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Spatiotemporal Distribution of Visceral Leishmaniasis with Consideration of Environmental Risk Factors, Minas Gerais, Brazil, 2012-2018

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By

Shelby Lyons

Bachelor of Arts
University of Louisville
2017

Thesis Committee Chair: Uriel Kitron, PhD, MPH

An abstract of
A thesis submitted to the Faculty of the
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Abstract

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A spatiotemporal ecological study utilizing confirmed cases of VL by municipality through the Brazilian Notifiable Disease Information System (SINAN) was conducted from January 2012 through December 2018. Maps of VL cumulative and annual incidence were created to evaluate the spatial heterogeneity of disease across the state. Multivariate negative binomial regression models were developed for the overall time period, as well as annually to evaluate the association between minimum temperature, maximum temperature, total precipitation, urban residence, and normalized vegetation, and VL incidence. Local indicator of spatial autocorrelation (LISA) and Kulldorff spatial scan statistics were conducted to evaluate clustering.

Over the study period 3,501 cases of VL were reported across Minas Gerais, for a cumulative incidence of 16.5 cases per 100,000 persons. The average annual incidence rate in MG was 2.1 cases per 100,000 persons. Maximum temperature and rainfall displayed significant association with VL incidence in MG in the overall model. Annual models differed with temperature, rainfall, and urbanicity being significant across different years. Significant clustering of VL was observed in northern MG.

This study offers insight into the distribution of VL in Minas Gerais, how environmental factors are impacting the disease in the state, and where clustering is most likely occurring. These results help offer guidance on prevention and control efforts for VL in Minas Gerais, Brazil.

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LITERATURE REVIEW

Leishmaniasis is a parasitic disease caused by protozoan parasites of the genus *Leishmania*, encompassing over 20 species, found mainly in the tropics, subtropics, and Southern Europe (1, 2). Leishmaniasis is transmitted to humans through the bite of an infected female phlebotomine sandfly. Over 90 sandfly species are known to be competent vectors for transmission. Leishmaniasis is classified as a neglected tropical disease and has 3 main forms: visceral leishmaniasis, cutaneous leishmaniasis, and mucocutaneous leishmaniasis. In Brazil, the primary form of the disease is cutaneous leishmaniasis. Cutaneous leishmaniasis causes skin sores that can range from bumps to ulcers, as well as swollen glands near the sores in some individuals. Mucocutaneous leishmaniasis results in either partial or full destruction of the mucosal membranes in the nose, throat, and mouth. However, visceral leishmaniasis, known as kala-azar in Southeast Asia, is also endemic in Brazil. Visceral leishmaniasis is a more severe disease and is fatal in 95% of clinically evident cases if left untreated. The disease manifests as fever, weight loss, enlargement of internal organs (such as the spleen, lymph nodes, and liver), and low blood counts. The most common symptoms presented by visceral leishmaniasis cases in Brazil are fever and enlarged spleen (splenomegaly) combined with weakness and weight loss (3). Illness typically develops within months to years of infection. However, many *Leishmania* infections remain asymptomatic or subclinical, meaning patients show no signs and are thus unaware of infection. Diagnosis of leishmaniasis is most often made by both symptomology and confirmatory laboratory tests, either serological or parasitological, depending on the manifestation.

Leishmaniasis is considered endemic in 97 countries and territories, with 65 of those being endemic for both visceral leishmaniasis and cutaneous leishmaniasis, and 10 countries endemic for only visceral leishmaniasis as of 2017 (4). In 2016, over 90% of visceral leishmaniasis cases reported came from seven countries: Brazil, Ethiopia, India, Kenya, Somalia, South Sudan, and Sudan. Of the 22,145 cases of visceral leishmaniasis reported in 2017 by the World Health Organization (WHO), Brazil contributed 4,297 (19.4%) cases (5). Within the Americas region of WHO, Brazil accounted for over 97% of the reported cases and reported a 28% increase compared to 2016 (4, 6). The most affected population in Brazil are men (64.6% of reported cases) and individuals aged 20-49 years (32.8%) (6).

Leishmaniasis is a complex infection and disease as varying transmission cycles, multiple reservoir hosts, numerous competent sandfly species, and multiple *Leishmania* species are present in the Americas (2). In Brazil, transmission of visceral leishmaniasis is zoonotic, occurring in both rural and urban areas with *Leishmania infantum* being the causative agent. Reporting of visceral leishmaniasis cases has increased in Southern Brazil, with a 5.1% increase in the number of municipalities reporting cases between 2002 and 2014. Minas Gerais itself had an increase in rate of visceral leishmaniasis from 2.1 cases per 100,000 residents for 2001-2006 (2,360 cases) to 2.3 cases per 100,000 residents for 2007-2014 (3,612 cases), although considered non-significant (3). In Belo Horizonte, the capital of Minas Gerais, morbidity of visceral leishmaniasis was on average 4.18 cases per 100,000 persons with a mortality rate of 11.16% between 2006 and 2013 (7). Furthermore, Governador Valadares, the ninth most populous city in Minas Gerais with 263,689 residents, presented 86 cases of visceral leishmaniasis alone from 2008 to 2011 with a 16.2% lethality rate (8). It is unknown if this was an increase from prior years, however. One previous study in Minas Gerais from 2002-2013 found the distribution of visceral leishmaniasis to be spatially heterogeneous at the mesoregion level, with Central Mineira, Jequitinhonha, Metropolitan area of Belo Horizonte, Northwest of Minas, North of Minas, and Vale do Rio Doce being the primary reason for expansion and maintenance of the disease in the state, with reported incidence rates up to 26 cases per 100,000 persons (9). This indicates that some regions of Minas Gerais may be important drivers of the visceral leishmaniasis incidence in the state and should be given additional attention for control and prevention.

The primary vector of visceral leishmaniasis in Minas Gerais is the *Lutzomyia longipalpis* (Diptera, Psychodidae, Phlebotominae) sandfly (10, 11). This particular sandfly has a wide distribution across South America and primarily occurs in tropical dry forests, between 0 and 1000 meters above sea level (12). *Lu. longipalpis* prefers areas with an average temperature of 24ºC and annual rainfall of 1,000-2,000 mm. Sandflies are primarily active when the weather is warm and bite during the night (12, 13). An entomological survey conducted in Governador Valadares captured 2,539 sandflies across 8 neighborhoods in the municipality, 84.5% of which were peridomicile, living in and around human habitations (8). The neighborhoods selected were based off favorable environments for the vector. Approximately 90% of the phlebotomines collected in that study were *Lu. longipalpis*. While this is the primary sandfly present, other sandfly species have been discovered in Minas Gerais such as *Migonemyia migonei*, *Evandromyia cortelezzi*, *Ev. sallesi*, *Nyssomyia whitmani*, *Psathyromyia lutziana*, *Ev. lenti*, *Brumptomyia* sp., and *Pressatia* sp. (10). The sandfly *M. migonei* is implicated in transmission of *Leishmania infantum* in other states of Brazil (14). Some of the other sandflies mentioned above have also been involved in the transmission of *Leishmania* across Brazil and other South American countries, raising even further concern for the potential for leishmaniasis to increase in Minas Gerais (15, 16).

