopRisk; HDI; PLAND; NP; Cane; MaxP; MeanTWint	386.95	10	-183.47	366.95
Atlantic Forest					
m7	PopRisk; HDI; PLAND; NP; Cane; TotalP; MeanT	982.55	9	-482.28	964.55
m3	PopRisk; HDI; NP; Cane; TotalP; MeanT	982.57	9	-482.28	964.57
m6	PopRisk; HDI; PLAND; Cane; MaxP; MeanT	983.95	9	-482.97	965.95
m4	PopRisk; HDI; PLAND; NP; Cane; MaxP; MeanT	984.04	10	-482.02	964.04
m2	PopRisk; HDI; PLAND; Cane; TotalP; MeanT	984.05	9	-	966.05

M	PopRisk; HDI; NP; Cane; MaxP; MeanT	984.1	10	483.02 - 482.05	964.1
m1	PopRisk; HDI; ED; Cane; TotalP; MeanT	984.54	9	- 483.27	966.54
m5	PopRisk; HDI; ED; Cane; MaxP; MeanT	984.8	9	-483.4	966.8

In both regions the same model was selected with the lower AIC. It was the model containing the variables population at risk, HDI, percent of habitat cover, number of fragments, amount of sugarcane, annual total precipitation and annual mean temperature (m7).

To be sure that our model contained all the best predictor variables, we also tested if the abundance of reservoir rodents of São Paulo had associations with Hantavirus incidence. To do so we extracted the abundance rodent data obtained in Prado (2015) in municipality levels (average abundance for each municipality for each reservoir rodent - *O. nigripes* and *N. lasiurus*).

Rodent abundance data were obtained through studies performed in Atlantic Forest region, conducted by Dra. Renata Pardini, Dra. Fabiana Umetsu and Dra. Adriana Bueno, from São Paulo University. The study area comprised areas of fragmented and continuously-forested landscapes characterized by different levels of remaining native forest (50, 30 and 10%), but similar with respect to climate, topography, type of forest and of human use, and distance to areas of continuous forest (Pardini et al. 2010). Small mammals were trapped with large pitfall traps in a total of 104 sampling points in 6 landscapes, of which 68 were collected in forest (18 in intact forest and 50 in forest fragments) and 36 in matrix areas. In control landscapes 6 points were sampled, being 3 in intact forest and 3 in intermediate forest. Greater details about point selection can be read in Bueno (2008), Umetsu (2010) and Puttker (2008). Total effort was 352 trap-night-point and 23.936 trap-night in the 68 points of the forest, and 176 trap-night-point, 6.336 trap-night in the 36 matrix points. Landscape metrics

(percent of forest cover, edge density and percent of forest cover at landscape level) were extracted in ArcGis 10.3 buffers (200, 500 and 800 m) of each sampled point. Multiple regression models, in an information-theoretic approach was performed to find the best model, and the best buffer size. From the best model, selected through AIC, the abundance of *O. nigripes* and *N. lasiurus* were extrapolated from the sampled landscapes to the entire state of São Paulo, using the São Paulo state Forest Inventory map from 2010 (more details in Prado 2015).

Again, we used generalized linear mixed models, considering municipality and year as random effects, and the presence of HCPS cases as the response variable. We performed a maximum likelihood model selection procedure, considering the second-order Akaike´s information criterion (AIC) (Burnham and Anderson 2002) to compare a set of 45 candidate models, for each biome, combining climate, social, landscape, agricultural and rodent variables, and including only variables with correlations lower than 0.4. Once rodent abundance data was extrapolated to São Paulo state based on landscape metrics, we never included abundance data and landscape metrics in the same model. All estimated parameters were standardized, centered on their means and divided by two standard deviations. The model with the lower AIC were used in the Bayesian analysis (Table S2 shows the results of the best models of exploratory analysis for both regions; for cerrado we also shown the first model with rodent included - once it does not appear in the best models selected).

Table S2. Exploratory analysis results made with generalized linear mixed model to reduce the number of predictor variables. Only the models that had an AIC value close to 2 are shown. For cerrado we also shown the first model with rodent included (once it does not appear in the best models selected). PopRisk = population at risk; HDI = Human Development Index; PLAND = percent of habitat cover; NP = number of fragments; ED= habitat edge density; Cane = amount of sugarcane; Pasture = amount of pasture; TotalP= annual total precipitation; MaxP= annual maximum precipitation; MeanT = annual mean temperature; MeanTWint = annual mean temperature of winter; NL = abundance of *Necromys lasiurus*; ON = abunandance of *Oligoryzomys nigripes*.

Cerrado

Model Nome	Predictors Variables	AIC	Df	logLik	Deviance
m7	PopRisk; HDI; PLAND; NP; Cane; TotalP; MeanT	384.79	9	-183.39	366.79
m11	PopRisk; HDI; NP; Cane; MaxP; MeanTWint	385.04	9	-183.52	367.04
m5	PopRisk; HDI; ED; Cane; MaxP; MeanT	385.6	9	-183.8	367.6
m9	PopRisk; HDI; ED; Cane; MaxP; MeanTWint	385.73	9	-183.87	367.73
m6	PopRisk; HDI; PLAND; Cane; MaxP; MeanT	385.91	9	-183.95	367.91
m10	PopRisk; HDI; PLAND; Cane; MaxP; MeanTWint	386.2	9	-184.1	368.2
m31	PopRisk; HDI; NP; Pasture; MaxP; MeanT	386.73	9	-184.36	368.73
m4	PopRisk; HDI; PLAND; NP; Cane; MaxP; MeanT	386.73	10	-183.37	366.73
m8	PopRisk; HDI; PLAND; NP; Cane; MaxP; MeanTWint	386.95	10	-183.47	366.95
r7	PopRisk; HDI; NL; Pasture; MaxP; MeanT	1360.84	10	-670.42	1340.84

Atlantic Forest

m7	PopRisk; HDI; PLAND; NP; Cane; TotalP; MeanT	982.55	9	-482.28	964.55
m3	PopRisk; HDI; NP; Cane; TotalP; MeanT	982.57	9	-482.28	964.57
m6	PopRisk; HDI; PLAND; Cane; MaxP; MeanT	983.95	9	-482.97	965.95
m4	PopRisk; HDI; PLAND; NP; Cane; MaxP; MeanT	984.04	10	-482.02	964.04
m2	PopRisk; HDI; PLAND; Cane; TotalP; MeanT	984.05	9	-483.02	966.05
M	PopRisk; HDI; NP; Cane; MaxP; MeanT	984.1	10	-482.05	964.1
r1	PopRisk; HDI; ON; Cane; MaxP; MeanT	984.51	9	-483.25	966.51
m1	PopRisk; HDI; ED; Cane; TotalP; MeanT	984.54	9	-483.27	966.54
m5	PopRisk; HDI; ED; Cane; MaxP; MeanT	984.8	9	-483.4	966.8

Again, in both regions the model containing the predictor variables population at risk, HDI, percent of habitat cover, number of fragments, amount of sugarcane, annual total precipitation and annual mean temperature (m7) was selected with the lower AIC. In that way these variables were included in the final Bayesian model. All set of candidate models analyzed in the generalized linear mixed models are shown in table S.3.

Table S3. All set of candidate models analyzed in the generalized linear mixed models.

Model Name	Predictor Variables
t1	Annual mean precipitation
t2	Annual total precipitation (A)
t3	Annual minimum precipitation
t4	Annual maximum precipitation (B)
t5	Annual mean precipitation of summer
t6	Annual minimum precipitation of summer
t7	Annual maximum precipitation of summer
t8	Annual mean precipitation of fall
t9	Annual minimum precipitation of fall
t10	Annual maximum precipitation of fall
t11	Annual mean precipitation of winter
t12	Annual minimum precipitation of winter
t13	Annual maximum precipitation of winter
t14	Annual mean precipitation of spring
t15	Annual minimum precipitation of spring
t16	Annual maximum precipitation of spring
t17	Annual mean temperature (C)
t18	Annual mean temperature of summer
t19	Annual mean temperature of fall
t20	Annual mean temperature of winter (D)
t21	Annual mean temperature of spring
M	Population at risk (E), HDI (F), Number of fragments, Sugarcane; (B) and (C)
m1	(E), (F), Edge density, sugarcane, (A), (C)
m2	(E), (F), Percent of habitat cover, sugarcane, (A), (C)
m3	(E), (F), Number of fragments, sugarcane, (A), (C)
m4	(E), (F), Percent of habitat cover, Number of fragments, sugarcane, (B), (C)
m5	(E), (F), Edge density, sugarcane, (B), (C)
m6	(E), (F), Percent of habitat cover, sugarcane, (B), (C)
m7	(E), (F), Percent of habitat cover, Number of fragments, sugarcane; (A), (C)
m8	(E), (F), Percent of habitat cover, Number of fragments, sugarcane; (B), (D)
m9	(E), (F), Edge density; sugarcane, (B), (D)
m10	(E), (F), Percent of habitat cover, sugarcane, (B), (D)
m11	(E), (F), Number of fragments, sugarcane, (B), (D)
m12	(E), (F), Percent of habitat cover, Number of fragments, Corn, (A), (C)

m13	(E), (F), Edge density, Corn, (A), (C)
m14	(E), (F), Percent of habitat cover, Corn, (A), (C)
m15	(E), (F), Number of fragments, Corn, (A), (C)
m16	(E), (F), Percent of habitat cover, Number of fragments, Corn, (B), (C)
m17	(E), (F), Edge density, Corn, (B), (C)
m18	(E), (F), Percent of habitat cover, Corn, (B), (C)
m19	(E), (F), Number of fragments, Corn, (B), (C)
m20	(E), (F), Percent of habitat cover, Number of fragments, Corn, (B), (D)
m21	(E), (F), Edge density, Corn, (B), (D)
m22	(E), (F), Percent of habitat cover, Corn, (B), (D)
m23	(E), (F), Number of fragments, Corn, (B), (D)
m24	(E), (F), Percent of habitat cover, Number of fragments, Pasture, (A), (C)
m25	(E), (F), Edge density, Pasture, (A), (C)
m26	(E), (F), Percent of habitat cover, Pasture, (A), (C)
m27	(E), (F), Number of fragments, Pasture, (A), (C)
m28	(E), (F), Percent of habitat cover, Number of fragments, Pasture, (B), (C)
m29	(E), (F), Edge density, Pasture, (B), (C)
m30	(E), (F), Percent of habitat cover, Pasture, (B), (C)
m31	(E), (F), Number of fragments, Pasture, (B), (C)
m32	(E), (F), Percent of habitat cover, Number of fragments, Pasture, (B), (D)
m33	(E), (F), Edge density, Pasture, (B), (D)
m34	(E), (F), Percent of habitat cover, Pasture, (B), (D)
m35	(E), (F), Number of fragments, Pasture, (B), (D)
Mg	(E), Gini, Number of fragments, sugarcane, (B), (C)
mg1	(E), Gini, Edge density, sugarcane, (B), (C)
mg2	(E), Gini, Percent of habitat cover, sugarcane, (B), (C)
mg3	(E), Gini, Number of fragments, sugarcane, (A), (C)
mg4	(E), Gini, Percent of habitat cover, Number of fragments, sugarcane, (B), (C)
mg5	(E), Gini, Edge density, sugarcane, (B), (C)
mg6	(E), Gini, Percent of habitat cover, sugarcane, (B), (C)
mg7	(E), Gini, Percent of habitat cover, Number of fragments, sugarcane, (A), (C)
mg8	(E), Gini, Percent of habitat cover, Number of fragments, sugarcane; (B), (D)
mg9	(E), Gini, Edge density, sugarcane, (B), (D)
mg10	(E), Gini, Percent of habitat cover, sugarcane, (B), (D)
mg11	(E), Gini, Number of fragments, sugarcane, (B), (D)
mg12	(E), Gini, Percent of habitat cover, Number of fragments, Corn, (A), (C)
mg13	(E), Gini, Edge density, Corn, (A), (C)
mg14	(E), Gini, Percent of habitat cover, Corn, (A), (C)
mg15	(E), Gini, Number of fragments, Corn, (A), (C)
mg16	(E), Gini, Percent of habitat cover, Number of fragments, Corn, (B), (C)
mg17	(E), Gini, Edge density, Corn, (B), (C)
mg18	(E), Gini, Percent of habitat cover, Corn, (B), (C)
mg19	(E), Gini, Number of fragments, Corn, (B), (C)
mg20	(E), Gini, Percent of habitat cover, Number of fragments, Corn, (B), (D)
mg21	(E), Gini, Edge density, Corn, (B), (D)
mg22	(E), Gini, Percent of habitat cover, Corn, (B), (D)
mg23	(E), Gini, Number of fragments, Corn, (B), (D)
mg24	(E), Gini, Percent of habitat cover, Number of fragments, Pasture, (A), (C)
mg25	(E), Gini, Edge density, Pasture, (A), (C)
mg26	(E), Gini, Percent of habitat cover, Pasture, (A), (C)

mg27	(E), Gini, Number of fragments, Pasture, (A), (C)
mg28	(E), Gini, Percent of habitat cover, Number of fragments, Pasture, (B), (C)
mg29	(E), Gini, Edge density, Pasture, (B), (C)
mg30	(E), Gini, Percent of habitat cover, Pasture, (B), (C)
mg31	(E), Gini, Number of fragments, Pasture, (B), (C)
mg32	(E), Gini, Percent of habitat cover, Number of fragments, Pasture, (B), (D)
mg33	(E), Gini, Edge density, Pasture, (B), (D)
mg34	(E), Gini, Percent of habitat cover, Pasture, (B), (D)
mg35	(E), Gini, Number of fragments, Pasture, (B), (D)
Mp	(E), Poverty, Number of fragments, sugarcane, (B), (C)
mp1	(E), Poverty, Edge density, sugarcane, (A), (C)
mp2	(E), Poverty, Percent of habitat cover, sugarcane, (A), (C)
mp3	(E), Poverty, Number of fragments, sugarcane, (A), (C)
mp4	(E), Poverty, Percent of habitat cover, Number of fragments, sugarcane, (B), (C)
mp5	(E), Poverty, Edge density, sugarcane, (B), (C)
mp6	(E), Poverty, Percent of habitat cover, sugarcane, (B), (C)
mp7	(E), Poverty, Percent of habitat cover, Number of fragments, sugarcane, (A), (C)
mp8	(E), Poverty, Percent of habitat cover, Number of fragments, sugarcane, (B), (D)
mp9	(E), Poverty, Edge density, sugarcane, (B), (D)
mp10	(E), Poverty, Percent of habitat cover, sugarcane, (B), (D)
mp11	(E), Poverty, Number of fragments, sugarcane, (B), (D)
mp12	(E), Poverty, Percent of habitat cover, Number of fragments, Corn, (A), (C)
mp13	(E), Poverty, Edge density, Corn, (A), (C)
mp14	(E), Poverty, Percent of habitat cover, Corn, (A), (C)
mp15	(E), Poverty, Number of fragments, Corn, (A), (C)
mp16	(E), Poverty, Percent of habitat cover, Number of fragments, Corn (B), (C)
mp17	(E), Poverty, Edge density, Corn, (B), (C)
mp18	(E), Poverty, Percent of habitat cover, Corn, (B), (C)
mp19	(E), Poverty, Number of fragments, Corn, (B), (C)
mp20	(E), Poverty, Percent of habitat cover, Number of fragments, Corn, (B), (D)
mp21	(E), Poverty, Edge density, Corn, (B), (D)
mp22	(E), Poverty, Percent of habitat cover, Corn, (B), (D)
mp23	(E), Poverty, Number of fragments, Corn, (B), (D)
mp24	(E), Poverty, Percent of habitat cover, Number of fragments, Pasture, (A), (C)
mp25	(E), Poverty, Edge density, Pasture, (A), (C)
mp26	(E), Poverty, Percent of habitat cover, Pasture, (A), (C)
mp27	(E), Poverty, Number of fragments, Pasture, (A), (C)
mp28	(E), Poverty, Percent of habitat cover, Number of fragments, Pasture, (B), (C)
mp29	(E), Poverty, Edge density, Pasture, (B), (C)
mp30	(E), Poverty, Percent of habitat cover, Pasture, (B), (C)
mp31	(E), Poverty, Number of fragments, Pasture, (B), (C)
mp32	(E), Poverty, Percent of habitat cover, Number of fragments, Pasture, (B), (D)
mp33	(E), Poverty, Edge density, Pasture, (B), (D)
mp34	(E), Poverty, Percent of habitat cover, Pasture, (B), (D)
mp35	(E), Poverty, Number of fragments, Pasture, (B), (D)
Mi	(E), Income, Number of fragments, sugarcane, (B), (C)
mi1	(E), Income, Edge density, sugarcane, (A), (C)
mi2	(E), Income, Percent of habitat cover, sugarcane, (A), (C)
mi3	(E), Income, Number of fragments, sugarcane, (A), (C)
mi4	(E), Income, Percent of habitat cover, Number of fragments, sugarcane, (B), (C)

mi5	(E), Income, Edge density, sugarcane, (B), (C)
mi6	(E), Income, Percent of habitat cover, sugarcane, (B), (C)
mi7	(E), Income, Percent of habitat cover, Number of fragments, sugarcane, (A), (C)
mi8	(E), Income, Percent of habitat cover, Number of fragments, sugarcane, (B), (D)
mi9	(E), Income, Edge density, sugarcane, (B), (D)
mi10	(E), Income, Percent of habitat cover, sugarcane, (B), (D)
mi11	(E), Income, Number of fragments, sugarcane, (B), (D)
mi12	(E), Income, Percent of habitat cover, Number of fragments, Corn, (A), (C)
mi13	(E), Income, Edge density, Corn, (A), (C)
mi14	(E), Income, Percent of habitat cover, Corn, (A), (C)
mi15	(E), Income, Number of fragments, Corn, (A), (C)
mi16	(E), Income, Percent of habitat cover, Number of fragments, Corn, (B), (C)
mi17	(E), Income, Edge density, Corn, (B), (C)
mi18	(E), Income, Percent of habitat cover, Corn, (B), (C)
mi19	(E), Income, Number of fragments, Corn, (B), (C)
mi20	(E), Income, Percent of habitat cover, Number of fragments, Corn, (B), (D)
mi21	(E), Income, Edge density, Corn, (B), (D)
mi22	(E), Income, Percent of habitat cover, Corn, (B), (D)
mi23	(E), Income, Number of fragments, Corn, (B), (D)
mi24	(E), Income, Percent of habitat cover, Number of fragments, Pasture, (A), (C)
mi25	(E), Income, Edge density, Pasture, (A), (C)
mi26	(E), Income, Percent of habitat cover, Pasture, (A), (C)
mi27	(E), Income, Number of fragments, Pasture, (A), (C)
mi28	(E), Income, Percent of habitat cover, Number of fragments, Pasture, (B), (C)
mi29	(E), Income, Edge density, Pasture, (B), (C)
mi30	(E), Income; Percent of habitat cover , Pasture, (B), (C)
mi31	(E), Income, Number of fragments, Pasture, (B), (C)
mi32	(E), Income; Percent of habitat cover, Number of fragments, Pasture, (B), (D)
mi33	(E), Income, Edge density, Pasture, (B), (D)
mi34	(E), Income, Percent of habitat cover, Pasture, (B), (D)
mi35	(E), Income, Number of fragments, Pasture, (B), (D)
Ml	(E), Life_expectancy, Number of fragments, sugarcane, (B), (C)
ml1	(E), Life_expectancy, Edge density, sugarcane, (A), (C)
ml2	(E), Life_expectancy, Percent of habitat cover, sugarcane, (A), (C)
ml3	(E), Life_expectancy, Number of fragments, sugarcane, (A), (C)
ml4	(E), Life_expectancy, Percent of habitat cover, Number of fragments, sugarcane, (B), (C)
ml5	(E), Life_expectancy, Edge density, sugarcane, (B), (C)
ml6	(E), Life_expectancy, Percent of habitat cover, sugarcane, (A), (C)
ml7	(E), Life_expectancy, Percent of habitat cover, Number of fragments, sugarcane, (A), (C)
ml8	(E), Life_expectancy, Percent of habitat cover, Number of fragments, sugarcane, (B), (D)
ml9	(E), Life_expectancy, Edge density, sugarcane, (B), (D)
ml10	(E), Life_expectancy, Percent of habitat cover, sugarcane, (B), (D)
ml11	(E), Life_expectancy, Number of fragments, sugarcane, (B), (D)
ml12	(E), Life_expectancy, Percent of habitat cover, Number of fragments, Corn, (A), (C)
ml13	(E), Life_expectancy, Edge density, Corn, (A), (C)
ml14	(E), Life_expectancy, Percent of habitat cover, Corn, (A), (C)
ml15	(E), Life_expectancy, Number of fragments, Corn, (A), (C)
ml16	(E), Life_expectancy, Percent of habitat cover, Number of fragments, Corn, (B), (C)
ml17	(E), Life_expectancy, Edge density, Corn, (B), (C)
ml18	(E), Life_expectancy, Percent of habitat cover, Corn, (B), (C)