The maintenance of the leishmaniasis transmission cycle is often reliant on animals in some areas. Natural animal hosts of leishmaniases include canids, armadillos, anteaters, rodents, primates, marsupials, and other mammals. The primary hosts are wild animals including rodents and canids. However, dogs are the main domestic reservoir and infection has been described in felines in endemic areas of Italy and other Mediterranean countries (17). In Greece, the population of stray dogs was one of the factors contributing to incidence of leishmaniasis (18). A survey in Brazil that included 16,529 domestic dogs across 35 districts showed an average of 30.2% (4, 992) seroprevalence for visceral leishmaniasis (8). Prevalence ratios ranged from 13.6% to 53.4% across the districts. In Belo Horizonte, researchers found that human visceral leishmaniasis cases appeared to be preceded by canine visceral leishmaniasis (19). Dogs thus play an integral role in the ongoing transmission of *L. infantum* within Brazil. More recently, bats have been investigated as potentially important in leishmaniasis dynamics. In Minas Gerais, samples of blood from 448 bats within Triângulo Mineiro and Alto Paranaiba were analyzed for the presence of *Leishmania* (20). Across the 8 districts studied, 36 bats within five districts tested positive, 41.6% of which were determined to be *L. infantum* infections. However, the role of bats in leishmaniasis transmission, if any, has not been determined.

Many factors have been found to be associated with prevalence of human visceral leishmaniasis cases. Climate changes such as rising temperature, rainfall, and humidity generate advantageous conditions for reservoirs, vectors, and the complex reservoir-vector-host interaction of vector-borne diseases (21-23). Projected increases in temperature could potentially increase biting of sandflies as well as human exposure to infection (24). These projected increases would reduce the incubation time of *Leishmania* in sandflies. Furthermore, this temperature change improves winter survival, allowing expansion of the vector into new areas. In the Mediterranean, the increase of temperature and humidity was determined to affect the distribution of sandflies capable of *Leishmania* transmission (21). Furthermore, in Morocco, bioclimatic changes as well as socio-economic factors were thought to contribute to the expansion of *Leishmania* (25). Across Argentina, increases in humidity and temperature preceded increases in the presence of *Lutzomyia* species (26). Those same global environmental factors are also drivers of infectious disease in Brazil, including weather oscillations such as El Niño and La Niña, droughts, flooding, temperature, humidity, rainfall, deforestation, and land use conversion (27, 28). These are particularly implicated in vector-borne diseases such as malaria, Chikungunya, West Nile, Dengue, and leishmaniasis, although its not always a straightforward relationship (29).

A study in Tocantins, Brazil found an increase in visceral leishmaniasis incidence rates with an increase in annual precipitation, humidity, Enhanced Vegetation Index, and nighttime temperature (30). When looking at projections for visceral leishmaniasis in Brazil in another study, precipitation had a strong relationship with leishmaniasis incidence (31). Furthermore, dogs who inhabit areas with sparse (>42.5%) vegetation displayed a prevalence of canine visceral leishmaniasis from *L. infantum* that was 5.72 times as high as the prevalence of dogs inhabiting areas with less vegetation (≤42.5%) (32).

In addition to climatic factors, land use modifications driven by agriculture and farming, deforestation, mining, and other industries are linked with increases in infectious diseases, including leishmaniasis. Ruiz et. al. found that urban forms and land use decisions made in the past had effects on transmission of West Nile virus in Chicago and Detroit (33). The West Nile incidence rate was highest in urban areas with 1940-1960 housing, moderate vegetation coverage, and high population density. In Columbia, deforestation was found to be one of the factors driving cutaneous leishmaniasis, also caused by *L. infantum*, and spread by *Lu. longipalpis*, as well as livestock production and mining activities (34).

Urbanization has been connected to the increase in vector-borne diseases worldwide. Over 70% of the population in South America lives in urban areas where the combination of human, animal, and vector populations increase infection incidence (35). While leishmaniasis was previously considered a rural disease, it has largely become urban since the 1980s, with around 70% of cases being urban residents (3, 36). Some drivers of the urbanization of visceral leishmaniasis in Brazil include environmental changes by human movement and unplanned urban occupations with poor sanitation (3, 37). For example, in north-eastern Brazil, prolonged drought and the associated outcomes have led to the abandonment of rural homes and re-settlement in slums on the outskirts of urban cities (35). In these settlements, sandflies and dogs are abundant and migrants often bring livestock that is kept in close proximity to living quarters. Combine that with poor sanitation and the perfect habitat for *Lu. longipalpis* is created.

While studies across the world have looked at the impacts of environmental factors on leishmaniasis, with one study finding spatial heterogeneity at the mesoregion level in Minas Gerais, no studies of these factors have been conducted at the municipality level across Minas Gerais. This is the first known study to review visceral leishmaniasis and environmental drivers at the municipality level within Minas Gerais. It is unclear what factors are driving visceral leishmaniasis increase in the state, how those factors are distributed, and how they have changed over time. Thus, the aim of this study is to describe the incidence rates of visceral leishmaniasis by municipality in Minas Gerais from 2012-2018; examine environmental factors impacting visceral leishmaniasis in Minas Gerais during that time frame, such as temperature, rainfall, normalized vegetation indices, and urbanization; and review areas of potential high risk and clustering.

One study predicted the incidence of visceral leishmaniasis in Brazil to increase over time, estimating an approximate 15% growth by the twenty-first century compared to 1992-2002 (31). Another study in São Paulo also predicted spread of visceral leishmaniasis in that state related to environmental and socioeconomic factors (38). Key control measures mentioned by the World Health Organization include early diagnosis and treatment, vector control, effective disease surveillance, control of host species, and social mobilization and strong partnerships (2). However, the vector control of sand flies heavily relies on the control of adults since the developmental habitats are nearly impossible to find (12). Knowledge of where visceral leishmaniasis cases are clustered in Minas Gerais could provide information on where vector control measures should be prioritized. In addition, the majority of visceral leishmaniasis cases between 2011 and 2016 were reported by referral hospitals, where patients visited an average of seven health services before receiving a confirmed diagnosis. The median time between symptom onset and case diagnosis was 25 days, usually longer for adult patients (39). Targeting high rate areas for provider knowledge and awareness campaigns could be highly beneficial in reducing the time to diagnosis and/or improve accuracy of diagnoses not just for leishmaniasis but other diseases presenting similar symptoms. Overall, understanding the environmental risk factors associated with leishmaniasis in Minas Gerais, how those are distributed throughout the state, and how they have changed over time, could assist in the enhancement of prevention and control strategies to delay or inhibit expansion and prevent future outbreaks of visceral leishmaniasis.