ml19	(E), Life_expectancy, Number of fragments, Corn, (B), (C)
ml20	(E), Life_expectancy, Percent of habitat cover, Number of fragments, Corn, (B), (D)
ml21	(E), Life_expectancy, Edge density, Corn, (B), (D)
ml22	(E), Life_expectancy, Percent of habitat cover, Corn; (B), (D)
ml23	(E), Life_expectancy, Number of fragments, Corn, (B), (D)
ml24	(E), Life_expectancy, Percent of habitat cover, Number of fragments, Pasture, (A), (C)
ml25	(E), Life_expectancy, Edge density, Pasture, (A), (C)
ml26	(E), Life_expectancy, Percent of habitat cover, Pasture, (A), (C)
ml27	(E), Life_expectancy, Number of fragments, Pasture, (A), (C)
ml28	(E), Life_expectancy, Percent of habitat cover, Number of fragments, Pasture, (B), (C)
ml29	(E), Life_expectancy, Edge density, Pasture, (B), (C)
ml30	(E), Life_expectancy, Percent of habitat cover, Pasture, (B), (C)
ml31	(E), Life_expectancy, Number of fragments, Pasture, (B), (C)
ml32	(E), Life_expectancy, Percent of habitat cover, Number of fragments, Pasture; (B), (D)
ml33	(E), Life_expectancy, Edge density, Pasture, (B), (D)
ml34	(E), Life_expectancy, Percent of habitat cover, Pasture; (B), (D)
ml35	(E), Life_expectancy, Number of fragments, Pasture; (B), (D)
Me	(E), education, Number of fragments, sugarcane (B), (C)
me1	(E), education, Edge density, sugarcane, (A), (C)
me2	(E), education, Percent of habitat cover, sugarcane, (A), (C)
me3	(E), education, Number of fragments, sugarcane, (A), (C)
me4	(E), education, Percent of habitat cover, Number of fragments, sugarcane, (B), (C)
me5	(E), education, Edge density, sugarcane, (B), (C)
me6	(E), education, Percent of habitat cover, sugarcane, (B), (C)
me7	(E), education, Percent of habitat cover, Number of fragments, sugarcane, (A), (C)
me8	(E), education, Percent of habitat cover, Number of fragments, sugarcane, (B), (D)
me9	(E), education, Edge density, sugarcane, (B), (D)
me10	(E), education, Percent of habitat cover, sugarcane, (B), (D)
me11	(E), education, Number of fragments, sugarcane, (B), (D)
me12	(E), education, Percent of habitat cover, Number of fragments, Corn, (A), (C)
me13	(E), education, Edge density, Corn, (A), (C)
me14	(E), education, Percent of habitat cover, Corn, (A), (C)
me15	(E), education, Number of fragments, Corn, (A), (C)
me16	(E), education, Percent of habitat cover, Number of fragments, Corn, (B), (C)
me17	(E), education, Edge density, Corn, (B), (C)
me18	(E), education, Percent of habitat cover, Corn, (B), (C)
me19	(E), education, Number of fragments, Corn, (B), (C)
me20	(E), education, Percent of habitat cover, Number of fragments, Corn, (B), (D)
me21	(E), education, Edge density, Corn, (B), (D)
me22	(E), education, Percent of habitat cover, Corn, (B), (D)
me23	(E), education, Number of fragments, Corn, (B), (D)
me24	(E), education, Percent of habitat cover, Number of fragments, Pasture, (A), (C)
me25	(E), education, Edge density, Pasture, (A), (C)
me26	(E), education, Percent of habitat cover, Pasture, (A), (C)
me27	(E), education, Number of fragments, Pasture, (A), (C)
me28	(E), education, Percent of habitat cover, Number of fragments, Pasture, (B), (C)
me29	(E), education, Edge density, Pasture, (B), (C)
me30	(E), education, Percent of habitat cover, Pasture; (B), (C)
me31	(E), education, Number of fragments, Pasture, (B), (C)
me32	(E), education, Percent of habitat cover, Number of fragments, Pasture, (B), (D)

me33	(E), education, Edge density, Pasture, (B), (D)
me34	(E), education, Percent of habitat cover, Pasture, (B), (D)
me35	(E), education, Number of fragments, Pasture, (B), (D)
R	(E), (F)/Gini/poverty/income/life-expectancy/education, NL/ON, sugarcane, (A), (C)
r1	(E), (F) Gini/poverty/income/life-expectancy/education, NL/ON, sugarcane; (B), (C)
r2	(E), (F) Gini/poverty/income/life-expectancy/education, NL/ON, sugarcane, (B), (D)
r3	(E),(F) Gini/poverty/income/life-expectancy/education, NL/ON, Corn, (A), (C)
r4	(E), (F) Gini/poverty/income/life-expectancy/education, NL/ON, Corn, (B), (C)
r5	(E), (F) Gini/poverty/income/life-expectancy/education, NL/ON, Corn, (B), (D)
r6	(E), (F) Gini/poverty/income/life-expectancy/education, NL/ON, Pasture, (A), (C)
r7	(E), (F) Gini/poverty/income/life-expectancy/education, NL/ON, Pasture, (B), (C)
r8	(E), (F) Gini/poverty/income/life-expectancy/education, NL/ON, Pasture, (B), (D)

Table S4. Predictor variables included in the model

Predictor variable	*Description*	*Years available*	*Source data*
People at risk	Number of men older than 14 years employed/ living in agricultural areas	1996/2006	IBGE
HDI	Human Development Index	1991/2000/2010	IBGE
Forest	Percentage of native vegetation cover in municipalities	2000/2010	São Paulo state Forest Inventory
NP	Number of native vegetation patches in municipalities	2000/2010	São Paulo state Forest Inventory
Sugarcane	Percent of municipality occupied by sugarcane	1993 to 2012	Agricultural Census of Institute of Agricultural Economics
Total Precipitation	Total annual precipitation	1993 to 2012	Climate Hazards Group Infrared Precipitation with Stations
Mean Temperature	Mean annual temperature per year	1993 to 2012	National Centers for

Table S5. Moran´s I test applied to the residuals of the Bernoulli models for cerrado (Table S5A) and Atlantic forest region (Table S5B) of São Paulo State. Moran´s I Test was performed for each year we had data (1993 - 2012), and used the spatial contiguity matrix based on the Queen´s case neighborhood relation. *: significant result.

(A) cerrado region

Year	Moran I statistic	p-value
1993	0.0891	0.0443*
1994	-0.015692173	0.5923
1995	-0.014679862	0.5743
1996	-0.018715981	0.6113
1997	-0.013574461	0.5471
1998	-0.024832829	0.6523
1999	-0.005903901	0.256
2000	-0.0519536464	0.729
2001	-0.037687182	0.717
2002	0.008955163	0.3917
2003	0.015124948	0.3521
2004	-0.096034078	0.913
2005	-0.076794304	0.8752
2006	0.055305607	0.1102
2007	-0.019493992	0.5687
2008	0.007485780	0.4118
2009	-0.046907146	0.7303
2010	-0.051158416	0.7268
2011	-0.023866604	0.5942
2012	-0.029805356	0.6324

(B) Atlantic forest Region

Year	Moran I statistic	p-value
1993	0.0077140407	0.263
1994	-0.0315037475	0.8504
1995	0.0173919765	0.2463
1996	-0.0085926427	0.6139
1997	-0.0298241028	0.8349
1998	-0.0050288202	0.5456
1999	-0.0209857310	0.7429
2000	-0.0073396152	0.5848
2001	-0.0017712202	0.4954
2002	-0.0151332196	0.6727
2003	-0.0253334696	0.7834
2004	-0.0064223055	0.5589
2005	-0.0071336002	0.5698
2006	-0.0099091010	0.6067
2007	-0.0216753551	0.7446
2008	-0.0074002665	0.5707
2009	0.0529313912	0.03095*
2010	-0.023258202	0.7621
2011	-0.0251251082	0.7802
2012	-0.0334661104	0.8626

Table S6. Moran´s I test applied to the number of HCPS cases for cerrado (Table S6A) and Atlantic forest region (Table S6B) of São Paulo State. Moran´s I Test was performed for each year we had data (1993 - 2012) and used the spatial contiguity matrix based on the Queen´s case neighborhood relation. *: significant result. Empty spaces means years with no diseases cases.

(A) cerrado region

Year	Moran I statistic	p-value
1993		
1994		
1995		

1996		
1997		
1998	-0.02002	0.7803
1999	-0.02002	0.7803
2000	-0.00967	0.645
2001	-0.00487	0.477
2002	-0.00439	0.46
2003	-0.00652	0.491
2004	-0.05601	0.7698
2005	-0.02008	0.5998
2006	0.000093	0.089
2007	-0.00611	0.4904
2008	-0.01426	0.5463
2009	-0.00652	0.491
2010	-0.01642	0.550
2011	-0.00696	0.4953
2012	-0.00480	0.4814

(B)Atlantic Forest Region

Year	Moran I statistic	p-value
1993	-00004.70	0.065
1994		
1995		
1996	-0.00653	0.58
1997		
1998	-0.00449	0.537
1999	-0.01885	0.7286
2000	-0.0099	0.9231
2001	-0.0139	0.6611
2002	-0.0126	0.6531
2003	-0.0164	0.7293
2004	-0.0127	0.6477

2005	-0.01299	0.6578
2006	-0.01147	0.6368
2007	-0.01096	0.634
2008	0.01208	0.3167
2009	0.0429	0.0626
2010	-0.0302	0.8484
2011	-0.0302	0.8421
2012	-0.00871	0.6071

Table S7. Average and range values of annual mean temperature and total precipitation for the municipalities of cerrado and Atlantic Forest regions, from 1993 to 2012.

	Cerrado		Atlantic Forest	
Year	Mean Temperature (°C)	Total Precipitation (mm)	Mean Temperature (°C)	Total Precipitation (mm)
1993	22.48 (18.8-24.22)	1340 (1089-2062)	22.49 (18.7-24.4)	1358 (1000-2338)
1994	22.73 (18.7-24.9)	1194 (948-1917)	22.77 (18.5-24.9)	1227 (922-2390)
1995	23.31 (18.6-25.5)	1411 (1170-2461)	23.25 (18.3- 25.6)	1443 (1115-2895)
1996	23.16 (19.1-25.2)	1389 (1053-2368)	23.17 (19-25.3)	1426 (1022-2728)
1997	22.86 (18.7-24.7)	1356 (1161-1891)	22.80 (18.4-24.7)	1396 (1103-2275)
1998	22.97 (19.1- 24.6)	1352 (1178-2171)	22.91 (18.8-24.6)	1400 (1150-2728)
1999	23.15 (19 - 25.1)	1187 (831-2087)	23.07 (18.9-25.1)	1186 (804-2325)
2000	22.71 (18.3 - 25)	1339 (1057-2244)	22.63 (18-25.2)	1356 (1053-2522)
2001	22.90 (19 - 25)	1414 (1148-2131)	22.76 (18.6-25.1)	1438 (1147-2426)
2002	23.01 (19.4- 25.1)	1270 (927-1891)	23.01 (19.3-25.2)	1286 (883-2349)
2003	23.87 (19.9-26.5)	1261 (915-1799)	23.86 (19.7-26.7)	1275 (892-2031)
2004	22.83 (19 - 25)	1204 (927-1983)	22.83 (18.7-25.2)	1235 (909-2310)
2005	22.44 (18.3-25.7)	1279 (1032-2169)	22.48 (18.2-26-2)	1333 (1018-2559)
2006	22.86 (18.5-26)	1312 (1101-1910)	22.90 (18.4-26.4)	1332 (1083-2309)

2007	23.05 (18.8-26)	1273 (1051-2058)	23.14 (18.8-27.6)	1315 (1038-2534)
2008	23.11 (18.8-26.7)	1219 (970-2490)	23.17 (18.7-27.6)	1302 (957-3079)
2009	22.91 (18.7-26)	1652 (1383-3218)	22.87 (18.5-26)	1729 (1322-3936)
2010	22.74 (19 - 24.9)	1188 (895-2637)	22.68 (18.8- 24.9)	1282 (846-3275)
2011	22.95 (19-24.4)	1384 (1139-2227)	22.81 (19-24.5)	1414 (1120-2703)
2012	22.67 (18.77-24.48)	1452 (1066-2631)	22.57 (18.7-24.4)	1483 (1068-3093)

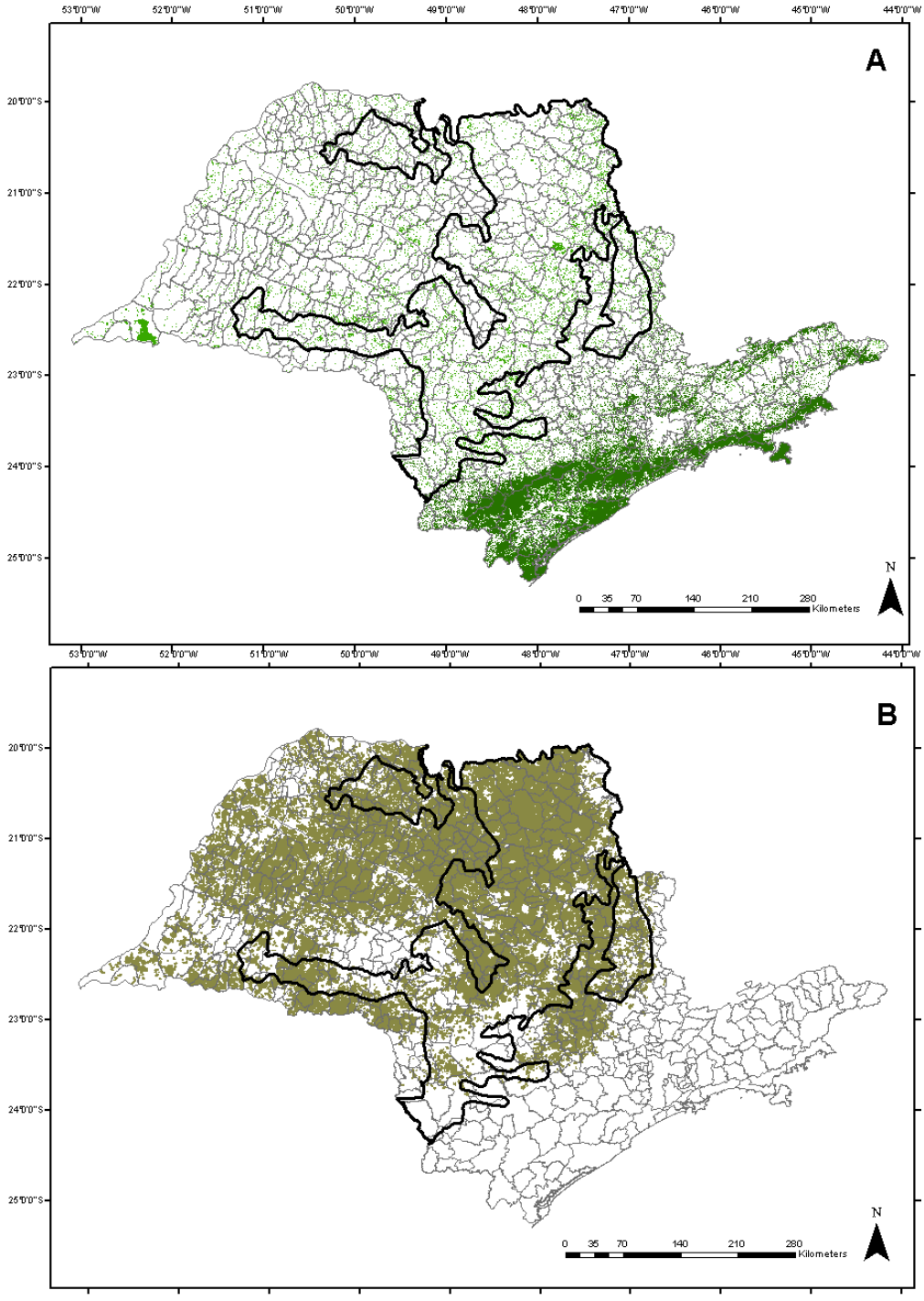

Figure S1. Amount of native vegetation (A) present in the state of São Paulo according to the Forestry Institute map of 2010, and (B) sugar cane plantation in 2011 according to the CaneSat/INPE 2011 (available at http://www.dsr.inpe.br/laf/Canesat/). Black line delineates the cerrado region.

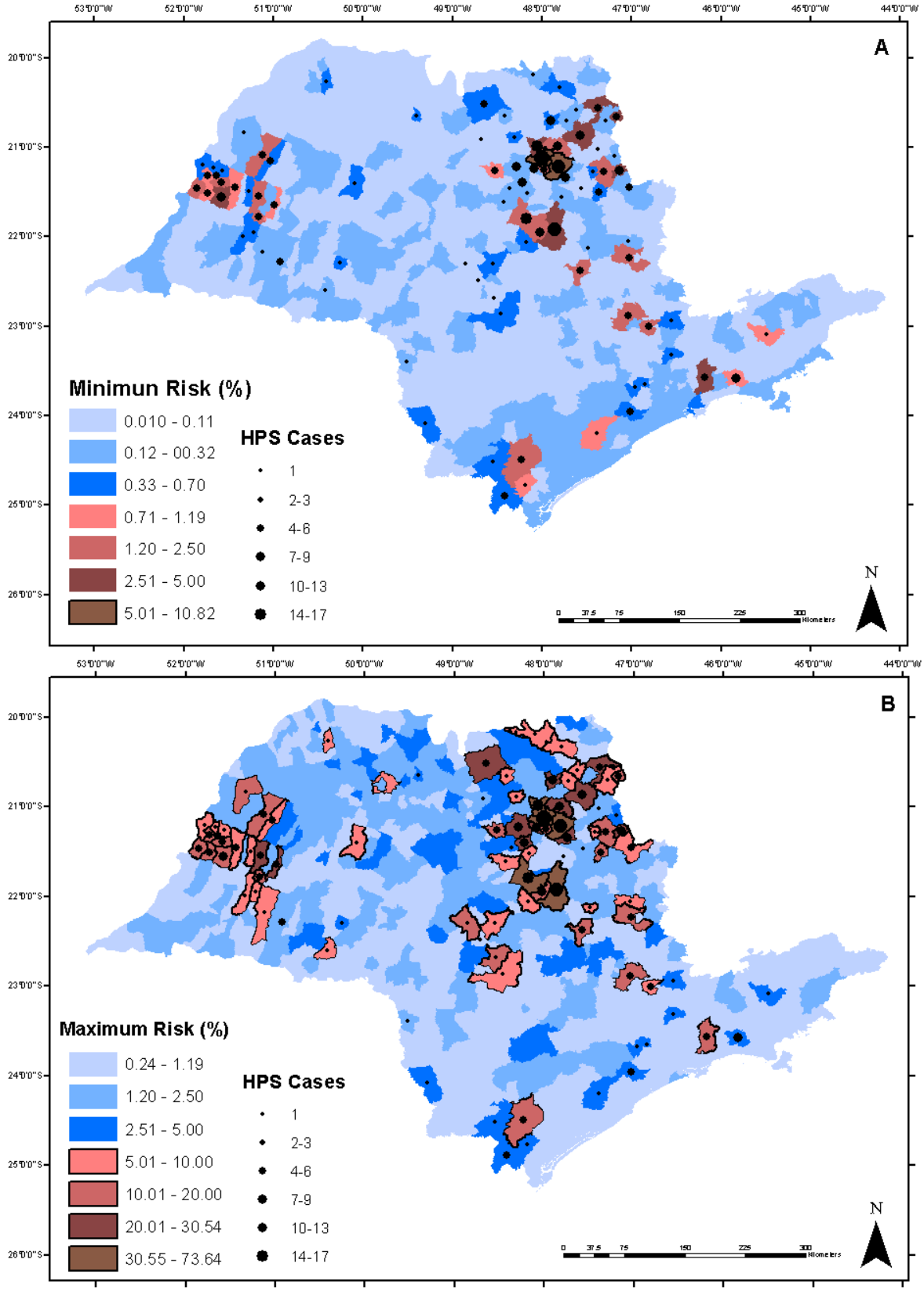

Figure S2. Spatial representation of the minimum (A) and maximum (B) probability of Hantavirus infection risk for São Paulo State and the number of HCPS reported cases from 1993 to 2012. The municipalities with no symbol means municipalities with no reported cases

of HCPS. Black outlines indicate municipalities with medium to high risk (> 5%) of Hantavirus infection and where preventive effort should be allocated.