MANUSCRIPT

ABSTRACT

Title: Spatiotemporal Distribution of Visceral Leishmaniasis with Consideration of Environmental Risk Factors, Minas Gerais, Brazil, 2012-2018

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to evaluate the association between minimum temperature, maximum temperature, total precipitation, urban residence, and normalized vegetation, and VL incidence. Local indicator of spatial autocorrelation (LISA) and Kulldorff spatial scan statistics were conducted to evaluate clustering.

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This study offers insight into the distribution of VL in Minas Gerais, how environmental factors are impacting the disease in the state, and where clustering is most likely occurring. These results help offer guidance on prevention and control efforts for VL in Minas Gerais, Brazil.

INTRODUCTION

Leishmaniasis, a protozoan parasitic disease, is considered endemic in Brazil. In 2017, over 19% of visceral leishmaniasis cases reported worldwide by the World Health Organization (WHO) and over 97% of the visceral leishmaniasis cases in the WHO Americas Region were in Brazil (4). Between 2016 and 2017, Brazil experienced a 28% increase in the number of visceral leishmaniasis cases (4, 6). In Minas Gerais, a state in Southern Brazil, the incidence rate of visceral leishmaniasis increased from 2.1 cases per 100,000 persons for 2001-2006 to 2.3 cases per 100,000 persons for 2007-2014. It is important to note, however, that authors did not find this to be significant. Urban areas in Brazil often experience higher incidence of VL, with Belo Horizonte, the capital of Minas Gerais, reporting 4.2 cases per 100,000 persons between 2006 and 2013. Visceral leishmaniasis is a more severe *Leishmania* infection and in Belo Horizonte the mortality rate from visceral leishmaniasis was approximately 11.2% (7). In some areas of Brazil, such as Governador Valadares, mortality rate has reached as high as 16.2% (8). Case fatality rates of visceral leishmaniasis range from 1.5% to 20% globally, with a 7.2% VL case fatality rate reported in Brazil during 2006 (40).

Leishmaniasis is a complex disease as transmission cycles differ based on location and multiple reservoirs, sandfly species, and *Leishmania* species are involved in transmission (2). In Brazil, both rural and urban zoonotic VL transmission occur, with *Lutzomyia longipalpis* being the primary vector for the

Leishmania infantum parasite (41). The primary hosts of VL are rodents and canids, with dogs being the primary domestic hosts. Dogs play an important role in the transmission of VL, particularly in urban areas (42). Seroprevalence of domestic dogs across Brazil has reached as high as 53.4%, with an average seroprevalence of 30.2% (8).

In addition to the transmission dynamics of VL, climatic factors have also been implicated in increased VL prevalence. Studies across the globe have found changes in temperature and humidity to precede expansion and increase of *Lutzomyia* species (21, 25, 26). In Brazil, studies have found associations between annual precipitation, humidity, Enhanced Vegetation Index, and nighttime temperature and VL incidence rates (30, 31, 43). Land use modifications, urbanization, and deforestation have also been shown to have implications in vector-borne disease transmission, including leishmaniasis (33-36). Such changes have been associated with higher prevalence of VL in dogs, higher cutaneous leishmaniasis, and optimal habitats for *Lu. longipalpis*.

To date, no known studies have assessed the impact of environmental factors of visceral leishmaniasis by municipality in Minas Gerais. It is unclear what factors are driving visceral leishmaniasis in Minas Gerais, how those factors are distributed across the state, and how they have changed over time. The aim of this study is to describe the incidence of visceral leishmaniasis by municipality in

Minas Gerais, determine environmental factors impacting Minas Gerais, and evaluate areas of high risk and potential clustering of VL cases across Minas Gerais from 2012-2018.

Previous research has predicted that the incidence of VL to increase over time, with an estimated 15% growth from 1992-2002 to the twenty-first century (31). Knowledge of where visceral leishmaniasis incidence is highest in Minas Gerais and what factors are associated with areas of high disease burden can provide valuable information on prevention and control strategies to delay or inhibit vector expansion and mitigate future outbreaks of visceral leishmaniasis.

METHODS

Ethical considerations

The protocol for this study was approved by Emory University Institutional Review Board (IRB: IRB00089431). No identifiable information was received as case data were aggregated by municipality and taken from a publicly available data source.

Study setting and population

This study was conducted in Minas Gerais, an inland state in southeastern Brazil. Minas Gerais is the fourth largest Brazilian state and contains the second largest population. The capital and largest city of Minas Gerais is Belo Horizonte, located in the south-central area of the state. The total population of Minas Gerais is 19,597,330, with 85.3% of the population living in urban areas. Minas Gerais terrain is primarily highlands, with an average elevation of 790 meters above sea level. Belo Horizonte is 853 meters above sea level with average temperature of the warmest month, February, being 22°C and the average temperature of the coldest month, July, being 17°C. Rainfall throughout Minas Gerais averages 1,000-1,500 mm per year. Populations in urban areas generally have better living conditions and access to healthcare.

Data collection

Surveillance for visceral leishmaniasis is passive, with healthcare providers reporting cases to the state. The total numbers of visceral leishmaniasis cases by municipality for the years 2012-2018 were obtained from Information System of Diseases Notification (Sistema de Informação de Agravos de Notificação—SINAN) open source data portal (44). Numbers of cases were obtained for reporting municipality and resident municipality. Additional variables obtained from the system included the residence type of cases (urban, rural, periurban) and the sex and age distribution of cases by municipality.

Social demographic data of interest such as population and percent urban population for each municipality, were obtained through the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatıstica, IBGE) (45).

Climate data were obtained through the WorldClim database for 2012-2018 (46). Variables obtained included minimum monthly temperature, maximum monthly temperature, and total monthly rainfall. Annual minimum temperature for the coldest month, annual maximum temperature for the hottest month, and total annual precipitation were calculated in Excel from these data. Monthly normalized difference vegetation index was obtained through MODIS using the AppEEARS platform for 2012-2018 (47, 48). From this data, average annual NDVI value was calculated, as well as minimum and maximum annual NDVI values.

Statistical analysis

All analyses were conducted using R, R Studio, and Microsoft Excel (49-51). Aggregated case data by municipality were reviewed by age, sex, urbanicity of residence, and year of diagnosis. A multivariable negative binomial regression model was developed accounting for overdispersion to analyze associations between environmental variables of interest and our outcome (incidence of visceral leishmaniasis) at the municipality level. Collinearity was assessed and corrected by removal of variables determined to be collinear (VIF over 10). Backwards elimination was conducted to reach all final models. Models were conducted for each individual year, as well as an overall model for 2012-2018. Annual models included minimum annual temperature, maximum annual temperature, total annual precipitation, percentage of the population considered urban, and average monthly normalized difference vegetation index as predictors. The overall model included average minimum temperature, average maximum temperature, average annual precipitation, percentage of the population considered urban, and average annual normalized difference vegetation index as predictors. Model fit was assessed using Nagelkerke R-squared values. Municipalities with no cases reported across the 7 year time-span of the study were treated as missing for statistical analyses.

Spatial analysis

Spatial analyses were conducted at an aggregated municipality level. Spatial distribution of annual incidence of visceral leishmaniasis by municipality per 100,000 persons was examined, as well as cumulative incidence mapping by municipality per 100,000 persons. In addition, significantly high risk areas were evaluated using standardized incidence ratios at a 5% significance level. Maps were also produced in R Studio using SAD69(96) UTM zone 25S spatial referencing.

Goodness of fit testing followed by Tangos test were conducted on total resident cases to evaluate spatial heterogeneity at municipality level, both aspatially and spatially. These statistics were then used to create a probability map for identified excess risk areas. Spatial autocorrelation was tested via Morans I statistics. Global Morans I for Poisson was evaluated, followed by Constant Risk

Local Indicators of Spatial Association (LISA) to visualize potential clusters of visceral leishmaniasis across Minas Gerais. Kulldorffs Scan Statistics were also applied to examine the most likely cluster of cases in Minas Gerais.

RESULTS

Study area and population

A total of 3,501 cases were identified through the Sistema de Informação de Agravos de Notificação (SINAN) database in Minas Gerais from January 2012 – December 2018. Minas Gerais is comprised of of 853 municipalities (Figure 1). Of those, 294 (34.5%) reported at least one visceral leishmaniasis case in residents of their municipality during the study period (Figure 2). The majority of cases were male (64.5%), urban residents (77.8%), and under the age of 40 (61.7%) (Table 1). The lowest incidence year was 2013, with only 347 cases reported. Twenty-five percent (875) of the cases reported during the study time occurred during 2017, a substantial increase compared to previous years and 2018. The total population of Minas Gerais was 21,168,791 in 2010, resulting in a cumulative incidence of 16.5 cases per 100,000 persons during the study period. The average annual visceral leishmaniasis incidence for the state was 2.1 cases per 100,000 persons. São João das Missões in northern Minas Gerais with a population of 13,014 (2019), reported the highest cumulative incidence (268.9 cases per 100,000 persons) and the highest average annual incidence (38.4 cases per 100,000 persons) during the study period, although only 35 cases were reported from 2012-2018.

Environmental conditions varied across the state and by year, with minimum temperatures ranging 3.1 to 17.0°C during the study period, maximum temperatures ranged from 24.1 to 34.9°C, total precipitation ranging 432 to 2,121 mm, and average NDVI ranging 2,722 to 8,029 (Table 2).

Multivariate analysis

For the overall model, maximum temperature and total precipitation were significantly associated with visceral leishmaniasis incidence in Minas Gerais at a 5% significance level (Table 3). Maximum temperature displayed a positive association (β=0.1154) with visceral leishmaniasis while total precipitation displayed a negative association (β=-0.0025). Annual model results varied, with different covariates being significantly associated with visceral leishmaniasis incidence throughout the study period (Table 4). Minimum temperature was significantly positively associated with VL incidence during the years of 2012 (β=0.2395) and 2014 (β=0.3108). Total precipitation was significantly negatively associated with VL incidence during 2014 (β=-0.0012), 2015 (β=-0.0026), 2016 (β=-0.0039), 2017 (β=-0.0032), and 2018 (β=-0.0020). Maximum temperature was significantly positively associated with VL incidence in 2016 (β=0.2435). The percent of the population considered urban was statistically significant for 2017 only (β=-0.0086), and borderline insignificant in 2018 (β=-0.0097, p = 0.05). Average NDVI was not significantly associated with VL incidence in any of the annual models. In addition, no covariates were considered statistically significant in 2013. The overall model accounted for 66% of the variation of visceral leishmaniasis incidence, while the annual models accounted for 14% to 56% of the variance.

Spatial analyses

A cloropleth map was generated for resident municipality prevalence (Figure 2), cumulative incidence (Figure 3) as well as annual incidence (Figure 4). These maps were also generated for reporting municipality (Figures S1-S3). Cumulative incidence across Minas Gerais appears to be spatially heterogeneous, with the majority of states in northern Minas Gerais reporting cases in residents, and few states in the southern half reporting cases (Figure 3). Annual incidence maps suggest differences in the distribution of visceral leishmaniasis throughout the state and by year. Aspatial and spatial Bayes smoothing were performed for annual incidence, with aspatial Bayes being a better representation than spatial Bayes (Figures S4 and S5), but neither method of smoothing with the current data is the optimal data representation.

Significantly high risk areas appear to be in the northern region, or more urban, especially in low incidence years such as 2013, with significant risk expanding to nearby municipalities during epidemic years, such as 2017 (Figure 5). However, the cumulative high risk areas exhibit a similar distribution to epidemic years in this study (Figure 6). The significantly higher risk areas are primarily in northern Minas Gerais, with other significant areas scattered across the state. Goodness of fit, Tangos test, and Morans I for clustering all indicated significant clustering of visceral leishmaniasis in Minas Gerais during the study period. Clustering of high incidence rates were present in northern Minas Gerais, with low rate clustering in the southern portion of the state (Figure 7). Discordant municipalities were also present, with high-low municipalities being identified. For low incidence years, such as 2013, less clustering of both high and low rates was observed (Figure 8). During epidemic years, such as 2017, the clustering closely matches the cumulative pattern, with significant high rate clustering in the north and some small clusters elsewhere in the state. Kuldorff scan statistics also identified the northern region as the most likely location for clustering of high incidence areas (Figure 9).

DISCUSSION

Visceral leishmaniasis (VL) is considered endemic in Brazil, with the country reporting 97% of cases in the World Health Organization's Americas region during 2018 (2). Several studies within Brazil have evaluated visceral leishmaniasis, primarily in northern states (3, 7-9, 19, 20, 27, 38, 42, 52-55). Only one study was found that evaluated the disease across Minas Gerais at a mesoregion level (9). That study indicated that the distribution of cases was spatially heterogeneous with a large portion of cases occurring in the northern region of the state. However, visceral leishmaniasis data in the state are still limited. This is the first known study to look at the spatiotemporal distribution and clustering of visceral leishmaniasis at the municipality level in Minas Gerais.