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Chapter 04

IMPACTS OF CLIMATE CHANGE AND SUGARCANE EXPANSION ON HANTAVIRUS INFECTION RISK IN THE STATE OF SÃO PAULO, BRAZIL

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Abstract

Hantavirus Cardiopulmonary Syndrome (HCPS) is a disease caused by Hantavirus, a collection of negative sense RNA viruses in the *Bunyaviridae* family that are highly virulent for humans. We know that the conversion of native vegetation to agriculture, particularly sugarcane, and temperature can influence rodent generalist species that serve as the principal reservoir host for HCPS, but our understanding of the compound effects of land use and climate change on HCPS incidence remains limited, particularly in tropical regions. Here we rely on a Bayesian model to fill this research gap and to predict the effects of sugarcane expansion and expected changes in temperature on Hantavirus infection risk in the state of São Paulo. Sugarcane expansion scenario was based on its past expansion (2000-2010), combined with a recent agro-environment zoning for the sugar and alcohol industry. Temperature anomalies were evaluated using 32 General Circulation Models from the IPCC 5th Coupled Model Intercomparison Project scenarios RCP4.5 and RCP8.5. Currently, average Hantavirus risk for the state is 1.3%, with 6% of the municipalities of the state being classified as high risk (HCPS risk ≥ 5%). Our results indicate that sugarcane expansion alone will increase average HCPS risk to 1.5%, and temperature anomalies alone will increase even more this risk (1.6% for RCP4.5 and 1.7%, for RCP8.5). Climate scenarios alone are responsible for the largest increase in the maximum risk of infection that goes from 46.1% to 51.4% under RCP4.5 and to 51.8% under RCP8.5. Sugarcane expansion and increasing temperatures combined are expected to increase the number of municipalities at risk to 7.0% for both experiments. In all scenarios, the largest increases in risk occurred in municipalities that already had a large risk of infection. Management actions and educational campaigns should thus be targeted to the municipalities with highest HCPS infection risk. Forecasting disease is critical for timely and efficient planning of operational control programs.

Key-words: Hantavirus, HCPS, environmental, climate change, sugarcane, risk map, RCP4.5, RCP8.5

Introduction

Global average temperatures are projected to increase between 1.7 and 4.8 ºC by the end of this century (Collins et al. 2013; IPCC 2013), with potential effects on human health, including mortality from extreme heat and cold, and changes in the ecology of infectious diseases (Kovats et al. 2001; Stott et al. 2004). Climatic variability and extreme weather events have profound impacts on infectious disease since fluctuations in temperature and precipitation influence both infectious agents (such as protozoa, bacteria and viruses) and population dynamics of their vectors (such as mosquitoes, ticks and rodents) (Kovats et al. 2001; Gubler et al. 2001; Calisher et al. 2005; Magnussom et al. 2010). Outbreaks of some

diseases such as Ross River virus disease (Woodruff et al. 2002), malaria (Bouma et al. 1996), meningits (García-Pando et al. 2014) and Hantavirus Cardiopulmonary Syndrome (HCPS) (Yates et al. 2002) have been associated with anomalies in climate events.

At the same time, increasing evidence suggests that land cover and land use change affect disease incidence by altering the interactions, abundance, and movement patterns of hosts, vectors, and people (McCallum 2008; Lambin et al. 2010). For instance, outbreaks of Hantavirus, Lyme disease and tick-borne encephalitis have been associated not only with climate-related changes in the density of host rodent and tick populations (Lindgren et al. 1988, 2000; Klempa 2009; Dearing and Dizney 2010), but also with shifts in the extent and type of land use (Brownstein et al. 2005; de Souza et al. 2008; Suzan et al. 2008a, 2008b; Dearing and Dizney 2010; Blasdell et al 2011; Lehmer et al. 2012).

Hantavirus (*Bunyaviridae*) is a virus transmitted by small mammals (Dearing and Dizney 2010) which causes two syndromes in humans: Hantavirus Cardiopulmonary Syndrome (HCPS), restricted to the Americas, and hemorrhagic fever with renal syndrome (HFRS) present in Eurasia and Africa (Terajima et al. 2004; Jonsson et al. 2010). HCPS was first identified in 1993 in both the United States and Brazil (Brazilian Ministry of Health 2013; CDC 2014). HCPS exhibits high lethality rates (Macneil et al. 2011; Brazilian Ministry of Health 2013). Unlike HFRS, a vaccine is not available for HCPS. Transmission to humans occurs through inhalation of the aerosolized form present in the urine, saliva and feces of infected rodents (Lee et al. 1981; Murany et al. 2005; Vapalahti et al. 2010).

Climate conditions can influence Hantavirus host population abundance and disease transmission dynamics (Prist et al. 2016). A number of studies in the arid and semi-arid region of U.S. have uncovered a positive association between precipitation, population size of rodent hosts and prevalence of Hantavirus (Meserve et al. 1995; Abbott et al. 1999; Mills et al. 1999; Luis et al. 2010). Anomalously high precipitation increases vegetation growth,

boosting rodent densities and enhancing the probability of encounters between humans and infected rodents and consequently Hantavirus transmission (Engelthaler et al. 1999; Yates et al. 2002). Temperature can influence rodent abundance and disease risk by altering vegetation growth (Jiang et al. 2011), reproduction and survival rates of small rodents (Calisher et al. 2005; Zeier et al. 2005; Jiang et al. 2011), and the time the virus remains infectious in the environment (Kallio et al. 2006). The capacity of Hantavirus to survive outside its host plays a critical role in transmission dynamics (Kallio et al. 2006). High temperatures have been associated with more frequent Hantavirus outbreaks (Engelthaler et al. 1999; Tersago et al. 2008; Clement et al. 2009; Klempa 2009), probably because high temperature leads to greater aerosolization of the virus and higher rates of inhalation by both humans and rodents (Lee et al. 1981; Doyle et al. 1998; Langlois et al. 2001; Vapalahti et al. 2010; Heyman et al. 2012). Nowadays there are evidences that only variations in temperature affect HCPS risk in Brazil, with precipitation showing no effect (Prist et al. 2016),

Sugarcane plantations may be also associated with increases in Hantavirus infection risk (Ruedas et al. 2004; de Souza et al. 2008; Armien et al. 2009; Prist et al. 2016). Experimental studies have shown that small mammal populations are frequently food-limited (Prevedello et al. 2013), thus the presence of an abundant, highly energetic food resource, such as sugarcane, which yields can be as high as 120 tons·year·ha−1 (Norman et al. 1995), might allow the increase and maintenance of large populations of these species, relative to other land uses, either natural or agricultural (de Souza et al. 2008; Gheler-Costa et al. 2012, Verdade et al. 2012). Furthermore, sugarcane still offers protective cover for feeding, burrowing and breeding activities, throughout the year (Parshad et al. 1986).

Many developing countries are expanding sugarcane plantation areas to produce biofuel, as a strategy to reduce their dependence on petroleum, to increase opportunities for the agricultural sector, and to mitigate global warming (Dufey 2006). In Brazil, the creation of

the pro-alcohol program, whose purpose was to replace a significant percentage of fossil-fuel consumption with ethanol produced from sugarcane (Moreira and Goldemberg 1999), was triggered by an increase of 428% in oil prices in 1973 (Barros 2007). This program and the recent interest in alternative energy sources has fostered an expansion in the extent of sugarcane cultivation, making the country the world's leader in ethanol production (Martines-Filho et al. 2006) and sugarcane (~ 490 million tons per year) exportation (UNICA 2014). Large part of this production (~74%) comes from the southeastern region, with the state of São Paulo producing 60% of the total (dos Santos and Sentelhas 2014; Ferreira-Filho and Horridge 2014).

In Brazil, the combined consequences of bio-energy expansion and climate change on Hantavirus infection risk remain unexamined. Understanding how these factors impact infectious disease risk is essential to fully evaluate the actual costs of the biofuel programs and is critical for timely and efficient planning of operational control programs. In this paper we analyze how sugarcane expansion and temperature changes under two climate scenarios will influence HCPS risk in the state of São Paulo until 2050. We tested the independent and combined effect of these two factors on HCPS risk. We hypothesize that HCPS incidence will show an increase under all scenarios because both changes in climate and sugarcane are supposed to increase HCPS risk through their positive effects on rodent abundance and virus survival and aerosolization on the environment, and that their combined effect can potentiate their individual effects.

Methods

Study Area

We focused our analyses in the state of São Paulo, the richest state of Brazil, where HCPS was first identified in 1993 and where the risk of disease increase is particularly high, due to both sugarcane expansion and climate change. São Paulo state is located in southeastern

Brazil, in an area of approximately 248.210 km^2 (Figure 1), and has a population of about 42 million, representing 21% of the Brazilian population (IBGE 2014).

Baseline model

Disease and social data

HCPS incidences were collected at the municipality level so we considered the 645 municipalities that compose the state as our sampling units (Figure 1). The number of reported HCPS cases in each municipality per year for the period between 1993 and 2012 is available from the Center for Epidemiological Surveillance of the State of São Paulo - CVE-SP (1993-2012), and the Health Portal SUS: http://portal.saude.gov.br/portal/saude/profissional/area.cfm?id_area=1558. Since the majority of cases were zeroes (98.71%) or ones (1.06%), we transformed the data into a binary variable, presence/absence of HCPS per year and per municipality.

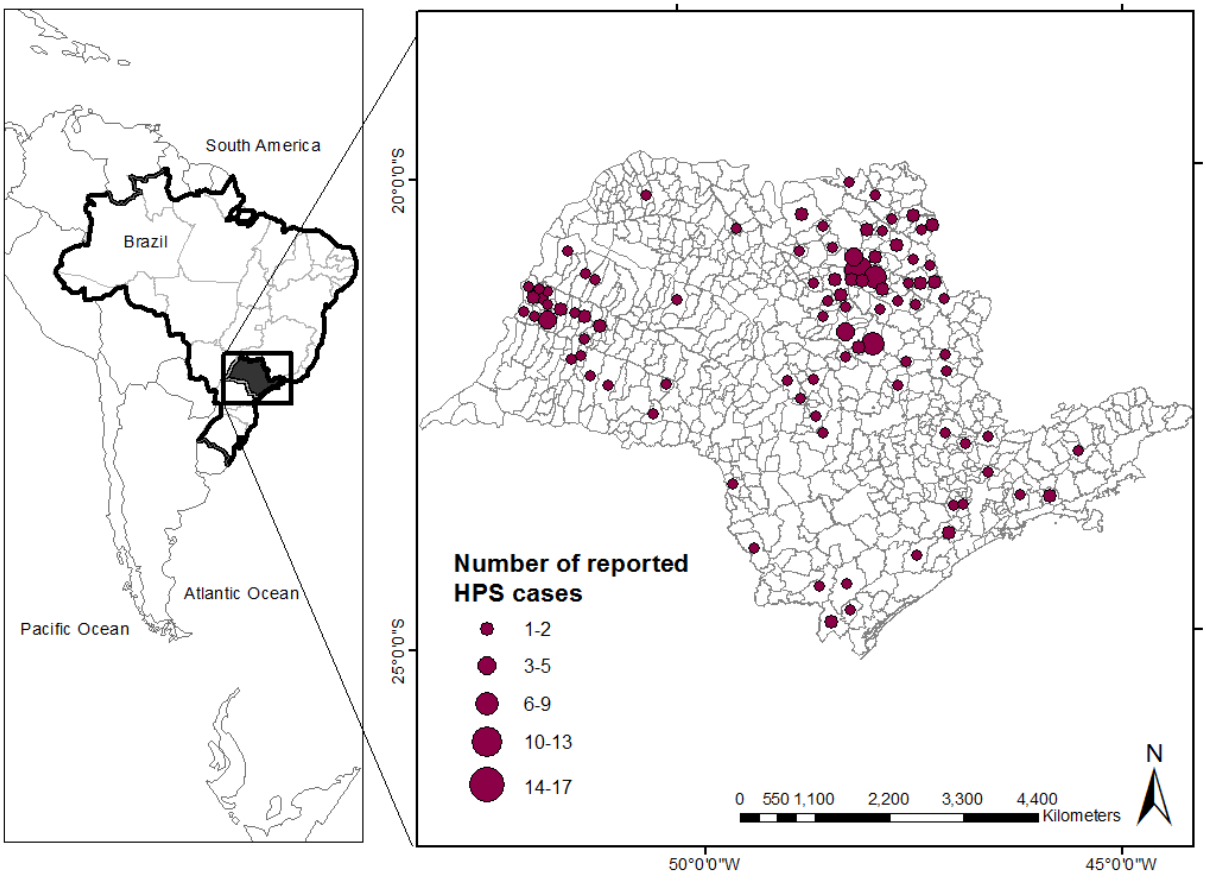

Figure 1. Number of HCPS reported cases between 1993 and 2012 in the state of São Paulo.

Epidemiologic data indicate that more than 70% of infected people work or live in agricultural areas, and around 93% are men over the age of twenty (Pincelli et al. 2003; Elkhoury et al. 2012). Because the available data are relatively coarse with respect to age distribution, we used the number of rural men older than 14 years in each municipality as the population at risk for HCPS. This information was extracted from the Brazilian National Institute of Geography and Statistics - IBGE website (www.ibge.gov.br) and is available for 1996 and 2006. For the analyses presented here, we used population data from 1996 to predict disease incidence from 1993 to 2001, and data from 2006 to predict disease from 2002 to 2012.

A number of socio-economic variables can contribute for a better understanding of HCPS transmission. The Human Development Index (HDI) is a summary measure of key dimensions of human development and it can be a good metric of the socio-economic factors that influence HCPS risk (Prist et al. 2016). Therefore, HDI was also included as a covariate in the model, and was extracted at municipality level from IBGE (accessible at www.ibge.gov.br), being available for 1991, 2000 and 2010. We used HDI data from 1991 to predict disease incidence from 1993 to 1998, data from 2000 to predict disease incidence from 1999 to 2005 and from 2010 to predict disease from 2006 to 2012.

Landscape composition and configuration metrics

We used the São Paulo State forest inventory maps (i.e. an habitat cover classification map - www.iflorestal.sp.gov.br) for 2000 and 2010 to calculate two landscape metrics (percentage of cover and density of fragments) for the native vegetation cover of each municipality. Native vegetation cover data encloses the two biomes of the state, the Atlantic forest (Joly et al. 2014) and the cerrado (Klink and Machado 2005). We performed the landscape analyses in ArcGis 10.0 and Fragstats 4.2. To match this information with available

disease data, we used metrics extracted from the 2000 native vegetation cover map as covariates to model incidence for 1993-2001, and metrics extracted from the 2010 map as covariates for period 2002-2012.

Since the1990s, rates of native vegetation loss have dropped dramatically, and an increase in the rates of natural regeneration has resulted in a small increase in native vegetation cover in the state of São Paulo (Kronka et al. 2005; Metzger et al. 2009; Lira et al. 2012). Since native habitat loss appears to have leveled off, we used the 2010 landscape metrics in evaluating future HCPS risk scenarios.

The proportion of sugarcane cultivated in each municipality was obtained from the Agricultural Census of the Institute of Agricultural Economics (www.iea.sp.gov.br) for the years 1993 to 2012. For the analyses presented here, we used annual agricultural data (1993 to 2012) to predict annual disease incidence from 1993 to 2012.

Current climatic variables

Current temperature and precipitation data, used in the baseline model, were obtained through the International Research Institute for Climate and Society (IRI) Data Library system (http://iridl.ldeo.columbia.edu/index.html). For more details see Prist et al. (2016).

Future scenarios

Sugarcane expansion

Our sugarcane expansion scenario combined data from past expansion of sugarcane with an Agro-environment Zoning for the sugar and Alcohol industry (AZA, http://www.ambiente.sp.gov.br). This zoning was created to minimize social and environment impacts of sugarcane (São Paulo 2007) and to regulate the expansion of this crop within the São Paulo territory (São Paulo 2008). We considered that sugarcane expansion in each municipality will (i) occur in the same proportion to what happened in the last ten years (2000-2010; i.e. we are considering a *business as usual scenario*), (ii) that this expansion will

occur only over pasture, once sugarcane expansion already occurs in areas occupied by pastures (Nassar et al. 2008; Sparovek et al. 2009; Ferreira-Filho and Horridge 2014; UNICA 2014), and (iii) the expansion will occur only in three zones considered as suitable for expansion according AZA determination (Figure S1).

Percentages of suitable and unsuitable areas for sugarcane for each municipality were extracted from AZA map using ArcGis 10.0 and Fragstats 4.2. Percentages of pasture and sugarcane, for each municipality, for the year of 2012 (the last year of our baseline model), were extracted from the Agricultural Census of Institute of Agricultural Economics. To calculate the proportion of sugarcane expansion of the last ten years we extracted the percentage of sugarcane for each municipality from the Agricultural Census of Institute of Agricultural Economics, for the years 2000 and 2010. The difference between these two percentages was considered as the 10-year sugarcane expansion, and was used as the maximum value (in percentage) of expansion for each municipality. This expansion was projected respecting the amount of suitable areas according AZA determination and the amount of pasture, and so not always reached the maximum value.

Climate Change Scenarios

The United Nations Intergovernmental Panel on Climate Change (IPCC) defines future climate change scenarios in terms of Representative Concentration Pathways (RCPs). The RCPs consider mitigation scenarios that assume policy actions will be taken to achieve certain greenhouse gases (GHG) emission targets (Taylor et al. 2012) consistent with a wide range of possible changes in future anthropogenic GHG emissions (Collins et al. 2013). Radiative forcing and mitigation policies are different for each RCP scenario. In the worst scenario, RCP8.5, radiative forcing increases throughout the twenty-first century before reaching a level of about 8.5 W m^{-2} at the end of the century. In the two intermediate scenarios, RCP4.5 and RCP6.0, radiative forcing stabilizes without overshoot at a level of 4.5

Wm-2 after 2100, while in the peak-and-decay scenario, RCP2.6, radiative forcing reaches a maximum near the middle of the twenty-first century before decreasing to an eventual nominal level of 2.6 W m^{-2} (Moss et al. 2010; Taylor et al. 2012). Global average temperatures show a similar increase of approximately 0.3-0.7°C for the period of 2016-2035 in all four scenarios. After this period, the magnitude of the projected climate change is substantially affected by the choice of scenario, with the highest global temperature increase estimated under RCP8.5 (Knutti and Sedlacek 2013).

We choose to evaluate the impacts of the high emission scenario (RCP8.5) and the intermediate pathway (RCP4.5) through 2050. The RCP4.5 was chosen instead of RCP6.0 because the predicted emission of RCP8.5 by the end of the century is close to the emission level of the RCP6.0 (Moss et al. 2010). Furthermore these two scenarios are the most commonly used in climate change analysis.

A previous study showed that only temperature (and not precipitation) had a significant effect on Hantavirus infection risk in São Paulo (Prist et al. 2016). Thus, we calculated land surface temperature (ts) averaged over the São Paulo state for the 2 climate change scenarios (RCP4.5 and RCP8.5) and for historical experiments. Historical experiments are necessary to calculate temperature anomalies relative to each model of climate change. Therefore, thirty-two General Circulation Models (GCMs), common to all 3 experiments (historical, RCP4.5 and RCP8.5), were used, and obtained from the Columbia University Lamont-Doherty Ocean and Climate Physics Data Library (http://strega.ldeo.columbia.edu:81/), for the state of São Paulo. Although climate-change projections are increasing in resolution, they are still not appropriate for analyzing disease patterns at scales smaller than a 250 km^2 grid (Patz et al. 2005). Therefore, we used the entire state of São Paulo as a basis for climate change scenarios data.

Temperature anomalies for RCP4.5 and RCP8.5 scenarios were calculated for each model individually as the difference between the future temperature predicted and the mean temperature during the 1976-2005 period of the historical experiment:

Temperature Anomalie = (Predicted Temperature RCP4.5/RCP8.5 for year X) - average temperature of last 30 years of historical experiment

We then calculated the average anomalies of all 32 models, for each scenario (RCP4.5 and RCP8.5), and this value was included in our predictions. Therefore we ended with one average of temperature anomalies of 32 models for RCP4.5 scenarios, from 2013 to 2050, and one average of temperature anomalies of 32 models for RCP8.5 scenario from 2013 to 2050, which we used to predict Hantavirus infection risk from 2013 to 2050. To diminish uncertainties that climate change analysis can provide, we used results of HCPS risk averaged for the 2040-2050 period as the final result to 2050 year.

To check the spread of the 32 GCMs anomalies used and the range of possible outcomes in HCPS risk that could come with the use of 32 models, we also predicted Hantavirus infection risk with the average ± one standard deviation of all GCMs temperature anomalies (see results and figures in Supplementary material- Figure S2).

Data analysis

The probability of Hantavirus infection risk in the state of São Paulo was calculated as a function of landscape, social and climatic factors using a Bayesian model, and is described in details in Prist et al. (2016). The model (baseline model) contained 7 predictor variables as fixed covariates: proportion of sugarcane, proportion of native vegetation cover, density of native vegetation patches, HDI, mean annual temperature (°C), total annual precipitation (mm), and rural male population >14 years old.

Municipality was included as a random effect to account for differences among these administrative units that are not captured in the fixed covariates. To facilitate interpretation, all estimated parameters were standardized by centering them on their mean and dividing by two standard deviations (Gelman and Hill 2007). To evaluate changes in Hantavirus infection risk, probability of HCPS infection under current conditions was compared to the predicted probability under five scenarios: two possible future climate change scenarios (RCP4.5 and RCP8.5); one possible sugarcane expansion scenario; and the combination of both climate and sugarcane expansion (RCP4.5 + sugarcane; RCP8.5+ sugarcane).

The covariates percent of native vegetation cover, number of patches, total annual precipitation, Human Development Index, and people at risk were assumed to be the same as the last year available of the baseline model (year of 2012) and kept constant for the predictions. These are reasonable assumptions considering that trends of urban-rural migration in São Paulo are constant (Cohen 2004), deforestation has been drastically reduced in the state (Kronka et al. 2005) and precipitation is not found to be relevant for HCPS risk (Prist et al. 2016). Despite the increase in sugarcane mechanization, manual harvest is still necessary and present in some parts of the process (Fredo et al. 2014). Additionally, skilled workers are replacing unskilled workers, while temporary workers are still being hired at the same rates as before (Baccarin et al. 2001). In that way, the number of people employed in sugarcane areas is not diminishing with sugarcane mechanization.

To obtain a clear view of the probability change on Hantavirus risk, we created a map with the change in infection risk for each scenario that was calculated using the difference between the current Hantavirus risk and the predicted risk for each scenario. We also used model predictions to generate a map of Hantavirus infection risk for the State of São Paulo for each scenario, where Hantavirus infection risk is classified as small (<5%), medium (≥5 and ≤10%), high (≥10 and ≤ 20%) and extremely high (≥20%). We considered that a

municipality with a risk higher than 5% should be target for preventive measures, due to the high disease lethality (maps are shown in supplementary material-Figures S3).

By associating the HCPS risk generated for each scenario with the population considered at risk for each municipality (rural men older than 14 years), and the disease lethality rates (41% - Brazilian Ministry of Health 2014), we predicted current and future human exposure to HCPS. We also calculated the percent increase in the number of people that could be infected in each scenario, by comparing each scenario with the baseline one.

Results

According to our sugarcane expansion scenario, this crop will increase ~30% on average in the state of São Paulo until 2050. Sugarcane area will increase from 26% to 34% of the Cerrado region (11.200 to 14.500 ha) and from 23% to 31% in the Atlantic Forest region (8.200 to 11.100 ha) (Figure S4).

Considering climate change scenarios, there is a general consensus among the 32 models evaluated, for both RCP4.5 and RCP8.5 scenarios, in the direction of the projected temperature change for São Paulo state. Also, RCP8.5 presents a smaller variation and a minor standard deviation between the 32 models analyzed than RCP4.5 models, especially from 2013 to 2050 (Figure 3). This is in part due to 2 models ACCESS1 and ACCESS3 (two blue lines presented above all other lines in figure 3), which showed larger anomalies in RCP4.5 than those of RCP8.5 in the earlier period. After 2050, the anomalies of RCP8.5 become larger than those from the RCP4.5 experiment, showing larger increases in temperature anomalies. Regardless of the time evolution of anomalies, both experiments present positive increases in temperature with respect to their historical experiments.

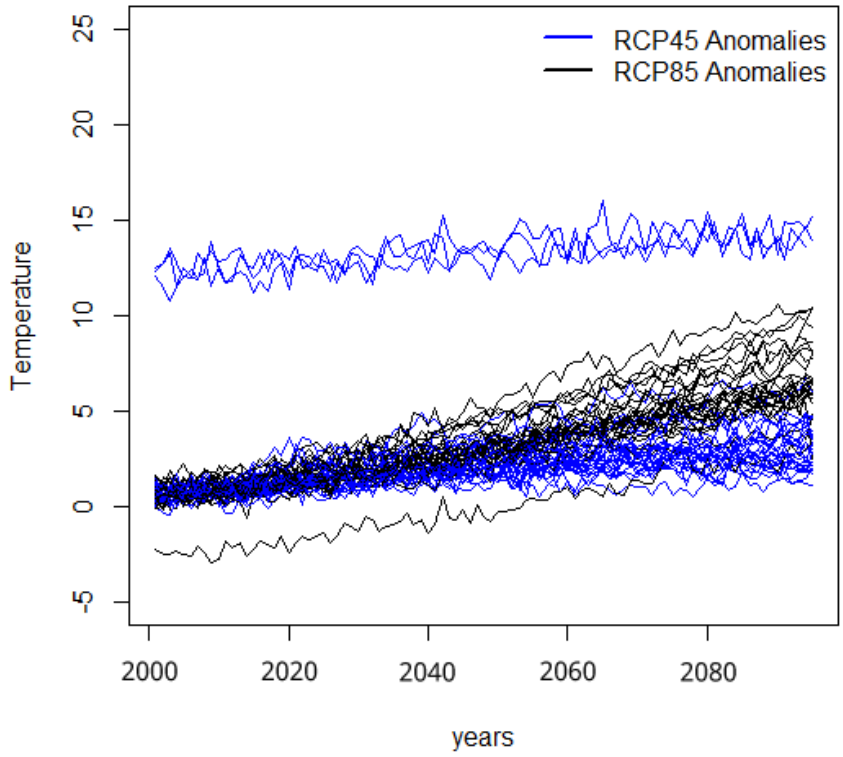

Figure 3. Annual temperature anomalies of São Paulo state, from 2006 to 2100, from the 32 RCP4.5 and RCP8.5 models used in climate change analysis.

Hantavirus Infection risk presents, on average, a small increase for all scenarios evaluated (0.25 to 0.37%) (Table 2). Sugarcane expansion is the scenario that predicted the smallest increase in Hantavirus risk, with 0.25% on average. The most pronounced changes are expected to occur in the west and mid-west parts of the state, where almost all municipalities exhibit an increase of 1.5% in HCPS infection risk (Figure 4B). Also, this scenario will lead to ~6.6% of all municipalities of the state to a risk greater than 5% for HCPS (43 municipalities).

Table 2. Average, minimum, maximum, standard deviation and increase in Hantavirus infection risk for all the municipalities (645) of the state of São Paulo for each scenario evaluated, and the current risk predicted through the baseline model.

Scenario	Average Risk	Minimum Risk	Maximum Risk	Standard deviation	Increase (%)
Current HCPS Risk (baseline model)	1.3%	< 0.1%	46.1%	3.4	-
Sugarcane Expansion	1.5%	< 0.1%	49.5%	3.6	0.25 (0-6.6)
RCP4.5	1.6%	0.1%	51.4%	3.8	0.35 (0-7.0)
RCP4.5 + sugarcane	1.6%	0.1%	50.4%	3.7	0.35 (0-6.9)
RCP8.5	1.7%	0.1%	51.8%	3.8	0.37 (0.-7.4)
RCP8.5 + sugarcane	1.6%	0.1%	50.7%	3.7	0.37 (0 - 7.3)

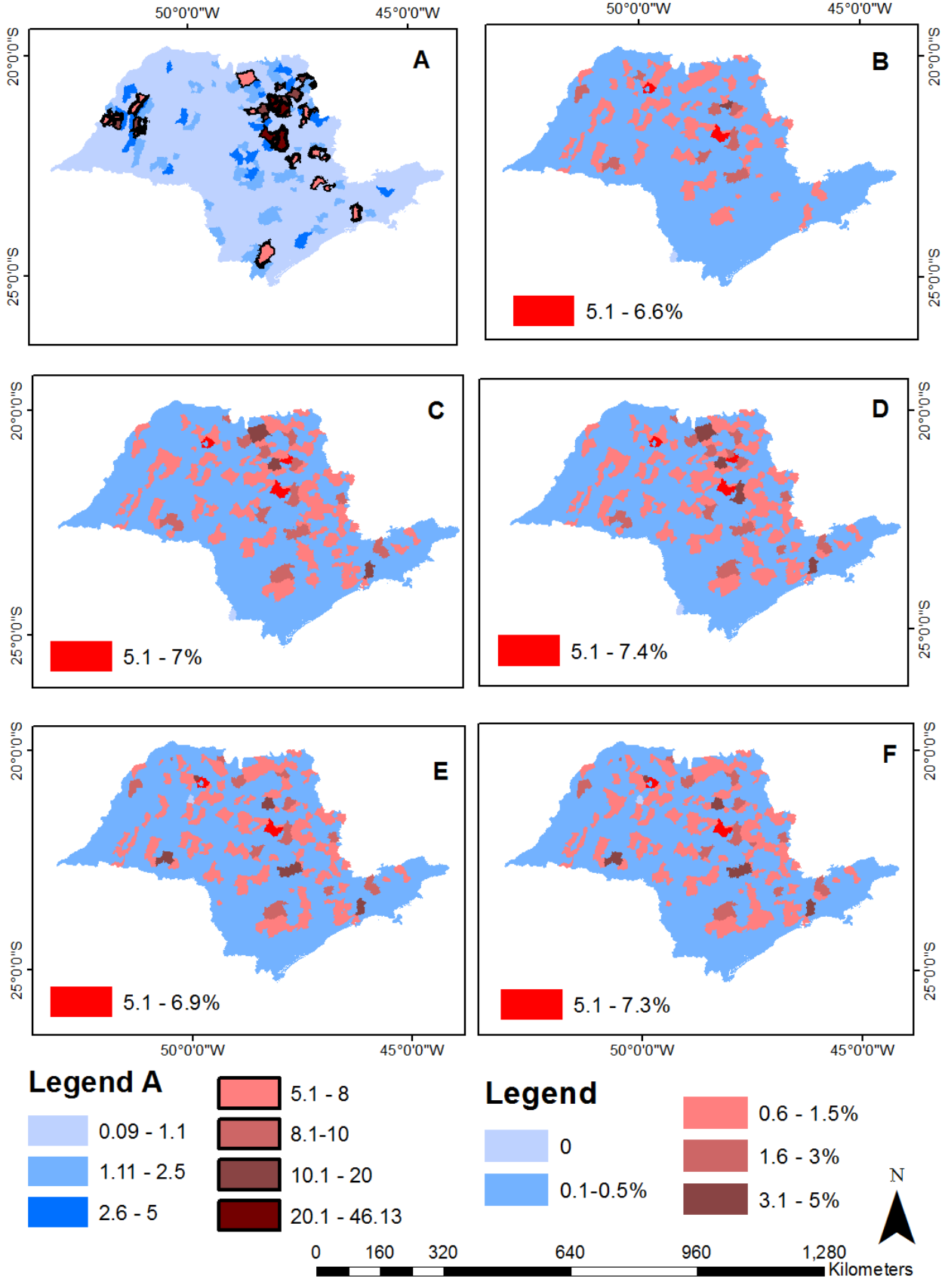

Figure 4. Current condition (baseline, A) and probability change in Hantavirus infection risk according to five scenarios: only sugarcane expansion (B), temperature anomalies of RCP4.5

(C) and RCP8.5 scenarios (D); combined effect of sugarcane expansion with RCP4.5 (E) and RCP8.5 (F) scenarios.

Projected temperature anomalies for both climate change scenarios predicted similar increases of HCPS in São Paulo state (0.35 and 0.37%), with larger increases concentrated in the northeast region, but with RCP8.5 predicting slight increases than RCP4.5 (Figure 4C, D). Moreover, there is a significant increase in the risk of infection for some municipalities that already had a large risk, especially in the mid-west region, with HCPS risk going to 51.4% in RCP4.5 and to 51.7% in RCP8.5. Municipalities of the state with a HCPS risk greater than 5% summed ~6.5% (42 municipalities) in RCP4.5 scenario and ~6.9% (44 municipalities) in RCP8.5 scenario. HCPS risk simulated using the temperature anomalies ± 1 standard deviation showed that the uncertainty of models simulations are small, and that disease risk are similar for all three experiments (mean temperature anomalies, mean temperature anomalies + 1 standard deviation and mean temperature anomalies - 1standard deviation). Thereby our confidence interval of predictions due to climate change analysis are narrow, showing a similar trend, and giving greater reliability to our predictions (Figure S2).

When combining climate change scenarios and sugarcane expansion, the average increase and the maximum HCPS risk for the state is the same as in the climate change scenarios alone (RCP4.5 and RCP8.5), showing that there is no additionality between these predictors. However, the increase in Hantavirus risk became more homogeneous throughout the state (Figure 4E and 4F), with the inclusion of ~7% of the municipalities of the state with HCPS risk greater than 5%.

When we consider the number of people that can be infected by HCPS (i.e. calculated based on the number of population at risk and the lethality rate for the disease - 41%), sugarcane expansion scenario alone presents an increase of 20% in the number of people that

can acquire HCPS. For RCP4.5 and RCP8.5 scenarios alones and combined with sugarcane expansion the number of people it´s the same, presenting an increase of 31 and 34% respectively (table 3).

Table 3. Size (indicated in number of people) and dynamic of the population at risk (average, minimum and maximum), averaged for all 645 municipalities of São Paulo state for current situation and the 5 considered scenarios of sugarcane expansion and climate change.

	Average number of people in risk for HCPS	Increase (%)	Maximum number of people	Standard Deviation
Current HCPS risk	11	-	1428	75
Sugarcane expansion	14	20	1529	81
RCP4.5	15.4	31	1615	86
RCP4.5 + sugarcane	15.4	31	1615	58
RCP8.5	15.7	34	1631	87
RCP8.5+ sugarcane	15.7	34	1631	59

Discussion

In accordance to our predictions, sugarcane expansion and temperature increase will lead to augments in HCPS risk, but with relatively low effects. Our results suggest that climate change effects can be more severe than those from sugarcane expansion, and surprisingly, there was no evidence of additive effects of sugarcane and climate in HCPS risk for São Paulo state.

First, the effects of sugarcane expansion and temperature anomalies on HCPS risk were smaller than initially expected, which may have occurred because transmission to humans is complex and involves a number of factors that are not yet fully understood, especially in the tropics. Hantavirus infection rates and prevalence in rodent populations are generally low, with transmission to rodents being density dependent (Calisher et al. 2007). In that way, the higher the density of reservoir rodents, the larger is the intraspecific encounter rates and virus transmission among rodents, and consequently the virus load in the environment and the human risk of acquiring infection increase (Khalil et al. 2014). However, high abundances of reservoirs alone do not guarantee that humans will become infected. To acquire HCPS, human exposure to infected rodents is also necessary, with disease transmission resulting from a combination of human behaviors (i.e., inadequate storage of grains and lack of protective measures), density and prevalence of reservoirs. For Hantavirus infection there is still another factor affecting disease transmission to humans, the climate, which affect virus survival and aerosolization in the environment (Kallio et al. 2006). In that way disease transmission to humans requires that four main factors interact in the same time and space, making its dynamic difficult and complex: an infected rodent; a certain abundance of reservoir rodents to proliferate the infection throughout the rodent population (in which prevalence is generally low); suitable climatic conditions in order to maintain virus in the environment and allow its aerosolization; and a susceptible human population. Due to all these factors, HCPS transmission to humans is difficult, and can be considered as a rare event,

with a low number of cases reported each year. However, even this numbers being low, this increase is extremely relevant given the high lethality rate of the HCPS, which is around 50% in São Paulo state.

Sugarcane expansion and HCPS risk

Our results confirmed previous studies showing that increases in the amount of sugarcane can augment HCPS risk (Ruedas et al. 2005; Armien et al. 2009; Prist et al. 2016). Our scenario predicted an augment of ~30% (or ~3.000 ha) of land occupied with sugarcane on average, for both cerrado and Atlantic forest regions, until 2050, predicting increases of ~15% in HCPS risk. This expansion, can be considered small, once São Paulo state tripled the area planted with sugarcane from 1990 to 2010, going from 3.000 to 9.000 ha on average (Ferreira-Filho and Horridge 2014; IEA 2015). Following this sugarcane boost, HCPS risk in São Paulo state, in the last 20 years, also increased almost 4 times on average (382%), going from 0.34 to 1.3% of average risk. Therefore, the increase in disease risk, predicted by our model, according to the expansion of sugarcane, is concordant with the historical increase in risk experienced from 1993 to 2012.

This increase, without any change in temperature, is on average low, but can reach up to 6.6% in some municipalities, leading to an increase of 20% in the number of people that can acquire HCPS. The main underlying mechanism to explain this pattern is that sugarcane provides a highly energetic food, leading to increased recruitment and a rapid population growth of rodents owing to food resources (Fitzgibbon 1997). Therefore, sugarcane plantations are a suitable habitat for these habitat generalist rodent species, as Hantavirus reservoirs, supporting greater abundances of rodents than other ecosystems, whether natural or agricultural (Gheler-Costa et al. 2012), with sugarcane becoming predominant in their diets (Takele et al. 2008).

Additionally, land-use changes indirectly influence local temperature (Loarie et al. 2011), and also alter albedo and evapotranspiration, which influence climate directly (Bonan 2008). In this sense sugarcane plantations have cooler temperatures and more moisture than pasture and other crops, being more similar to areas of natural vegetation (Loarie et al. 2011). This microclimate changes may contribute to the increase in HCPS risk, since it makes sugarcane an even more suitable habitat to rodents, contributing to rodents thermal comfort. This climate aspects can also affect the indirect path of transmission, extending the time the virus remains infectious in this environment and augmenting HCPS risk, once virus inactivation happens only in dry conditions and above 37°C (Kallio et al. 2006).

Climate change and HCPS risk

Climate change scenarios lead to larger increases in HCPS risk when compared with sugarcane expansion alone, however here also the effects were low. Increase in temperature may be larger than the sugarcane ones because temperature interact with disease transmission through multiple mechanisms, having a more complex effect than this crop: it affects positively vegetation growth (Luis et al. 2010; Wang et al. 2011), what can lead to increases in the abundance of reservoir rodent species, once small mammal populations are food-limited (Prevedello et al. 2013); it affects reproduction and survival of small rodents (Zeier et al. 2005; Luis et al. 2010), which may have a positive or negative effect depending on the magnitude of temperature change (Luis et al. 2010; Carver et al. 2015); and also temperature affects the direct process of HCPS transmission, determining virus survival and aerosolization in the environment.

There is a lack of studies involving reservoir rodent species and Hantavirus related to HCPS and climate variables, but for HFRS, mild temperatures (10-25° C) are most favorable for reservoirs rodents breeding (Liu et al. 2012), and for increases in the time the virus

remains infectious in the environment (Kallio et al. 2006). Increases in temperature lead to greater aerosolization of the virus and higher rates of inhalation by both humans and rodents (Langlois et al. 2001). In that way, augments in temperature may have a positive effect on reservoir rodent abundance and virus survival and aerosolization until reaching a certain threshold (around 40° C), from where it will exert a negative effect.