Overall, the results of this study support the previous study performed in Minas Gerais at the mesoregion level. Municipalities in the northern half of the state generally appear to have higher cumulative incidence throughout the study period than southern municipalities, as shown by the figures presented. The municipalities in southern Minas Gerais reporting high cumulative incidence are primarily near cities such as Belo Horizonte and Governador Valadares. The average annual incidence for Minas Gerais in this study was consistent with prior studies at 2.1 cases per 100,000 persons (3). One municipality in the northern region, São João das Missões, had the highest average annual incidence rate at 38.4 cases per 100,000 persons, despite having a relatively small population (13,1014 people). It is however, one of the poorest municipalities in the state and relies primarily on agricultural practices. The areas of significant risk in the north remain, even in low incident years such as 2013, but expand significantly to neighboring municipalities during epidemic years, as observed in 2017. In response to these dynamics, control and prevention measures should consistently be focused on the high risk areas observed in 2013, but neighboring municipalities as identified in 2017 should also be prepared for outbreaks in order to most effectively utilize resources, mitigate and respond to outbreaks, and reduce visceral leishmaniasis burden in the state.

Interest and research in environmental change and infectious diseases such as visceral leishmaniasis have increased, with many studies evaluating the relationships across the world (15, 23, 25-27, 42, 55-58). However, the environmental factors driving visceral leishmaniasis in Minas Gerais remain unclear. This is also the first known study to assess environmental factors such as temperature, rainfall, urbanicity, and vegetation on the disease in the state by municipality. Environmental conditions across the state varied by year, as anticipated. For the overall model, only maximum temperature and total precipitation were significantly associated with visceral leishmaniasis in Minas Gerais from 2012-2018. The model did account for 66% of the variance of visceral leishmaniasis however, indicating that the factors considered (minimum temperature, maximum temperature, rainfall, urbanicity, and NDVI) were drivers of visceral leishmaniasis in Minas Gerais during the study period. For the overall model, maximum temperature was shown to be positively associated with visceral leishmaniasis, meaning that as temperature increased, so did the incidence of visceral leishmaniasis on average throughout Minas Gerais during the study period. Total precipitation however, was shown to be negatively associated with visceral leishmaniasis incidence, meaning that as the total amount of rain increased, visceral leishmaniasis decreased on average throughout the study period. This is an interesting finding in that the range of precipitation values are within the preferred rainfall range of *Lutzomyia longipalpis.* While minimum temperature, urbanicity and NDVI were not found significant in the overall model, they were significant in annual models. In 2012 and 2014, higher minimum temperature was shown to be associated with increases of visceral leishmaniasis incidence on average. In 2017, as percentage of the population considered urban increased, the incidence of visceral leishmaniasis decreased on average, another interesting finding as urban transmission in Brazil and Minas Gerais is common (3, 35-37). This suggests that non-urban transmission might also be common in Minas Gerais. In addition, as NDVI increased in 2017, visceral leishmaniasis decreased on average, meaning lower NDVI was associated with higher incidence of visceral leishmaniasis. During the lowest incidence year, none of the variables were significantly associated with visceral leishmaniasis in Minas Gerais, but it is possible that a lack of data led to those results. The year with the highest incidence (2017) showed significant associations between the total precipitation and urbanicity and visceral leishmaniasis incidence. That model accounted for 56% of the variance in visceral leishmaniasis incidence during that year, indicating these factors potentially contributed to the increase in cases observed during that year. Adding additional variables, such as humidity could enhance the models performed in this study. These findings are in agreement with studies conducted in other parts of Brazil suggesting that environmental conditions contribute to visceral leishmaniasis in the country. However, the factors determined significant and their directionality vary. Prior studies have shown increases in temperature, humidity, Enhanced Vegetation Indices, and precipitation to lead to increases in

Lutzomyia longipalpis, thus increasing incidence of visceral leishmaniasis (27, 30, 31). Studies have also shown that urbanization leading to unplanned occupations has resulted in more suitable habitats for the vector and thus transmission (35-37). As urbanization and land use modification continue, and climate changes, these factors may continue to have implications on visceral leishmaniasis in the state through the creation of more favorable conditions for *Lutzomyia longipalpis*, although more research is needed.

The main limitation of the current study is the reliance on passive surveillance. Depending on healthcare provider reporting often leads to underreporting, especially in underserved areas (53, 59). Therefore, it is likely that the number of visceral leishmaniasis cases and true burden of disease in Minas Gerais are much higher. The difference between cases in reported municipality and resident municipality indicate that access to health care may be a factor in the treatment and thus in reporting of visceral leishmaniasis cases. It is also a potentially important confounder in the relationship between environmental and social factors and visceral leishmaniasis incidence in Minas Gerais. Therefore, seroprevalence studies are needed for better estimates of the true prevalence and distribution of disease in the state. Future studies should also consider how other social factors, such as access and distance to health care facilities, impact visceral leishmaniasis incidence, given that a previous study mentioned social marginality impacting cutaneous leishmaniasis (60).

In addition, potential misclassification of location is highly possible in this study, as it is virtually impossible to ascertain where individuals were infected with *Leishmania*. With many roads linking municipalities in Minas Gerais, and many residents around cities such as Belo Horizonte and Governador Valadares commuting to the city, it is likely that some of the cases were actually infected somewhere other than residence. Ascertaining travel history of cases could alleviate some of this misclassification, especially in municipalities outside of high transmission areas, such as south or west Minas Gerais.

This is the first known study to evaluate the spatiotemporal distribution of visceral leishmaniasis in Minas Gerais at the municipality level, with considerations of environmental risk factors. The results highlight that environmental factors do play a role in visceral leishmaniasis in Minas Gerais, with clustering of cases in the northern region of the state. Research efforts should continue to evaluate visceral leishmaniasis in the state and factors driving incidence. Specifically, studies should be conducted to assess additional environmental factors, such as humidity, and social factors such as access to care in combination with the factors evaluated in this study. In addition, further review of the northern region of Minas Gerais and evaluation of factors in that region specifically could increase understanding of the dynamics driving incidence in the high risk areas, and potentially reduce overall disease burden in the state. Despite the above mentioned limitations, the results observed in this study can help health officials in Minas Gerais better allocate resources to prevent and control visceral leishmaniasis, by contributing to understanding of the distribution of disease in the state, and of the factors that are potentially driving incidence.

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TABLES

Table 1. Descriptive Characteristics of Visceral Leishmaniasis Cases in Minas Gerais, Brazil, 2012-2018.