The low effect of climate change on HCPS risk, despite all the points previously cited, may also occur due to the fact that temperature anomalies, until 2050, can be considered small and similar for both RCP4.5 and RCP8.5, with larger increases being observed after 2050. Anyway, it is important to highlight that increases in temperature anomalies lead to augments in HCPS risk, even if small, in all the 645 municipalities of the state of São Paulo. Therefore, higher increases for disease risk are expected after 2050, if carbon emissions are not controlled and climate change mitigation actions are not successful.

Combined effect of climate change and sugarcane on HCPS risk

Individual evaluation of climate and sugarcane could have resulted in a better understanding of the individual contributions of each factor on disease risk. However, evaluating these scenarios together is a more realistic approach of future predictions, given that indeed they will occur and act together, and that temperatures are increasing due to both global climate changes and local expansion of sugarcane expansion.

Sugarcane expansion and temperature anomalies together showed no additionality, predicting the same average increase in HCPS risk for the state of São Paulo as climate change scenarios alone. This may have happened, as mentioned before, because there are multiple mechanisms through which temperature influences HCPS risk, some of which overlaps sugarcane mechanisms (e.g., effect on rodent densities). Particularly, even in conditions where rodent abundances and prevalence are high (due to sugarcane expansion), if

temperature conditions are not ideal for virus survival and aerosolization, transmission to humans will not occur. Therefore, the capability of the virus to survive outside the host is critical for the transmission within rodent populations and to humans, with temperature being one of the determining factors of this survival. This effect, may have contributed to the lack of additionality between temperature and sugarcane, once sugarcane effects will only occur when temperature conditions are also adequate.

Conservation and public health implications

Land cover and land use change are at the origin of the outbreaks of Hantavirus, and can also be an important component to reduce or mitigate its spread. Given that temperature growths will lead to increases in HCPS risk, forest restoration can be an alternative to attenuate the effects of increased temperature in HCPS risk for three main reasons. First, forest regrowth, especially in tropical regions, can remove anthropogenic carbon through net growth, absorbing about 30% of all CO_2 emissions from fossil fuel burning and net deforestation (Canadell et al. 2007; Bonan 2008), contributing to climate mitigation. Second, forest regeneration can mitigate the creation of warmer and drier climate in agricultural systems (Bonan 2008), reducing those ideal conditions to Hantavirus survival. Third, increasing forest cover could also reduce HCPS risk arising from sugarcane expansion, once it would lead to increased suitable habitat for habitat specialist species, leading to a more diverse community, with decreased abundance of habitat generalist species (Umetsu and Pardini 2007), as Hantavirus reservoir species.

Regarding health questions, considering the expected increase in temperature and sugarcane expansion, costs in public health will increase. At least part of this cost should be included in sugarcane production, including expenditures associated with rodent control, educational and preventive campaigns targeting how to avoid virus inhalation and contact

with infect rodents excretes. Additionally, rodents can also bring damages to sugarcane once they are considered major pests of this crop (Takele et al. 2008), leading to a loss of 825.000 tones of sugarcane in one year in India (Rao 2003). Also, states and municipalities that are starting sugarcane expansion, should plan for costs involved with educational campaigns and preventive measures, for example educating workers and residents from rural areas how to avoid Hantavirus inhalation and contact with infect rodents excretes. This could be crucial in order to avoid disease propagation to places where HCPS risk is low or absent until now. This type of information should be incorporated into the costs of land use decisions. Sugarcane expansion can provide a solution to one specific problem, as supplying oil market, but can also create a human health problem, increasing risks of acquiring HCPS.

Similarly, our results reinforce the links between climate change and augments in different kind of diseases, such as Lyme disease, West Nile Virus and *Echinococcus* (Thevenet et al. 2005; Ogden et al. 2008; Hahn et al. 2015). Those evidences should be considered as an additional argument to encourage governments, companies and citizens to sign agreements and start massive campaigns in order to mitigate climate change.

Final remarks

Our scenarios of future sugarcane expansion and climate change RCP4.5 and RCP8.5 predicted a low but relevant increase in HCPS risk in the state of São Paulo until 2050. Despite the lack of additive effects of sugarcane and climate in HCPS risk, we suggest that prevention and mitigation actions should act in both components, through land use planning and forest restoration programs, and by concentrating healthcare effort in areas with higher HCPS risk.

To better explore the underlying mechanisms of the observed pattern, we stimulate futures studies testing the relationships between sugarcane production with rodent reservoir

densities and virus prevalence, as well as studies concerning the effects of temperature and moisture on reservoir rodents population dynamics and on virus survival and aerosolization. Understanding those relationships is crucial to better comprise HCPS transmission dynamics in different environments and situations, being important to the effective design of preventive health strategies.

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Suppporting information

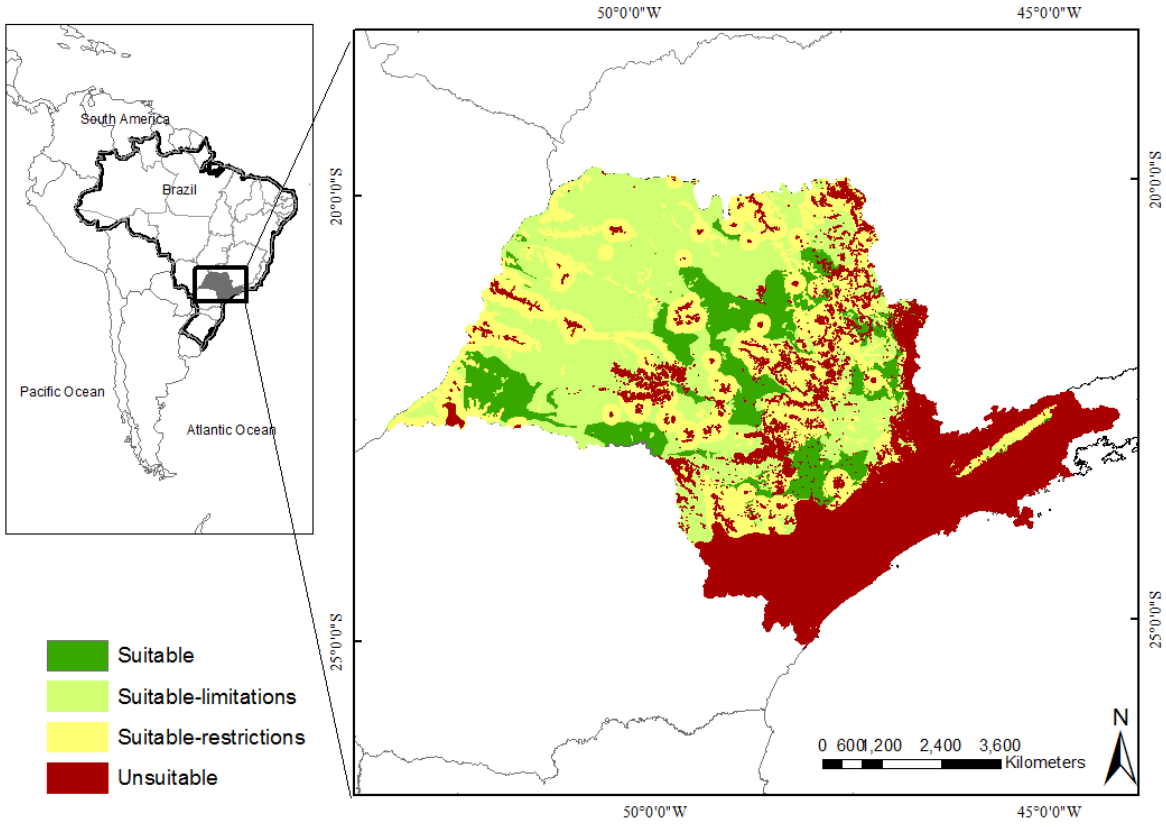

Figure S1. The Agro-environmental Zoning for the sugar and Alcohol Industry (AZA). The suitability classification is based on edapho-climate conditions and biodiversity protection. Source: Environment Secretary of the state of São Paulo.

AZA divides the State in four zones, according to their suitability for sugarcane: suitable areas (favorable edaphic and climatic conditions, with no environmental constraints); suitable areas with environmental limitations (favorable edaphic and climatic conditions, but includes some protected areas; simple measures of environmental regulation and agricultural management are necessary); suitable areas with environmental restrictions (favorable edaphic and climatic conditions, but include buffer zones of Protected Areas, requiring complex measures of environmental preservation and protection to fauna and flora); and unsuitable

areas (unfavorable edaphic and climatic conditions; slope higher than 20%, which prevent mechanization). This zoning was developed using multiple data: climate, air quality, slope, soil, water availability and water quality, protected areas and important fragments for biodiversity connectivity (São Paulo 2008). We considered all three zones of suitable areas as suitable for sugarcane expansion, because it simulates an occupation pattern that is already happening in the state of São Paulo: 50% of the new areas occupied with sugarcane are in suitable areas with environmental limitations, 30% occur in suitable areas with environmental restrictions, and 18% in suitable areas; while 0.8% occur in unsuitable areas (Gomes and Montaño 2012).

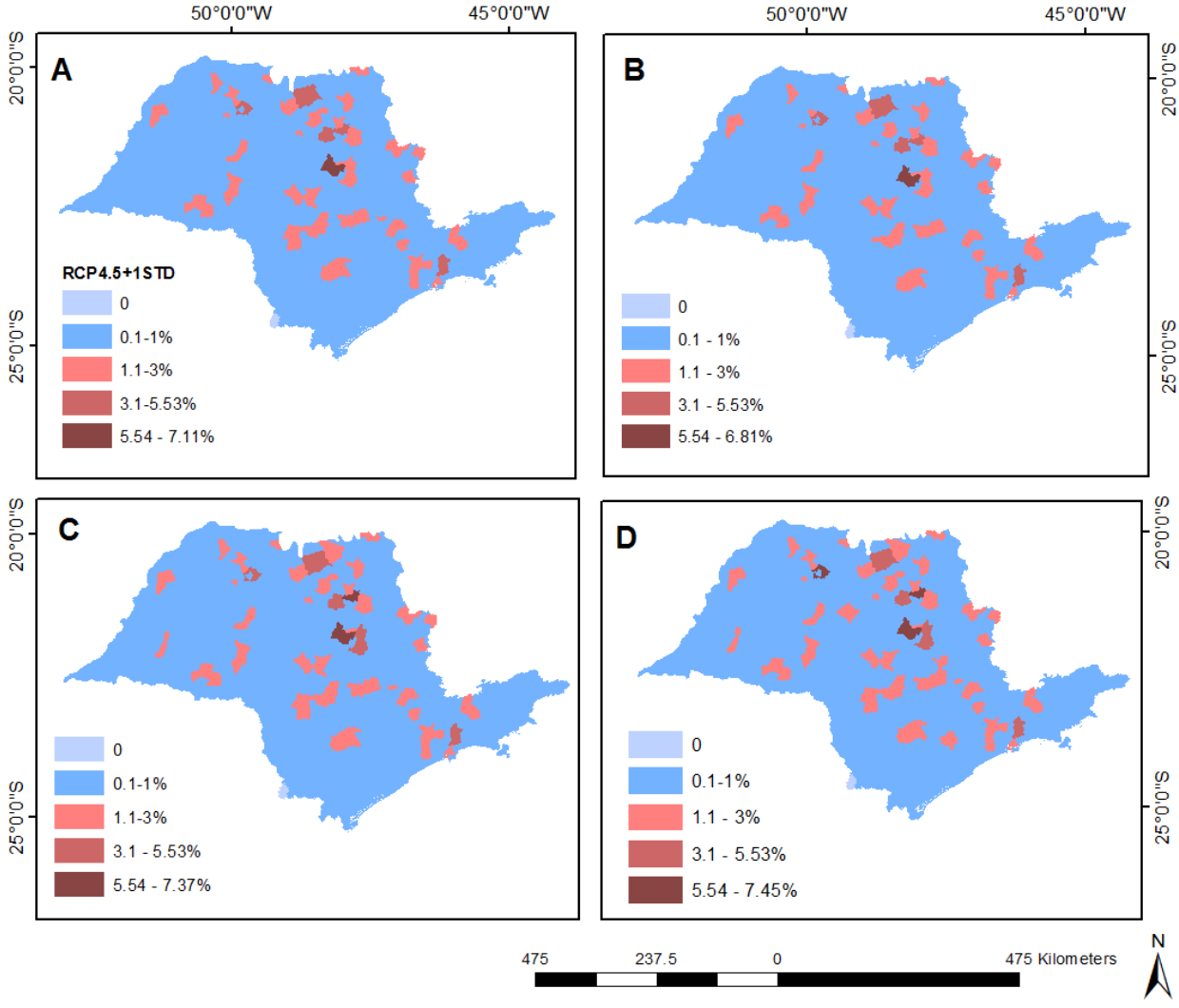

Figure S2. Map of change in Hantavirus infection risk according to four scenarios: (A) average temperature anomalies + 1 standard deviation of RCP4.5 (B) average of temperature anomalies - 1 standard deviation of RCP4.5 (C) average of temperature anomalies + 1 standard deviation of RCP8.5; (D) average of temperature anomalies - 1 standard deviation of RCP8.5. The risk predicted by the average temperature anomalies ± 1 standard deviation, for both RCP4.5 and RCP8.5 scenarios, showed similar patterns of trend, with both predicting the same average of increase for São Paulo state (0.36%) and a small difference in the maximum risk for some municipalities (6.8 to 7.11 for RCP4.5; and 7.37 and 7.45 for RCP8.5). They also show a similar pattern of increase with the risk predicted by the anomalies averaged of

the 32 models, indicating a small range of possibilities in the predicted HCPS risk and a large confidence in our predictions.

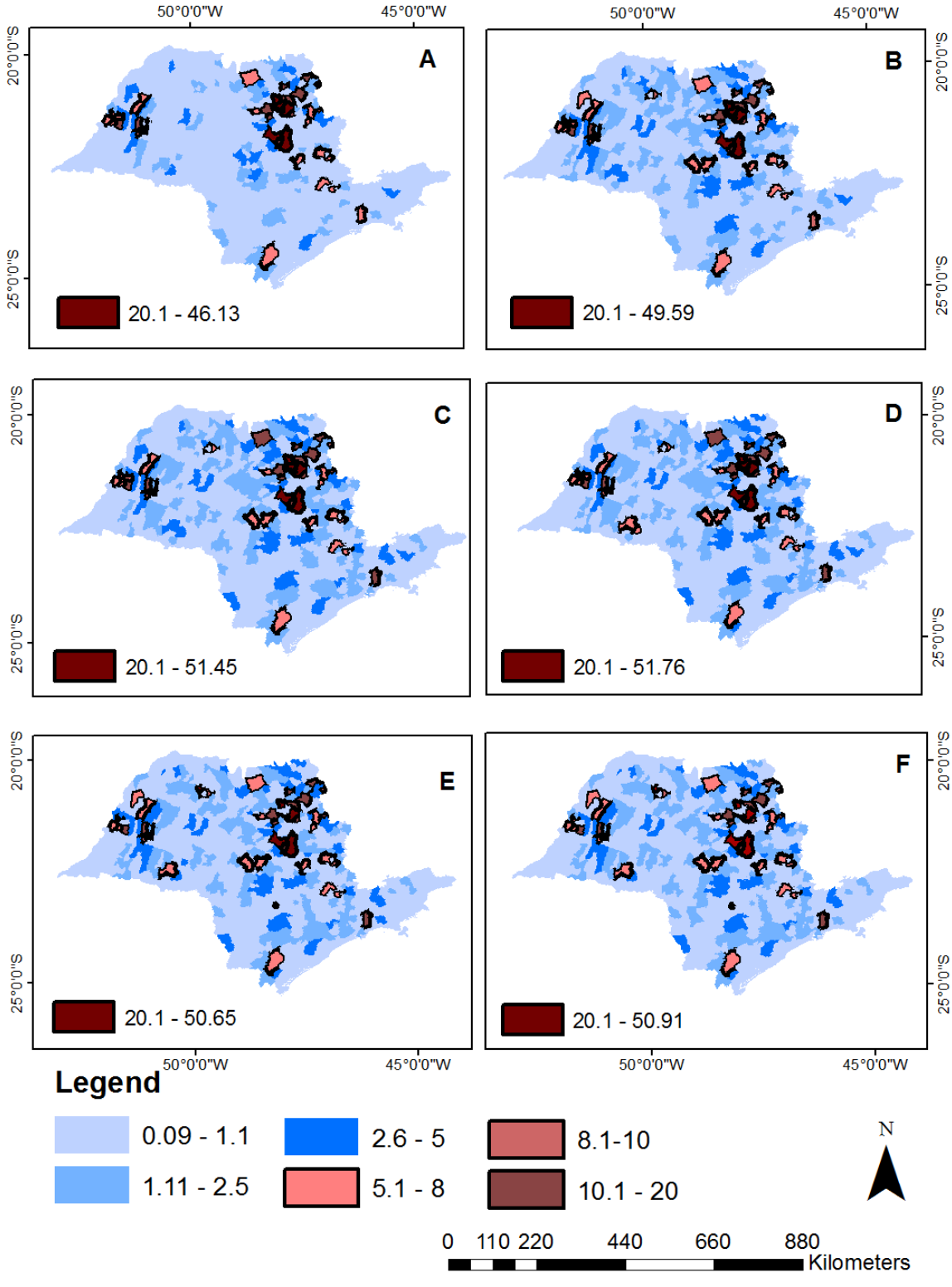

Figure S3. Map of Hantavirus infection risk according to current condition (baseline, A) and five scenarios: sugar cane expansion (B), temperature anomalies of RCP4.5 (C) and RCP8.5 scenarios (D); RCP4.5 and RCP8.5 scenarios combined with sugar cane expansion (E and F, respectively). Local values (municipalities) are indicated in each map, as well as maximum values for HCPS risk.

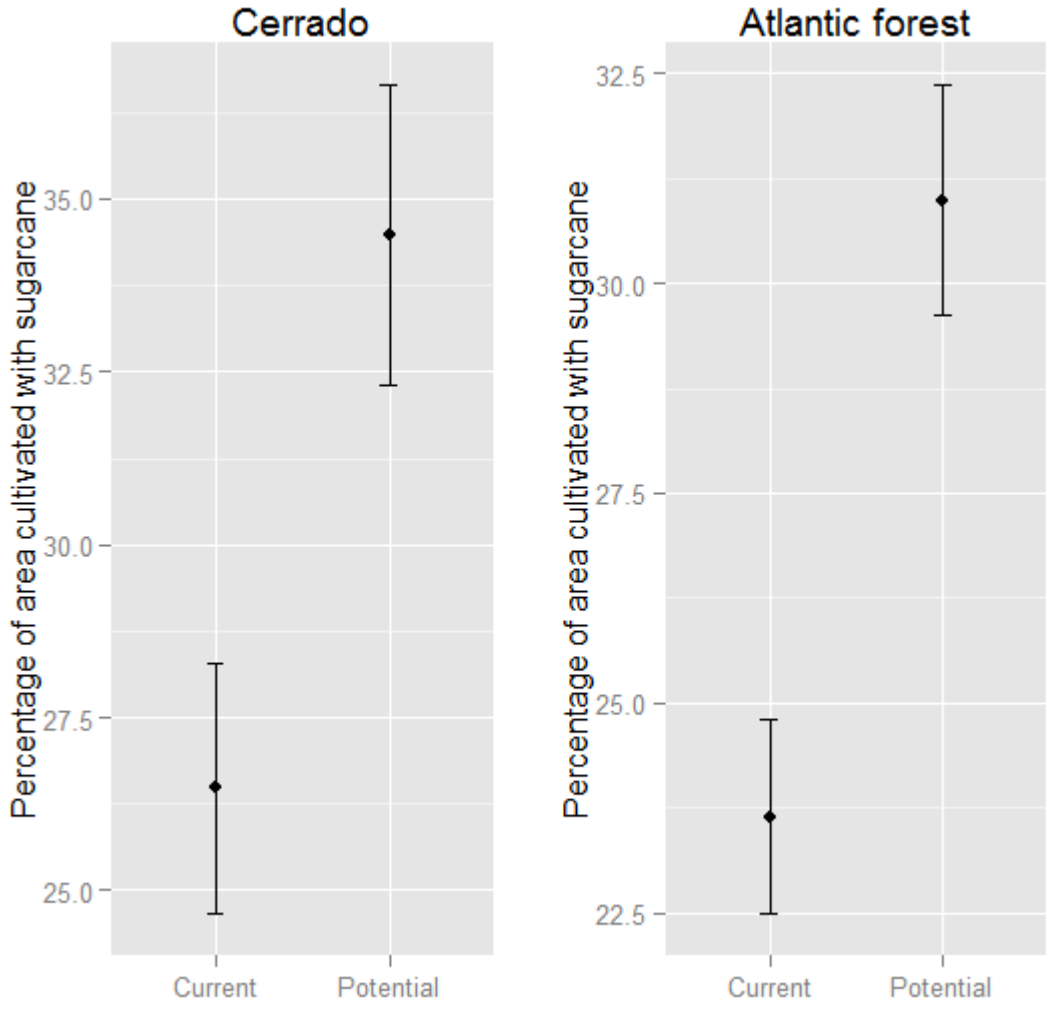

Figure S4. Mean sugarcane cover (in %) for municipalities with Cerrado and Atlantic forest vegetation in 2012 (“current”) and 2050 according to our model expansion scenario (potential). Black points represent the means, and horizontal bars represent standard errors.

Chapter 05

GENERAL DISCUSSION AND CONLCUSIONS

General Discussion and Conclusions

This study is the first one that aims to understand the relationship of landscape structure, social and climate variables with Hantavirus Cardiopulmonary Syndrome (HCPS) infection risk in Brazil, and that uses climate models and sugarcane expansion scenarios to predict the future effects of climate change and land use on HCPS infection risk. Our results indicate that HCPS risk is positively associated to increases in the amount of sugarcane cultivated area, and to the human development index (HDI). For the Atlantic Forest region, average annual temperature and population at risk also had positive relationships with disease risk. Also, according to our model, the average HCPS infection risk for the state of São Paulo is 1.3%, with 6% of the state's municipalities being classified as having medium to high HCPS risk.

If temperature increases according to the rates projected by the IPCC RCP4.5 and RCP8.5 scenarios, and sugarcane expands according to our scenario, HCPS risk will increase. Sugarcane expansion alone increases the average risk of infection from 1.3% to 1.5%, while for RCP4.5 and RCP8.5 scenarios the risk of infection goes to 1.6% and 1.7% respectively. These scenarios are also responsible for the largest increase in the maximum risk of infection presented by some municipalities, ranging from 46.1% to 51.4% in RCP4.5 and to 51.7% in RCP8.5. The expansion of sugarcane combined with RCP4.5 and RCP8.5 scenarios did not increase average or maximum risks of infection, showing no additionality of predictors. However, they are responsible for the further expansion of the risk throughout the state, with 7% of the municipalities being classified as medium to high risk for these scenarios.

Risk factors for HCPS transmission include agricultural, domestic and leisure activities, and low living conditions in rural areas, all related to human exposure to rodents excreta (MSB 2014). In this sense, it would be expected that municipalities with low HDI and a higher number of people living in inadequate conditions, or involved in agricultural and

rural activities, would have a greater chance of contact with rodents and their excreta. However, our results showed an opposite result: the higher the HDI the greater the chance of HCPS infection in a municipality. This is probably due to the fact that much of the HDI of a municipality is linked to the amount of sugarcane planted. Thus, municipalities where sugarcane is dominant economically have better socioeconomic conditions, and stronger social welfare indicators (Camargo and Toneto 2008; Martinelli et al. 2010), even outperforming the greater São Paulo Metropolitan Region (Camargo and Toneto 2008).

The population at risk (i.e., males in rural activities older than 14 years) had a positive relationship with HCPS risk only in the Atlantic forest region, with no effects being observed for the Cerrado region. HCPS transmission requires an effective contact between humans and infected rodents or with its aerosolized excreta, which is most likely to occur in individuals that participate in agricultural or forestry activities (Elkhoury et al. 2012; Watson et al. 2014), as indicate by the disease risk factors (MSB 2014). For Cerrado, the lack of effect may occur because this region have more land proportionally cultivated for sugarcane plantations than Atlantic forest, and this crop brings large numbers of temporary workers from other states who do not end up in the official population statistics (Ficarelli and Ribeiro 2010). Therefore, this region must have larger number of temporary workers, working directly in the sugarcane fields than Atlantic forest, which may have masked the effects of population at risk for the Cerrado biome.

The percentage of area planted with sugarcane in each municipality is the most important predictor of HCPS risk in both Atlantic Forest and Cerrado regions. According to our model, the larger the area occupied with sugarcane, the greater the chances of a person becoming infected with HCPS. This is probably due to the fact that this culture supports greater abundances of rodents than any other ecosystems, whether natural or agricultural (Gheler-Costa et al. 2012, Verdade et al. 2012). The dynamics of the host population and its

relationship with environmental conditions determine the extent to which a pathogen can persist or disappear, affecting the risk of transmission to humans (Mills and Childs 1998). Due to its preference for agricultural environments, and the fact that sugarcane provides food and shelter for rodents, this crop can increase the contact rates between humans and reservoir rodents, providing opportunity for pathogens to "overcome" the species barrier and infect humans (Gortázar et al. 2014).

This is of most concern once sugarcane is expanding considerably, especially in the state of São Paulo, which is responsible for 62% of Brazil´s ethanol production. From 1990 to 2010, the state of São Paulo tripled the area planted with sugarcane (Ferreira-Filho and Horridge 2014; IEA 2015). According to our expansion scenario, which used the agri-environmental guidelines, pasture areas and the percentage of expansion of the last ten years, sugarcane can increase almost 30% in the state of São Paulo until 2050, going from 24.3% to 31.8% on average. Expanding the amount of land occupied by this crop also lead to small increases in Hantavirus infection risk. Therefore, management practices before the harvest should be implemented in order to avoid contact between workers and rodent excreta, diminishing the risk of transmission, with this costs being included in sugarcane production.

Temperature also had positive effects on Hantavirus transmission risk, especially in the Atlantic Forest. This was observed before for other diseases, as in West Nile Virus (Reisen et al. 2006), where decreases in temperature leads to fewer outbreaks, and for Hantavirus infection in Asia and Europe, where higher temperatures increased the virus prevalence in reservoir rodents (Bi et al. 2002). The majority of disease transmission occurs during hot periods of the year, because pathogen replication rates inside the vector typically increases as a curvilinear function of ambient temperature (Reisen et al. 2006). Additionally, the majority of pathogens has a thermal development threshold below which replication and transmission do not occur (Reisen 2010).

Temperature can also affect vegetation growth (Jiang et al. 2011) and the survival rate of rodents (Zeier et al. 2005), with mild temperatures (10-25°C) being most favorable for rodent breeding (Liu et al. 2012). Furthermore, climate variables are important for the survival and virulence of the virus, once it is spilled in the environment through the urine and feces of rodents, where it remains active until it is aerosolized, a condition which also depends on climatic conditions (drier climates facilitate its aerosolization). Therefore, reservoir rodents normally exhibit a peak in Hantavirus infection during warmer months (Teixeira et al. 2014), probably because high temperature leads to greater aerosolization of the virus and higher rates of inhalation by both humans and rodents (Lee et al. 1981; Vapalahti et al. 2010).

Atmospheric concentrations of greenhouse gases are increasing and leading to a rise in temperature, mainly due to human activities (Watson et al. 1996; Patz 2001). According to the 32 models evaluated, by 2050, temperatures are expected to increase by 3.2°C for RCP4.5 and by 2.8°C RCP8.5, augmenting HCPS risk by 0.35% to 0.37%, on average.

Climate change scenarios RCP4.5 and RCP8.5 combined with sugarcane expansion showed no differences in predicted risk when compared to climate scenarios alone, and mostly climate scenarios had larger effects on HCPS risk than sugarcane expansion. However, when considering the two factors together, they presented the larger expansion of Hantavirus risk through the state. Originally, 6% of the state was classified as high risk for Hantavirus infection, but in combined scenarios, this number become 7.0%. The lack of additionality may have happened, because there are multiple mechanisms through which temperature influences HCPS risk, some of which overlaps sugarcane mechanisms (e.g., effect on rodent densities). Particularly, even in conditions where rodent abundances and prevalence are high (due to sugarcane expansion), if temperature conditions are not ideal for virus survival and aerosolization, transmission to humans will not occur. Therefore, the

capability of the virus to survive outside the host is critical for the transmission within rodent populations and to humans, with temperature being one of the determining factors of this survival. This effect, may have contributed to the lack of additionality between temperature and sugarcane, once sugarcane effects will only occur when temperature conditions are also adequate.

It is clear that temperature has a positive effect on HCPS risk. However few studies tested so far the effects of this variable on rodent breeding and survival, and on virus survival and aerosolization. Studies with Puumala virus in Europe showed that after 37° the virus is no longer viable. Similar studies in Brazil are needed in order to elucidate this question. Similarly, virus aerosolization and infection happens in dry conditions, which indicates that moisture probably has an important role in the transmission path. Understanding this effect may support the adoption of new preventive measures to avoid disease propagation. If moisture is important to avoid virus aerosolization, simply keeping sugarcane humid could diminish contact between humans and virus and decrease diseases transmission.

Similarly, sugarcane effects were only tested in rodent abundances. It would be interesting to understand the effects of this crop on Hantavirus prevalence, and compare with different agricultural fields, as well as with different growth phases of sugarcane. Additionally, understand if there is a landscape context in which this crop is inserted to promote larger abundances and prevalence in reservoir rodents would be important to create management strategies. Sugarcane can be a suitable habitat for Hantavirus reservoir species, but are these conditions maintained in all sugarcane stages of growth? And how Hantavirus reservoir species change along the year, specially after the harvest when resource offer in the agricultural matrix is drastically reduced?

Our results showed that landscape composition, specifically the amount of sugarcane, is the most important predictor of Hantavirus infection in the state of São Paulo. Landscape

configuration (i.e., natural vegetation fragmentation) appeared not to be so important in this case, as predicted by other authors, that claimed that forest fragmentation had a positive association with Hantavirus infection risk in the Neotropics (Suzan et al. 2008a,b). However, those Neotropical studies compared forest fragments and pastures, and because pastures did not represent a favorable habitat to rodents as sugarcane, fragmentation may have played a more important role than in our landscapes. Therefore the type and amount of crop that exist in a landscape may be important and ascertain the kind of relation between HCPS and the landscape structure.

Concerns about the ecosystem services provided by forests are growing, especially because deforestation rates continues to amazing levels of 2-3%/year of global forest loss (Wolfe et al. 2000). Tropical forests rich in species are converted to agricultural and pasture uses, which are poor in species richness, and dominated by few species, that increase in abundance (Patz et al. 2004), which often constitute diseases reservoirs. With this, there is an increased exposure from humans to pathogens (Wolfe et al. 2000).

The importance of zoonotic diseases should be emphasized. Zoonotic pathogens are the most significant cause of infectious emerging diseases affecting humans (Patz et al. 2004). More importantly, a number of those diseases has a high rate of mortality and no treatment or vaccination, as HCPS. As the threat to the natural environment is extensive and continues, many of the major diseases are the result of anthropogenic changes to the natural environment. The state of São Paulo is highly fragmented, and is being dominated by sugarcane, which continue to expand. This can lead to propitious conditions of infection, especially when combined with temperature, which appears to be ideal for Hantavirus infection.

In the last years the number of HCPS reported cases in the state of São Paulo has increased. This can be a result of sugarcane expansion, which has double its area in the state,

but can also be attributed to the improvement of diagnosis and increased awareness, which are getting better along the years. Nevertheless, the loss, fragmentation and conversion of forest habitats, brings humans into ever-closer contact with rodent populations that serve as Hantavirus reservoirs, generating serious risks to public health (Araujo et al. 2011). In the state of São Paulo the area occupied with sugarcane is the most important predictor of Hantavirus infection, leading workers in close contact with rodent excreta and HCPS risk. Management programs and rodent surveillance should be implemented, especially in the municipalities that are dominated by this crop. Easy and cheap measures, as education programs, the use of masks, wetting the cane before harvesting, and closing places where food is stocked, can diminish transmission risk and avoid contact with rodent excreta.

In short, our results demonstrate that:

- The amount of land cultivated with sugarcane has positive effects on HCPS risk;
- Human development index is positive related with HCPS risk;
- Temperature has marginally positive effects for HCPS risk in the Atlantic forest of São Paulo;
- The average HCPS risk for the entire state of São Paulo is 1.3%, with 6% of the municipalities being classified as medium to high risk;
- Temperature anomalies, predicted by RCP4.5 and RCP8.5 climate change models increases HCPS risk to 1.6 and 1.7%;
- Sugarcane expansion increases HCPS risk to 1.5% on average;
- Sugarcane expansion combined with temperature anomalies did not presented increases in HCPS risk, however boosted the number of municipalities being classified as medium to high risk.

It is worth mentioning that our study was exploratory and aimed to identify which variables must affect Hantavirus infection in the state of São Paulo. It was performed in a

coarser spatial scale, at municipality level, and in order to confirm our results, studies in smaller spatial scales, exploring the underlying processes that can explain those general observed patterns, should be developed in the future.

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Attachments

USING DIFFERENT PROXIES TO PREDICT HANTAVIRUS DISEASE RISK IN SÃO PAULO STATE, BRAZIL

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Abstract

Recent studies predict disease risk using different proxies, such as pathogen prevalence in hosts, abundance of the main hosts, and the number of reported disease cases. These proxies are used to build risk maps that can aid the prevention of new disease outbreaks. To date, these proxies have not been widely tested for differences in their predictions and effectiveness, which could have great implications for disease control measures. In this study we aimed to compare two different proxies inferring Hantavirus disease risk in the state of São Paulo (Brazil) by comparing risk distribution and accuracy of risk maps constructed using (a) rodent reservoir abundance data (RRA) sampled between 2002 and 2008 and (b) C Cardiopulmonary Syndrome (HCPS) cases reported between 1993 and 2012. RRA data were collected within forest fragments and matrix of six landscapes and was extrapolated for São Paulo state through regression models. Throughout Bayesian models, we created HCPS risk map using annual HCPS incidence and climate, landscape structure metrics and social factors. We validates RRA and HCPS risk maps with actual reported HCPS cases (2013–2015). These data were categorized according to risk levels (low, medium, high and very high) and compared through histograms and correlation tests. The two risk maps (RRA and HCPS) had a low correlation (0.038) and a low covariance (0.016), indicating high uncertainty in the predictions between these two proxies. The RRA map predicted that 68% of the municipalities in the state are in the medium to high risk categories, while the HCPS risk map predicted only 6%. This indicates that the RRA risk map might be overestimating high risk areas. The RRA map also had a higher sensitivity than the HCPS risk map to newly reported cases, correctly identifying 82% of the cases in medium to high risk areas. On the other hand, the HCPS risk map had a higher specificity (91%), leading to a much better prediction of the low risk areas (31% for RRA risk map). Our results draw attention to the fact that different proxies can give different results and predict different risk levels, and should be used carefully in disease studies.

Keywords: diseases; HCPS; predictive power; risk maps; rodent host abundance

Introduction

Several studies use different proxies to infer disease risk via risk maps, which have become more common in the last 20 years (Kitron 1998). The proxies used generally range from the presence/abundance of a reservoir or of the main hosts in the environment (Frank *et al.* 1998; Guerra *et al.* 2002), pathogen prevalence in these hosts (Boone *et al.* 2000; Ostfeld *et al.* 2005; Goodin *et al.* 2006; Xiao *et al.* 2016), and the number of reported cases of the disease of interest (Graham *et al.* 2004, Bhatt *et al.* 2013). This information, when coupled with

information on vector/host ecological requirements, can lead to the prediction of patterns of disease emergence, spread and control (Biek & Real 2010). Therefore, the methods by which these proxies infer disease distribution vary from the simple mapping of disease vector distribution – such cases includes Lyme disease (Frank *et al*. 1998; Guerra *et al*. 2002), malaria (Martens *et al.* 1999), and dengue (Little *et al*. 2011), where distribution maps of their vectors are used to infer disease risk - to the use of the number of infected hosts or reported disease cases in Bayesian models, regression or niche modeling analysis to model disease risk, as in the cases of Hantavirus Cardiopulmonary Syndrome (HCPS) (Glass *et al.* 1992, 2000; Boone *et al.* 2000; Rogers & Randolph 2000; Donalisio & Peterson 2011; Glavanakov *et al.* 2001) and bluetongue in France (Guis *et al*. 2007).

Vector or reservoir biology dictates the dynamics of pathogen transmission and pathogen persistence (Reisen 2010). For example, Lyme disease and HCPS hosts (and vectors for Lyme) may serve as effective long-term reservoirs (Talleklint *et al*. 1995), with the effective dispersal of the pathogen being limited to the dimensions of reservoir home ranges (Madhav *et al.* 2004). However, the presence or high abundance of hosts alone do not guarantee that humans will become infected with the pathogen. To acquire a disease, human exposure to infected vectors/hosts is also necessary, with disease transmission resulting from a combination of human behaviors and vector/host risk. Generally, human behaviors include the use of vector/host habitats and protective clothing, while vector/host risks include the density of vectors and hosts and the proportion of these that are infected (Horobik *et al*. 2007). Additionally, anthropogenic influences such as expanding agriculture or urbanization are affecting the epidemiology of several diseases and transmission dynamics by altering landscape structure (reduction of habitat amount, patch size and connectivity; increase of edge influences and patch isolation; see Fahrig 2002) and expanding ecotonal areas. These effects provide mechanisms that enhance vector/host breeding success, and promote rapid pathogen

dispersal by juxtaposing vectors, reservoir hosts, domestic animals, and humans – enhancing disease transmission (Reisen 2010).

Usually, disease risk assessment comes with several caveats (Ostfeld *et al.* 2005), particularly because it is hard to incorporate all important risk factors in a single model. The use of spatially referenced and temporal data - when properly linked to health data - adds strength to epidemiological analyses. Additionally, remote sensing data has been widely used for monitoring, mapping and predicting diseases (Hay 2000, Hay *et al.* 1998, 2006, 2013). However, to date, no study has evaluated the use of different data (host presence/abundance; infection prevalence; diseases cases) to infer disease risk, neither have incorporate these information with data derived from remote sensing. Often, the only available information relates to cases but not to host abundance (Woodbury 2003), or to host distribution but not to pathogen prevalence (Martens *et al.* 1995), or it is limited to human cases with unknown mechanisms of viral transmission in space and time (Glass *et al*. 2002).

HCPS ranks among the major emerging diseases of the last century, with great potential to become a public health threat into the near future (Pereira *et al*. 2007). It was first recognized in 1993, both in USA (CDC 2014) and Brazil (Brazilian Ministry of Health 2013). Rodents of the family Cricetidae are the primary hosts of Hantavirus (Mills *et al.* 1999, Jonsson *et al*. 2010, family *Bunyaviridae*), a virus that causes two syndromes in humans: HCPS in the Americas and hemorrhagic fever with renal syndrome (HFRS) in Eurasia and Africa (Jonsson *et al*. 2010). Transmission to humans occurs via inhalation of aerosolized virus particles derived from the urine, saliva, and feces of infected rodents (Lee *et al.* 1981; Vapalahti *et al.* 2010). HCPS has a high lethality rate (US 35%, Macneil *et al.* 2011; Canada 38%, Drebot *et al*. 2000), especially in Brazil, where it reaches ~40% (Brazilian Ministry of Health 2013; de Oliveira *et al.* 2015).

Hantavirus shows high host specificity and, therefore, each Brazilian region has different reservoir species that host distinct virus strains (Pereira *et al*. 2007; Guterres *et al.* 2015). In the south and southeast of Brazil, the rodents *Oligoryzomys nigripes* and *Necromys lasiurus* are the main reservoirs for human HCPS cases of the Juquitiba and the Araraquara viruses, respectively (Suzuki *et al*. 2004, de Oliveira *et al*. 2013). Both species have a high degree of adaptability to anthropic environments, such as pastures (Martin *et al*. 2012), crops (Goodin *et al*. 2006, Umetsu & Pardini 2007, Gheler-Costa *et al.* 2012), and to native habitat edges (Santos-Filho *et al.* 2008, de Oliveira *et al*. 2015); hence, they may vary in the use of space.