Characteristic	Number of Cases (%) N = 3,501
Sex	
Female	1,242 (35.5)
Male	2,259 (64.5)
Age	
≤1 – 19	1,340 (38.3)
20 – 39	820 (23.4)
40 – 59	852 (24.3)
60 – 79	409 (11.7)
≥80	80 (2.3)
Urbanicity Reported	
Rural	594 (17.0)
Periurban	22 (0.6)
Urban	2,723 (77.8)
Ignored/Blank	162 (4.6)
Year of Diagnosis	
2012	409 (11.7)
2013	347 (9.9)
2014	393 (11.2)
2015	477 (13.6)
2016	566 (16.2)
2017	875 (25.0)
2018	434 (12.4)

Table 2. Descriptive Characteristics of Environmental Factors Considered in Minas Gerais, Brazil, 2012-2018.

Environmental Factor	Median (IQR)
Minimum Monthly Temperature	
2012	11.2 (9.7 – 12.6)
2013	11.0 (9.4 – 12.7)
2014	10.8 (9.3 – 12.2)
2015	11.5 (10.3 – 13.0)
2016	10.8 (9.3 – 12.7)
2017	10.0 (8.7 – 11.4)
2018	11.6 (10.2 – 12.9)
Maximum Monthly Temperature	
2012	29.6 (28.7 – 30.9)
2013	29.3 (28.2 – 30.7)
2014	29.7 (28.8 – 31.0)
2015	30.2 (29.3 – 31.6)
2016	30.1 (29.1 – 31.4)
2017	29.8 (29.0 – 31.4)
2018	29.3 (28.4 – 30.8)
Total Precipitation	
2012	1,259.7 (1,067.5 – 1,487.0)
2013	1,361.1 (1,221.7 – 1,440.1)
2014	941.6 (852.0 – 1,195.2)
2015	1,004.0 (797.5 – 1,413.4)
2016	1,168.5 (992.3 – 1,390.1)
2017	1,099.0 (1,002.3 – 1,334.6)
2018	1,435.9 (1,241.0 – 1,513.1)
Average Monthly NDVI	
2012	6,488 (5,959 – 6,850)
2013	6,662 (6,083 – 7,003)
2014	6,155 (5,715 – 6,545)
2015	6,561 (5,968 – 6,962)
2016	6,450 (5,824 – 6,795)
2017	6,349 (5,825 – 6,762)
2018	6,642 (6,142 – 7,005)

Abbreviations: NDVI – Normalized difference vegetation index; IQR – Interquartile range

Table 3. Overall Multivariate Analysis of Association between Environmental Risk Factors and Visceral Leishmaniasis Incidence in Minas Gerais, Brazil, 2012-2018.

Covariate	**Coefficient (β)**	**Std. Error**	**P Value**	**R squared**
Overall Model				**0.66**
Intercept	-8.20	1.70	<0.001*	
Average Minimum Temperature	0.0157	0.0528	0.77	
Average Maximum Temperature	0.1154	0.0530	0.03*	
Average Total Precipitation	-0.0025	0.0003	<0.001*	
Percent Population Urban, 2010	-0.0025	0.0030	0.42	
Average NDVI	-0.0001	0.0001	0.11	

*Statistically significant at 5% significance level ($p < 0.05$)

Table 4. Annual Multivariate Analysis of Association between Environmental Risk Factors and Visceral Leishmaniasis Incidence in Minas Gerais, Brazil, 2012-2018.

Covariate	Coefficient (β)	Std. Error	P Value	R squared
2012				**0.14**
Intercept	-9.66	3.48	0.005*	
Minimum Temperature	0.2395	0.1065	0.02*	
Maximum Temperature	-0.0404	0.1074	0.71	
Total Precipitation	-0.0008	0.0005	0.09	
Percent Population Urban, 2010	0.0063	0.0069	0.36	
Average NDVI	-0.0003	0.0002	0.08	
2013				**0.16**
Intercept	-16.45	3.642	<0.001*	
Minimum Temperature	0.1207	0.1180	0.31	
Maximum Temperature	0.1967	0.1212	0.11	
Total Precipitation	-0.0007	0.0008	0.35	
Percent Population Urban, 2010	0.0052	0.0070	0.46	
Average NDVI	-0.0002	0.0002	0.34	
2014				**0.23**
Intercept	-9.14	2.84	0.001*	
Minimum Temperature	0.3108	0.0888	<0.001*	
Maximum Temperature	-0.0719	0.0953	0.45	
Total Precipitation	-0.0012	0.0004	0.007*	
Percent Population Urban, 2010	-0.0033	0.0053	0.53	
Average NDVI	-0.0003	0.0002	0.09	
2015				**0.46**
Intercept	-10.86	2.58	<0.001*	
Minimum Temperature	0.0331	0.0089	0.71	
Maximum Temperature	0.0992	0.0783	0.21	
Total Precipitation	-0.0026	0.0004	<0.001*	
Percent Population Urban, 2010	-0.0012	0.0050	0.80	
Average NDVI	-0.0001	0.0001	0.45	
2016				**0.55**
Intercept	-10.77	2.77	<0.001*	
Minimum Temperature	-0.1367	0.0858	0.11	
Maximum Temperature	0.2435	0.0833	0.003*	
Total Precipitation	-0.0039	0.0006	<0.001*	
Percent Population Urban, 2010	0.0016	0.0047	0.74	
Average NDVI	-0.0002	0.0001	0.13	
2017				**0.56**
Intercept	-8.63	2.30	<0.001*	
Minimum Temperature	0.0954	0.0717	0.18	
Maximum Temperature	0.0672	0.0713	0.35	
Total Precipitation	-0.0032	0.0005	<0.001*	

Percent Population Urban, 2010	-0.0086	0.0040	0.03*	
Average NDVI	-0.00003	0.0001	0.79	
2018				**0.47**
Intercept	-10.82	2.61	<0.001*	
Minimum Temperature	-0.0063	0.0894	0.94	
Maximum Temperature	0.1466	0.0829	0.08	
Total Precipitation	-0.0020	0.0004	<0.001*	
Percent Population Urban, 2010	-0.0097	0.0050	0.05	
Average NDVI	-0.0001	0.0001	0.52	

*Statistically significant at 5% significance level ($p < 0.05$)

FIGURES

Figure 1. Map of Minas Gerais, Brazil.