Evaluating how different data sources predict disease risk and determining which data source to use in related studies can enable greater accuracy in predictions of future outbreaks. Better inferences of risk are critical to define effective surveillance, control, and mitigation programs. Additionally this will help researchers to choose the best data source in their inference studies. Some authors indicate the need for studies that include reservoir/host data in disease risk analysis (de Oliveira *et al.* 2015). However, the differences and effectiveness in disease risk predicted by these different proxies (abundance data and disease cases) remains widely unexamined until now. Here, we fill this research gap by using rodent reservoir abundance data and the number of reported HCPS cases in a case study focused on São Paulo State, Brazil. We created risk maps from both datasets and tested the following predictions:

a. The risk map based on reported HCPS cases will have more accuracy than the risk map based on abundance reservoir data, since contracting a disease requires not only the presence of the reservoir species but also a risk behavior by humans;

b. The risk maps based on abundance reservoir data and disease data will have a high similarity, since a high abundance of reservoir rodents is necessary for people to acquire HCPS.

Material and Methods

Study area

This study used data from São Paulo State, Brazil, which includes both the Cerrado and Atlantic Forest biomes and covers an area of ~248,210 km^2, with a population of ~42 million (21.5% of Brazil's population; IBGE 2014). This region is a good model for this type of study because it presents a gradient of potentially important factors of disease risk, such as landscape attributes important to the presence of rodent reservoirs – a marked gradient of forest amount (Ribeiro *et al.* 2009) and gradients of human population size and different crops (see Disease and Social data session).

Rodent abundance data

We used data on rodent abundance collected in six landscapes located in the Atlantic Forest in São Paulo State: three fragmented landscapes and three within continuous forest; each of them cover about 10,000 hectares (Pardini *et al.* 2010, Umetsu 2010). The three fragmented landscapes have 11%, 31% and 49% native forest cover, respectively (here called FClandscapes). Adjacent to each of the fragmented landscapes are the corresponding continuous forest landscapes, which we sampled as controls; these control areas contain more than 90% native forest cover (Figure 1). The landscapes include native forest fragments on intermediary successional stage; the continuous forests are a mosaic of mature and native forest in intermediate successional stage. The main types of anthropogenic matrix in the fragmented landscapes are crops, pastures, urban areas, forestry, and native vegetation in early stages of regeneration.

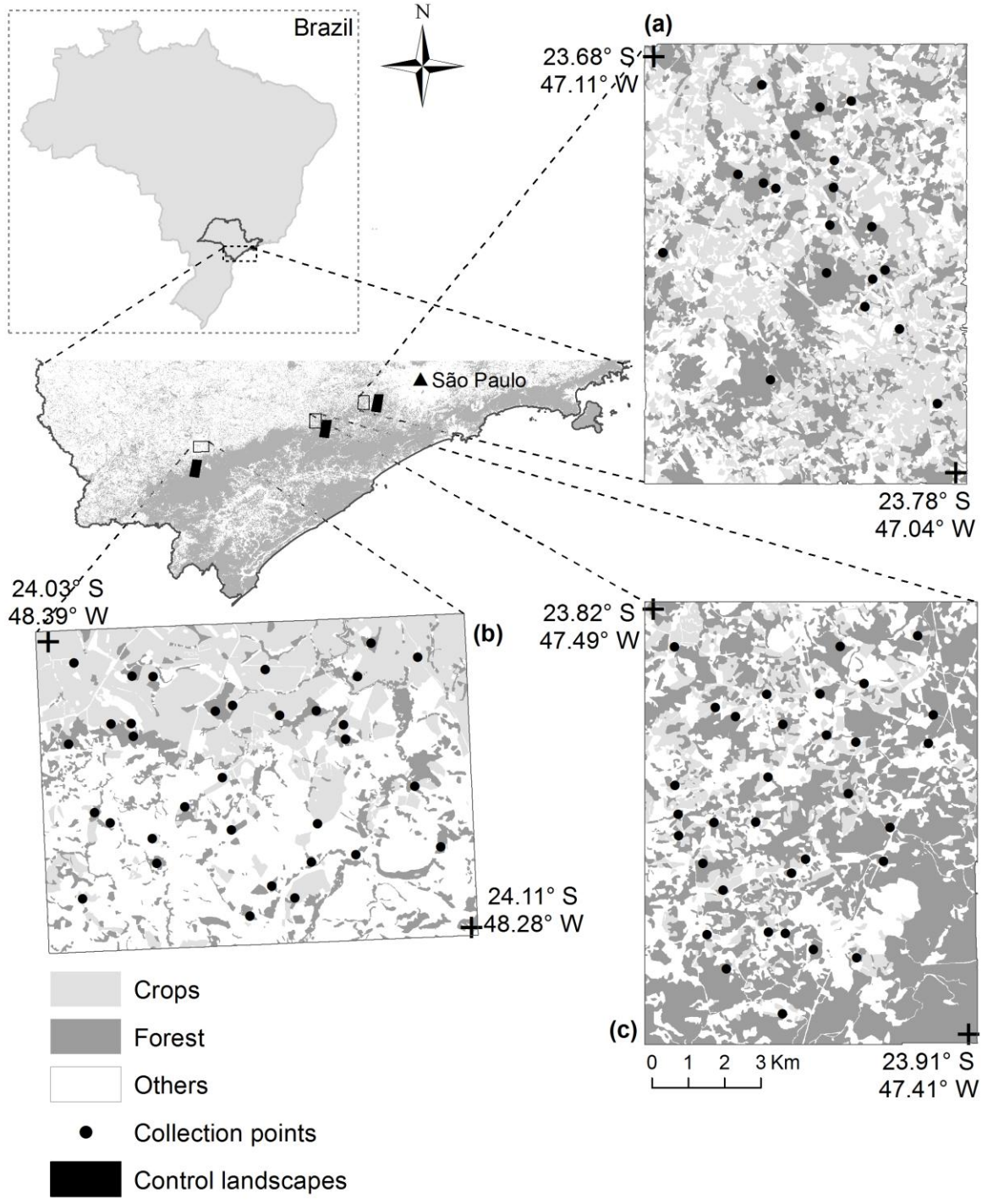

Figure 1. Location of the studied landscapes in São Paulo State, southeastern Brazil used to build model that extrapolates the abundance of rodent hosts (RRA model). (a) 31% forest cover landscape, (b) 11% forest cover landscape, and (c) 49% forest cover landscape.

Black dots show collection points and the black rectangles are the control landscapes of continuous forest sites. Adapted from Pardini *et al.* (2010).

Rodents were sampled in five summers from 2002 to 2008 at 104 sites within the six landscapes: 68 sites were in native forest, 50 of which were in fragments (15 sites in 11% and 49% native forest cover landscapes, and 20 sites in 31% native forest cover). The other 18 sites were sampled in continuous forest (six in each control landscape - see Pardini *et al.* 2010); the remaining 36 sites were in annual crops, such as corn, zucchini,, yam and tomato (18 sites in 11% and 49% native forest cover landscapes - see Umetsu 2010).

At each site, a 100-m sequence of 11 pitfalls traps (60 L) spaced 10 m apart from each other and connected by a 50-cm high plastic fence were set. For continuous forest and fragment sites, four capture sessions of eight days each (consecutive) were conducted during two consecutive summers (2002 and 2003 in 31% forest cover landscape and 2006 and 2007 in 11% and 49% forest cover landscapes), totaling 32 capture days per site, 352 trap-nights per site and 23,936 trap-nights in the 68 forest sites (Pardini *et al.*, 2010). For the sites in agricultural areas, we conducted two capture sessions of eight days each (consecutive) during the summer of 2008, totaling 16 capture days per site, 176 trap-nights per site and 6,336 trap-nights at all 36 matrix sites (Umetsu 2010). In all sites, traps were checked daily and animals were marked with numbered tags at first capture (Small animal tag-size 1; National Band and Tag Co., Newport, Kentucky), following the guidelines of the American Society of Mammalogists (Sikes *et al.* 2011).

*O. nigripe*s is a habitat generalists species found in forest and in several types of matrix (Umetsu & Pardini 2007). This species was collected in matrix, fragments and in continuous forest (here called Collection Context). For matrix sites, we calculated the abundance per site as the sum of all individuals captured over all capture sessions. For *O.*

*nigripe*s collected in forest sites, we calculated the abundance per site as the sum of all individuals captured over all capture sessions divided by 2, because the collect effort was doubled at forest in comparison to matrix sites. However, for *N. lasiurus* – typically found in open areas (Mares *et al*. 1986, Gheler-Costa *et al*. 2012) – we calculated the abundance of all sites, but we used only the 36 matrix sites for analysis because the number of individuals collected in fragments and continuous forest was very small, crippling the analysis for these sites. Therefore for this species we consider only data collected in matrix sites. The rodents collected did not had their infection rates tested, and because we did not have access to information on infection rates in hosts along a gradient of environmental conditions, we worked only with host abundances. Therefore, a limitation of our methods is the absence of information about the infection rates in rodent hosts, which we consider that should be taken into account in future studies. However, rodents were collected always in the summer and in the same period of the epidemiologic data (i.e. 1993-2012), which match with the periods of our predictor variables. Additionally, there is a lack of studies collecting small mammals in a forest cover gradient, and we could not validate the rodents host abundance extrapolations. We therefore compared our RRA model with the results of other studies conducted in São Paulo State using abundance standardized by sampling effort of the two rodent host species separately.

Disease and social data

HCPS incidences were collected at the municipal level, so we treated the 645 municipalities within São Paulo State as our sampling units. The number of reported HCPS cases in each municipality per year for the period 1993-2012 were obtained from the website of the Center for Epidemiological Surveillance of the São Paulo State (CVE-SP) and the Health Portal SUS (http://portal.saude.gov.br/). Since the majority of the municipalities had

none (98.39%) or only one case per year (1.61%), we decided to use the data as binary, reflecting presence *vs.* absence of HCPS.

Epidemiologic data indicate that more than 70% of HCPS-infected people were working or living in agricultural areas, and more than 75% were men over the age of 20 (Elkhoury *et al*. 2012, de Oliveira *et al.* 2014, Willemann & de Oliveira 2014). Because the available data are relatively coarse with respect to age distribution, we used the number of rural men older than 14 years in each municipality as the population at risk for HCPS. This information was obtained from the National Institute of Geography and Statistics (IBGE) website (www.ibge.gov.br) and was available only for 1996 and 2006. Since we wanted to model the incidence of HCPS from 1993 to 2012, thus we used the 1996 data as covariates to predict disease incidence for 1993-2001, and 2006 data to predict incidence for 2002-2012.
A number of socio-economic variables can contribute for our better understanding of HCPS transmission. HCPS occurs mostly in poorly built human dwellings and close to agricultural areas (Pereira *et al.* 2007). Its incidence reflects the living conditions in rural communities, the absence of preventive measures such as the use of masks when cleaning houses (Ferreira 2003), and the poor storage of grains in warehouses or residential houses (Pereira *et al.* 2007, CDC 2014, de Oliveira *et al.* 2015). The Human Development Index (HDI) can be used as a proxy for human development. Because of this, HDI are an appropriate metric of the socio-economic factors that influence HCPS risk, particularly for including life expectancy, income (GDP/capita) and education (literacy). Therefore, the HDI was also used as a covariate in the model. HDI data at the municipal level was obtained from IBGE (accessible at www.ibge.gov.br) and is available for 1991, 2000 and 2010. We used HDI data from 1991 as covariates to predict incidence for 1993-1998, data from 2000 to predict incidence for 1999-2005, and data from 2010 to predict incidence for 2006-2012.

Native vegetation and land-use metrics

HCPS risk map

We used the São Paulo State forest maps (www.iflorestal.sp.gov.br) for 2000 and 2010 to calculate landscape metrics (percentage of native habitat cover, and number of habitat fragments) for the native vegetation cover of each municipality in São Paulo State. This native vegetation inventory was generated at a 1:50.000 scale, with a minimum mapped area of 2.5 ha, being able to identify small fragments, which are very common in the São Paulo state. Additionally, using this inventory made possible to use information with same spatial resolution and mapping method for both cerrado and Atlantic Forest biome in the state. Native vegetation data aggregate both Atlantic Forest and Cerrado patches, and a municipality was considered as part of one biome or the other depending on the percentage of its area that overlapped with the original distribution of these biomes (see *Statistical analyses*).

We performed the landscape analyses in ArcGis 10.0 and Fragstats 4.2 (McGarigal *et al.* 2012). To match this information with available disease data, we used metrics extracted from the 2000 map as covariates to model incidence for 1993-2001, and metrics extracted from the 2010 map as covariates for period 2002-2012.–The proportion of sugarcane cultivated in each municipality was obtained from the Agricultural Census of the Institute of Agricultural Economics (www.iea.sp.gov.br) for the years 1993 to 2012. For the analyses presented here, we used annual agricultural data (1993 to 2012) to predict annual disease incidence from 1993 to 2012.

Rodent abundance risk map

We used the São Paulo State forest map for 2010 (the same used in the analysis for the HCPS risk map) to calculate landscape metrics (percentage of native habitat cover, and the amount of edge density) for the native vegetation cover of each scale, 200-m, 500-m and 800-m, radius circles around the rodent´s point of collection. These scales were set according to the species rate of movement (ca. 50 to 100 m) and the study of Jackson and Fahrig (2012),

which suggests that the appropriate range for a species is between four a nine times its average distance dispersal. Analysis were performed in ArcGis 10.0.

Climatic variables

We obtained the meteorological data from the International Research Institute for Climate and Society (IRI) Data Library system (http://iridl.ldeo.columbia.edu/index.html). Gridded land surface temperature data were obtained from National Centers for Environmental Prediction (NOAA NCEP), from the combined GHCN and CAMS station data at 0.5-degree (~50 km) spatial resolution and monthly time step from 1948 to the present. Annual mean temperature for 1993-2012 was calculated for each municipality. The temperature value of the grid cell closest to each municipality's geographic coordinates was used as representative of that location. The relatively coarse resolution of the NOAA-NCEP dataset likely resulted in more than one municipality being represented by the same gridded temperature value.

We used the University of California Santa Barbara Climate Hazards Group Infrared Precipitation with Stations (CHIRPS) dataset. Both spatial and temporal resolutions of CHIRPS are higher than those of temperature data, with 0.05-degree (~ 5 km) and 10-day precipitation average respectively. We calculated annual total amount of precipitation for 1993-2012 for each municipality. Therefore, we used precipitation and temperature annual data (1993 to 2012) to predict annual disease incidence from 1993 to 2012.

Statistical analysis

Rodent abundance risk map

We used a model selection approach (package *MuMIn* in R, Barton 2015) to analyze the influence of landscape variables (percentage of native habitat cover , and the amount of edge density) at different scales (200, 500 and 800 m) for both *O. nigripes* and *N. lasiurus* abundance data. We fitted generalized linear models for *O. nigripes* and *N. lasiurus*. For *O.*

nigripes, we fitted a normal distribution and used the following predictor variables: the percentage of native habitat cover (FC) and the amount of edge density at each scale (200, 500 and 800 m); the collection context (fragments, continuous forest, and matrix); and the percentage of native forest cover at landscape level (FClandscape; with 11, 31, 49 and ~90% native forest cover). For *N. lasiurus*, we fitted a negative binomial distribution and used the following predictor variables: FC at each scale; FClandscape; and type of crops, which was separated into 'corn' and 'others'. We added this last variable for *N. lasiurus* once Hantavirus cases are frequently associated with corn plantations (Figueiredo *et al*. 1999). All predictor variables included in the models had a correlation lower than 21%.

For both rodents and at each scale (200, 500 and 800 m), we compared the following candidate models: (1) simple models for each variable; (2) additive models for two, (3) three and (4) four variables and (5) interactive models composed by two or three variables plus one interaction (all possible interactions between two variables were considered). For both species, we constructed four null models: (1) species abundance is constant, and geographic null models; (2) only the latitude has influence; (3) only the longitude has influence; and (4) both latitude and longitude have influence. In total, there were 95models for *O. nigripes* and 35 for *N. lasiurus*.

The best models selected for each species were those with the lowest AICc values (FC at 800 m scale, FClandscape, and the interaction between collection context and FClandscape for *O. nigripes*; and FC at 800 m scale for *N. lasiurus*; Table 1). From the best models, we extrapolated the abundance of the two species to the entire state of São Paulo, using the São Paulo State forest map of 2010. The *N. lasiurus* and *O. nigripes* abundance maps were summed to build a unique abundance map for both rodents using ArcGIS 10.0.

Table 1. Slope and standard error (±SE) for each variable (FC = % native habitat cover, FClandscape = % native habitat cover in landscape, Context = context in which rodents were collected, represented by matrix, forest fragments (FR) or continuous forest (CF)), from the best models explaining the abundance of *Oligoryzomys nigripes and Necromys lasiurus* (RRA model). The value in parentheses in front of variables represents the extent of scale.

Species	*O. nigripes*	*N. lasiurus*
Predictor Variables	Slope (±SE)	Slope (±SE)
FC (800)	0.008 (±0.004)	-0.033 (±0.011)
FCLandscape	$-1.9x10^{-4}$ (±0,007)	
Context (FR)	2.472 (±0.331)	
Context (CF)	-7.273 (±17.825)	
Context (FR): FClandscape	-0.043 (±0.008)	
Context (CF): FClandscape	0.081 (±0.198)	

Reported HCPS cases risk map

The probability of Hantavirus infection risk for the state of São Paulo was calculated as a function of landscape, social and climatic factors, using a Bayesian model (Prist *et al. 2016*). The procedure to calculate the probability of Hantavirus infection risk model for São Paulo State is described in detail on (Prist *et al.* 2016). HCPS infection risk was predicted using a Bernoulli distribution and containing seven predictor variables as fixed covariates: proportion of sugarcane, proportion of native vegetation cover, number of native vegetation patches, HDI, mean annual temperature (°C), total annual precipitation (mm), and rural male population >14 years old. The likelihood of an HCPS case in municipality *i* in year *j* for the full data set across all *M* (645) municipalities in each biome (Cerrado or Atlantic Forest) is:

$$p(w) = \prod_{i=1}^{M} \prod_{j=1}^{20_p} Binomial\left(w_{ij}\right) \qquad \text{[Eqn. 1]}$$

The probability of an HCPS case in municipality *i* in year *j* is the logit:

$$logit\left(w_{ij}\right) = X_{ij}\beta \qquad \text{[Eqn. 2]}$$

where $\boldsymbol{X}_{ij}$ is the design vector of factors and covariates, and β is the corresponding vector of parameters. Municipality was included as a random effect to account for differences among these administrative units that are not captured in the fixed covariates. To facilitate interpretation, all estimated parameters were standardized by centering them on their mean and dividing by two standard deviations (Gelman *et al.* 2006). All priors were assigned as uninformative distributions. We used the *rjags* package in R (Plummer *et al.* 2014, R Core team 2014) and examined model convergence and performance via Gelman-Rubin diagnostics.

We used model results to generate a map of Hantavirus risk areas for the state of São Paulo. For each municipality, we calculated the mean of the simulated results for study years and imported the values into ArcGIS 10.0 for visualization.

Risk map comparison and validation

The Hantavirus infection risk map was classified as small (<5%), medium (≥5 and ≤10%), high (≥10 and ≤20%) and extremely high (≥20%) risks. The abundance risk map was classified as small (< 30%), medium (30≥ and ≤40%), high (40 ≥and ≤ 45%) and extremely high (>45%) risks, allowing paired comparison. Classifications were done in ArcGIS using break classes according to quantities based on natural groupings inherent in the data.

Risk maps were then compared through histograms illustrating the differences in their distributions for each category of risk, and through correlation (i.e., indicating the relationship between two datasets; calculated as the ratio of the covariance between the two layers,

divided by the product of their standard deviation) and variance (i.e., statistical measure of variance from the mean) to check their similarities and dissimilarities, using the Band Collection Statistics tool in Spatial Analyst-Multivariate. Continuous data were also compared through Pearson's correlation (between RRA and HCPS models). Thus, we ascertained whether the maps were comparable in predicting the levels of risk in the same municipalities. Subsequently, risk maps were validated with actual reported HCPS cases (2013-2015) to see which proxy had the best sensitivity (e.g., proportion of positives that are correctly identified) and specificity (e.g., proportion of negatives that are correctly identified) (Brooker *et al.* 2002) in predicting new disease cases, which we considered as indicators of accuracy. We measured sensitivity as the percentage of municipalities with actual disease cases in higher risk categories (medium, high and extremely high), and specificity was the percentage of municipalities without actual infection and/or disease that were correctly categorized as lower (small) risk.

Results

The two Hantavirus risk maps obtained from rodent reservoir abundance data and HCPS cases have a low Pearson´s correlation considering risk categories, with r=0.038 of accordance and 0.017 of covariance. Considering risk data without categorization, Pearson's correlation between HCPS and RRA maps, also shows that the maps had a low correlation: (r=0.11, p-value = 0.003). The results from the statistical model generated through rodent abundance data classified 208 municipalities as low risk (32% of the state) and 437 as medium or high risk (68%) for HCPS infection (Figure 2). According to this RRA risk map, the highest infection risk is present in municipalities in the west and north region, while the eastern part of the state, where the Serra do Mar is situated, is classified as low risk for HCPS infection (Figure 3).

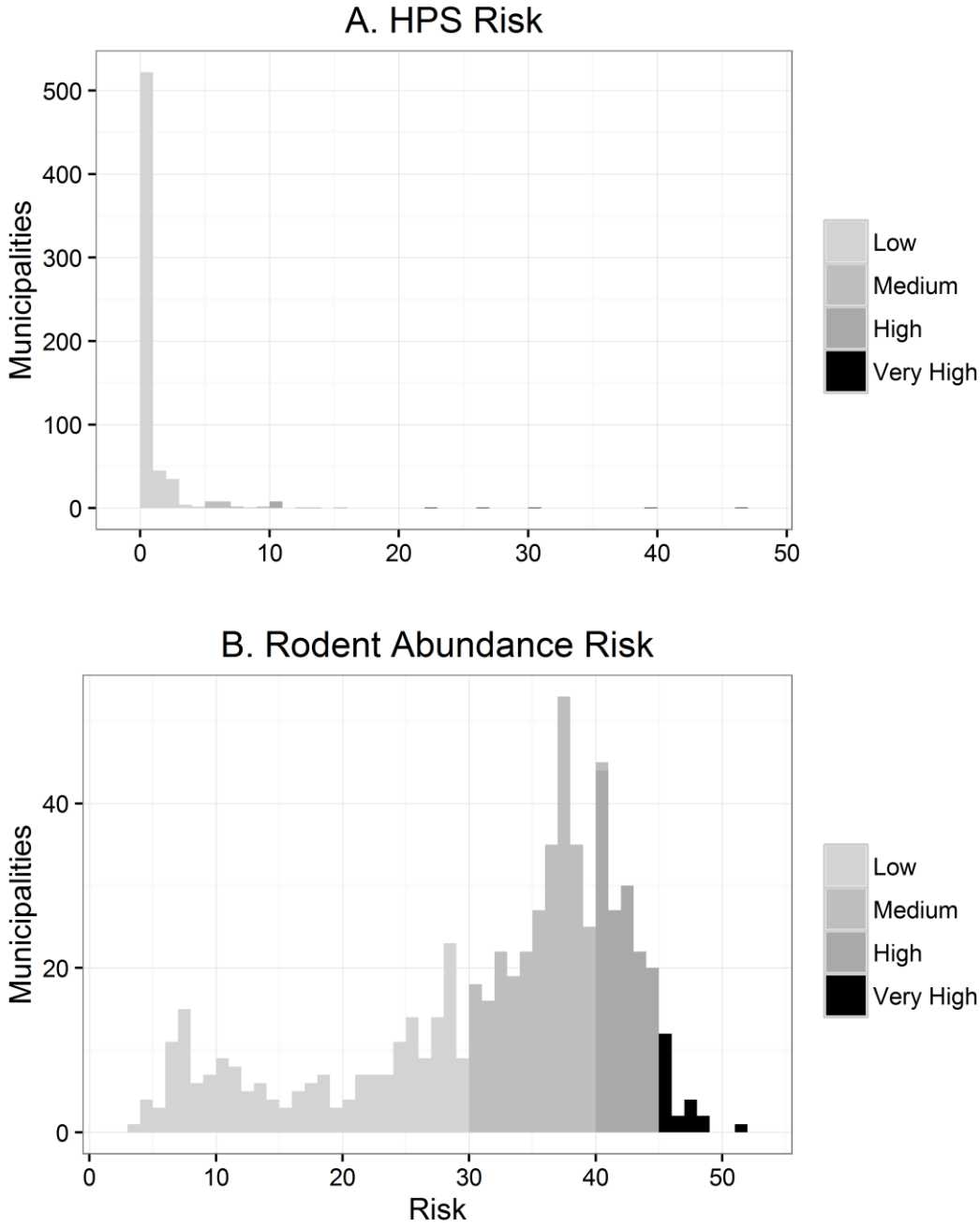

Figure 2. Number of municipalities classified in each risk category (low, medium, high and very high) according to (A) the rodent abundance and (B) Hantavirus Cardiopulmonary Syndrome risk map, São Paulo, Brazil

The results from the statistical model generated through reported HCPS cases classified 6% of the state in the medium (5-10%) or high (> 10%) risk category for HCPS infection, and 94% was classified in the low risk (<5%) category. These numbers means that 606 (94%) out 645 municipalities were classified as low risk, 21 (3.2%) as medium, 13 (2%) as high risk and 5 (0.8%) as very high risk (Table 2; Figure 2). According to this risk map, the highest infection risk is present in municipalities in the northeast region, followed by some municipalities in the east, close to Serra do Mar, and in the western part of the state (Figure 3).

Table 2. Number of municipalities out 645 classified in each risk category in each risk map predicted using reported Hantavirus Cardiopulmonary Syndrome cases (HCPS) and Rodent Abundance data (RRA), São Paulo State, Brazil.

	HCPS Cases		**Rodent Abudance**	
Risk category	**Risk Map**	**New cases**	**Risk Map**	**New cases**
Low	606	21	208	6
Medium	21	4	273	12
High	13	2	143	14
Very High	5	6	21	1

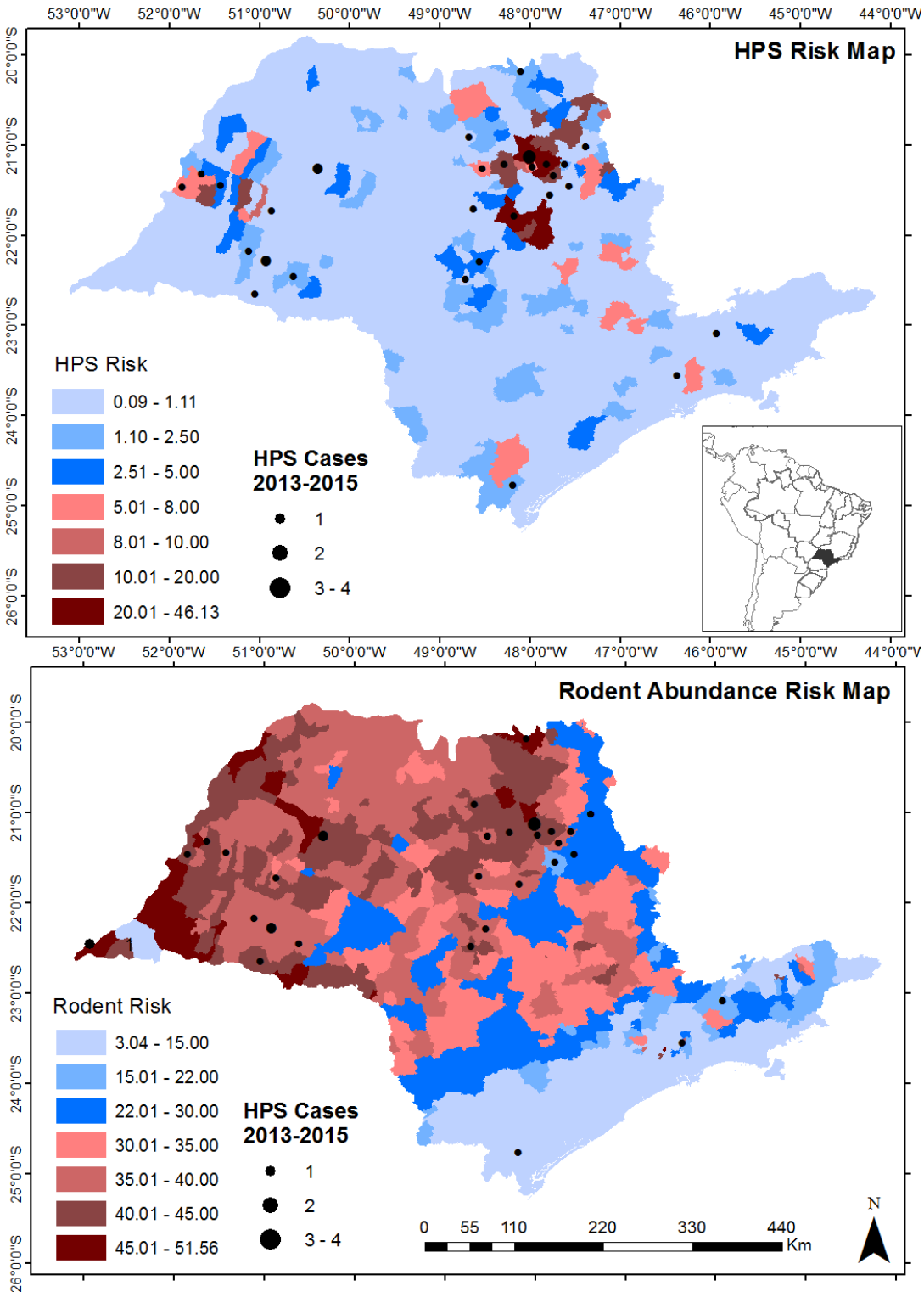

Figure 3. HCPS risk map (A) and rodent abundance risk map (B) generated through reported Hantavirus Cardiopulmonary Syndrome cases and rodent abundance data from São Paulo State, Brazil. Municipalities with black outline are medium to very HCPS high risk.

From 2013 to 2015, 33 new HCPS cases were recorded in the state of São Paulo. The HCPS risk map hit 36% (=sensitivity) of the new cases, which were reported in municipalities classified as medium to extremely high disease risk. The other 64% of cases (21) were reported in municipalities with low risk for HCPS. From these 21 cases, 15 were reported in municipalities with up to 2% risk. From the 606 municipalities classified as low risk, only 19 had HCPS cases during these two years – an error of 3.13%. Additionally, HCPS risk maps predicted that 587 (91%) municipalities would be at low risk for HCPS infection, and these presented zero new cases of the disease.

The RRA risk map identified 82% (=high sensitivity) of the new cases, which were reported in municipalities classified as medium to high risk. From the 208 municipalities classified as low risk, only six had actual HCPS cases – an error of 2.88%. Furthermore, RRA risk map predicted that 202 municipalities (31%) would be at low risk for HCPS infection. Both risk maps classified municipalities in the eastern part of the state, close to Serra do Mar region, in the low risk category, and some of the municipalities in the northwest region were categorized as high risk.

Discussion

This study is a first attempt to evaluate how different data predict disease risk, using as model the HCPS disease. On the contrary of our predictions, risk maps presented low similarities and correlation, predicting a large number of municipalities in different levels of risk category. Accuracy aspects also varied between risk maps, with this variation reflecting the non-uniformity on distribution of risk levels in the State, predicted by the use of different data. HCPS reported cases identified ~6% of the state of São Paulo (39 municipalities), while rodent abundance data (RRA) identified 68% (437 municipalities), as presenting medium-to-high risk of Hantavirus transmission. Reservoir Rodent Abundance (RRA) risk map had a better sensitivity than HCPS risk map, however HCPS risk map performed better in the

specificity. It is noteworthy that the majority of municipalities are classified as medium to high risk in the RRA risk map, making easier the chance to hit the new HCPS cases. Withal, the HCPS risk map also classified the majority of the municipalities as low risk (96%), increasing the chances to hit municipalities with no cases. In that sense both risk maps are overestimating one class of disease risk, and failing to predict others classes.

In our Reservoir Rodent Abundance (RRA) risk map, we hypothesized that areas with high reservoir abundance were associated with a higher risk of infection, which is similar to what was explored by Ryan *et al* (2004) and Diallo *et al.* (2011). Despite having a large sensitivity and identifying a great number of new cases (82%), this risk map had a higher incidence of cases than actually occurred throughout the state. Therefore, it seems that our RRA risk map is strongly over estimating high risk municipalities, which may be occurring because this risk map was extrapolated to the entire São Paulo State, through the original abundance data, which was collected specifically in Atlantic forest landscapes. Comparison of our rodent abundances extrapolations with data from published articles (Bonvicino *et al.* 2002, Briani *et al.* 2001, Gheler-Costa *et al.* 2013, Naxara *et al.* 2009, Martin et al. 2012), showed that extrapolated abundances of *N. lasiurus* differed from observed studies by an average of 2.27 individuals. For *O. nigripes* the mean difference was 3.36 individuals (Table 3). All studies but one differed from the extrapolated abundance by less than five individuals (N=11), but only in four cases they differed for less than one individual.

Table 3. Comparison of rodent host abundances (*Necromys lasiurus* = NL and *Oligorizomys nigripes* = ON) between studies performed in São Paulo state and extrapolated by this study (RRA model). Differences between sampled and RRA model abundances smaller than one are highlighted in boldface. Sampling efforts are shown in trap-nights; Standardized abundance = (individuals/trap-night)*100; Sampling effort RRA= sampling effort of data used to extrapolate rodent abundance data;

Species	**Municipality**	**Sampling effort**	**N° individuals captured**	**Standardized abundance**	**Sampling effort RRA**	**N° individuals predicted RRA**	**Standardized abundance predicted RRA**	**Difference between sampled and RRA model**	**Reference**
NL	Pedreira	1,830	24	1.31	6,336	5.98	0.09	1.22	Bonvicino *et al*. 2002
NL	Sertãozinho	5,376	157	2.92	6,336	6.89	0.11	2.81	Gheler-Costa *et al*. 2013
NL	Angatuba	1,030	23	2.23	6,336	3.21	0.05	2.18	Martin *et al*. 2012
NL	Angatuba	893	36	4.03	6,336	3.87	0.06	3.97	Martin *et al*. 2014
NL	Angatuba	3,054	38	1.24	6,336	6.34	0.10	1.14	Martin *et al*. 2016
ON	**Pedreira**	**1,830**	**15**	**0.82**	**30,272**	**25.65**	**0.08**	**0.73**	**Bonvicino *et al*. 2002**
ON	**Rio Claro**	**911**	**5**	**0.55**	**30,272**	**50.54**	**0.17**	**0.38**	**Briani *et al* 2001**
ON	**Sertãozinho**	**5,376**	**29**	**0.54**	**30,272**	**20.2**	**0.07**	**0.47**	**Gheler-Costa *et al.* 2013**
ON	Cotia	2,700	34	1.26	30,272	1.1	0.00	1.26	Naxara *et al*. 2009
ON	Angatuba	1,030	122	11.84	30,272	29.3	0.10	11.75	Martin *et al*. 2013
ON	Angatuba	893	42	4.70	30,272	3.47	0.01	4.69	Martin *et al*. 2015
ON	Angatuba	3,054	130	4.26	30,272	2.2	0.01	4.25	Martin *et al*. 2017

These differences point the level of uncertainties of our RRA model, and together with the lack of data on cerrado areas in São Paulo, they can be considered a source of bias; thus, more accurate measures of abundance that take into account typical cerrado-dominated landscapes could raise the performance of RRA risk maps. The bias might also explain some disagreements between the two risk maps, especially in the west of the state, were there is predominance of the Cerrado domain (Fig 2B). Also, the abundance of *O. nigripes* and *N. lasiurus* is only partly explained in our models by Forest Cover (FC), collection context, FC landscape and the interaction between collection context and FClandscape, and FC, respectively, as both models have a low r^2 (0.45 for *O. nigripes* and 0.14 for *N. lasiurus*). Although not examined here, other variables that influence abundance, such as habitat quality, would likely enhance and increase the predictive value of our model, and the accuracy of our disease risk map.

The spatial dynamics of hosts and reservoirs are important for understanding disease epidemiology as well as for formulating the most appropriate control strategy, because they indicate where and how far an outbreak could spread (Diallo *et al*. 2011). However, the presence and abundance of hosts and vectors alone is not enough to explain the circulation and persistence of the virus within the reservoir population (Maroli *et al*. 2015), nor does it guarantee that the disease will occur. For example, HCPS is a horizontally transmitted disease, which requires not only that a certain threshold abundance of reservoir rodents is present in a landscape, but also that a certain level of infection is circulating in this population and that an effective contact occurs between an infected rodent and a human. Disease transmission to a human requires that three main factors interact in the same time and space: an infected rodent; a certain abundance of reservoir rodents to proliferate the infection throughout the rodent population; and a susceptible human population. Therefore, the emergence of human diseases is often found to be more spatially restricted than the

distribution of the reservoir host (Schmaljohn & Hjelle 1997). This seems to be the case for HCPS in São Paulo, as it was for southern Argentina, where only 28% of the area with the highest probability for HCPS occurrence also represented the highest probability area for the reservoir rodent (Andreo *et al.* 2014). Other study also failed to detect a relationship of HCPS cases and reservoir abundance or Hantavirus antibody prevalence (Carver *et al.* 2015).

The HCPS risk map, which used reported HCPS cases as a proxy, involves all the three interacting factors, since a person can acquire HCPS only through an effective contact with the excreta of an infected rodent; in other words, this proxy is based on a more complete data. However, the HCPS risk map had a low sensitivity (36%), and a great specificity (91%), better predicting the municipalities with zero cases. Thereby, it seems that this risk map is strongly over estimating low risk municipalities, which may be occurring because HCPS can be considered a rare event in São Paulo state, and have only a few cases reported (~200 in 20 years of data available).

In a study on the West Nile Virus, the infection incidence in the vector population and the degree of clustering of human cases was consistent with the distribution, abundance and behavior of the predominant host species (Reisen *et al.* 2006). However, they did not use the abundance of host species as risk maps to guide the allocation of resources and to help implement preventive measures, as has occurred in other studies. We advertise that risk maps should be used carefully, and, whenever possible, different kinds of relevant data should be compared to test for the best model.

Due to the limitations on the use of our RRA risk map, we suggest that data on viral infection and presence of infected rodents could be associated with abundance of reservoir data. The former may be the main determinant of direct transmission (Ostfeld *et al.* 2005), but it is a proxy that is hard to obtain or extrapolate in space. Normally disease reported cases involve all aspects of the disease transmission, and should be a more complete data to be used

in modeling. However, our HCPS risk maps were modeled with reported HCPS cases, which are rare events in São Paulo State and may therefore be underestimating high risk areas and overestimating low risk areas. In that way, new studies using other diseases should compare predictions made with different data sources, in order to confirm our results. Moreover, the use of viral infection as a proxy to infer disease risk still need to be tested and compared with host abundances and disease cases, to see if it is a better inference for disease risk than these last two.

As a first and novel approach, the analyses presented here were based on types of two easily accessible epidemiological data: official reported HCPS cases and reservoir rodent abundances. This study presents an effort to initiate the discussion on which data is most appropriated for inferring disease risk. Since these models suffer from several limitations, further studies comparing these proxies needs to be done, especially with other diseases. This will help to determine whether models based only on host presence are enough to predict disease risk or if we need reported cases of disease, which involves an effective contact between the infected host and a human. Although we used HCPS as an example, the overarching concept can be extrapolated to other diseases. This is an opportune moment for the use of proxies and its validity to be further investigated, given the large number of emerging diseases affecting populations globally (Jones *et al*. 2008).

We conclude that disease predictions using different proxies also differ with respect to their modeling approaches, and their results are highly variable, also varying in their sensitivity and specificity in predicting new cases of HCPS throughout São Paulo State. According to our results, each proxy has a bias, and is good only to predict certain levels of disease risk. This represents a risk to public policies once these different results are normally used to guide the allocation of resources in preventive measures programs, educational campaigns and even to the collection of reservoir and hosts in order to implement vector/host

control policies. The use of different proxies in modeling disease risk should be used in other studies, in other regions and with different diseases and vectors/hosts, in order to discover which is the best data to be used in studies about disease inference. Our study is a first attempt in disease ecology to show that different data produce different risk maps. Despite, our models lack to be better validated because, as HCPS is a rare disease, we had few records both for the model as the validation. However, their validity can be further investigated over time, as new HCPS cases are reporting and new studies with reservoir rodents are made. Moreover, the use of different proxies should be treated carefully for predicting disease risk, and a discussion of which is the best data to use should start, once several studies are using different data to produce risk maps for several diseases around the world, and according to our results they give different results.

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