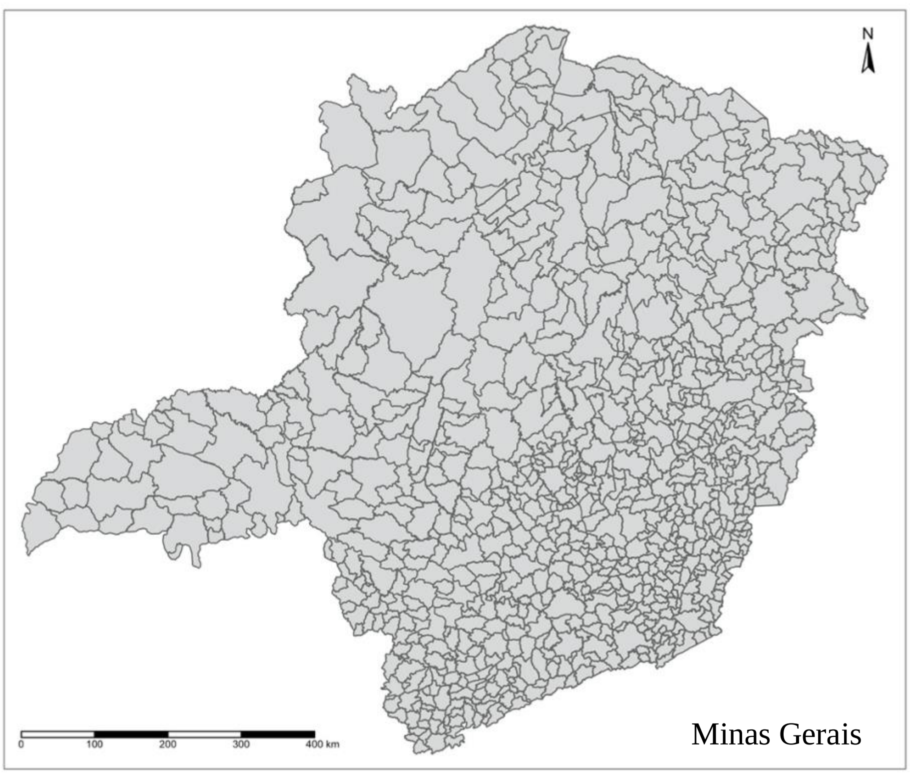


Figure 2. Distribution of Visceral Leishmaniasis Cases by Resident Municipality, Minas Gerais, Brazil, 2012-2018.

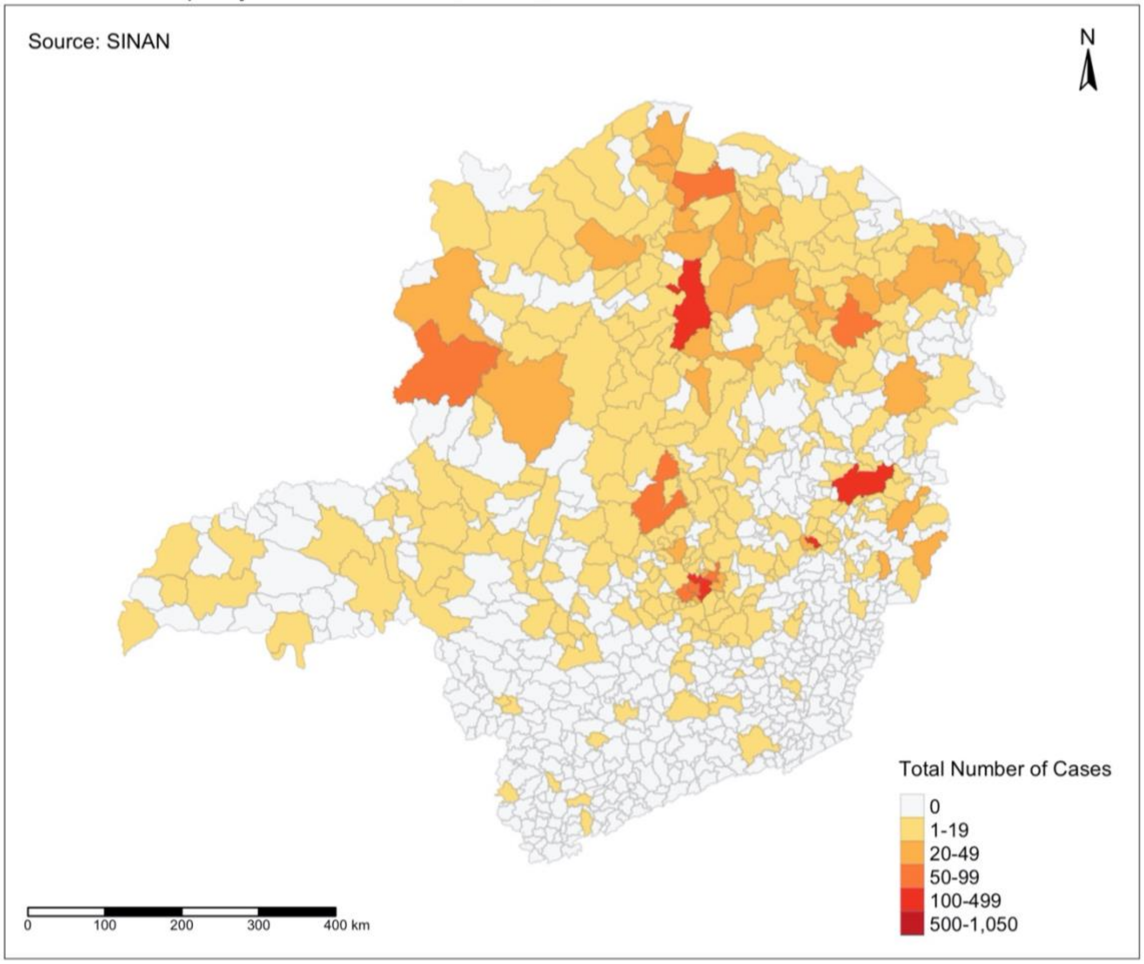


Figure 3. Cumulative Incidence of Visceral Leishmaniasis by Resident Municipality in Minas Gerais, Brazil, 2012-2018.

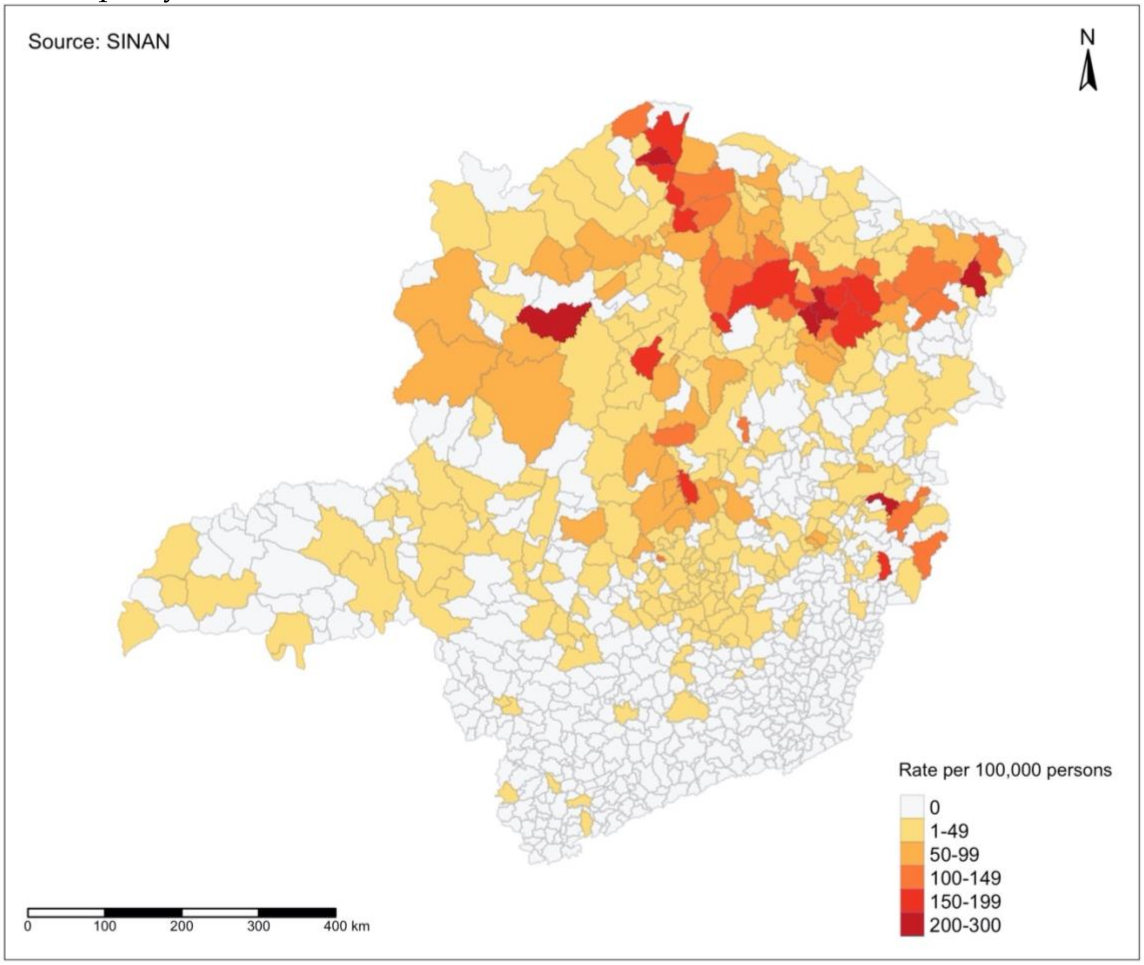


Figure 4. Annual Visceral Leishmaniasis Incidence by Resident Municipality in Minas Gerais, Brazil, 2012-2018.

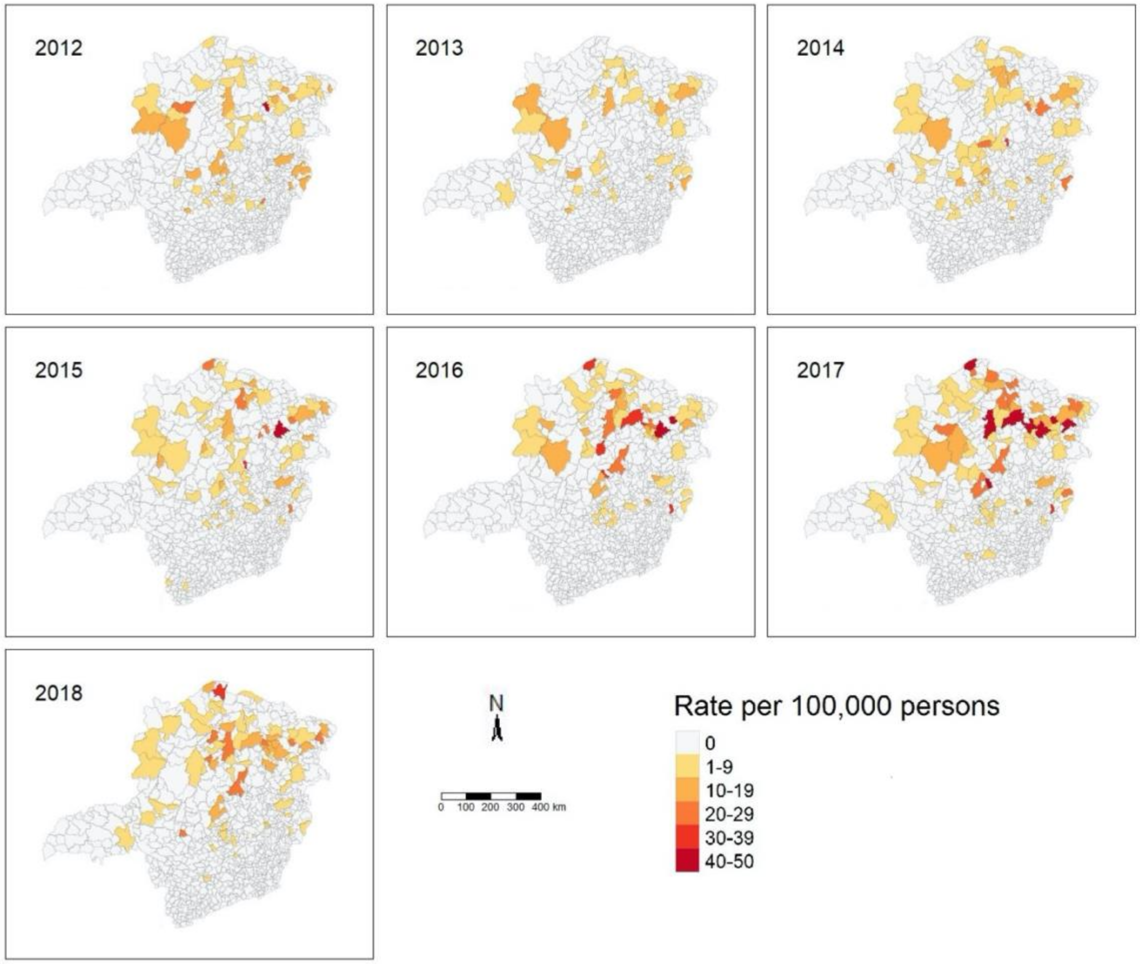


Figure 5. Municipalities Considered Signficiant High Risk Areas for Visceral Leishmaniasis in Minas Gerais, Brazil, 2012-2018.

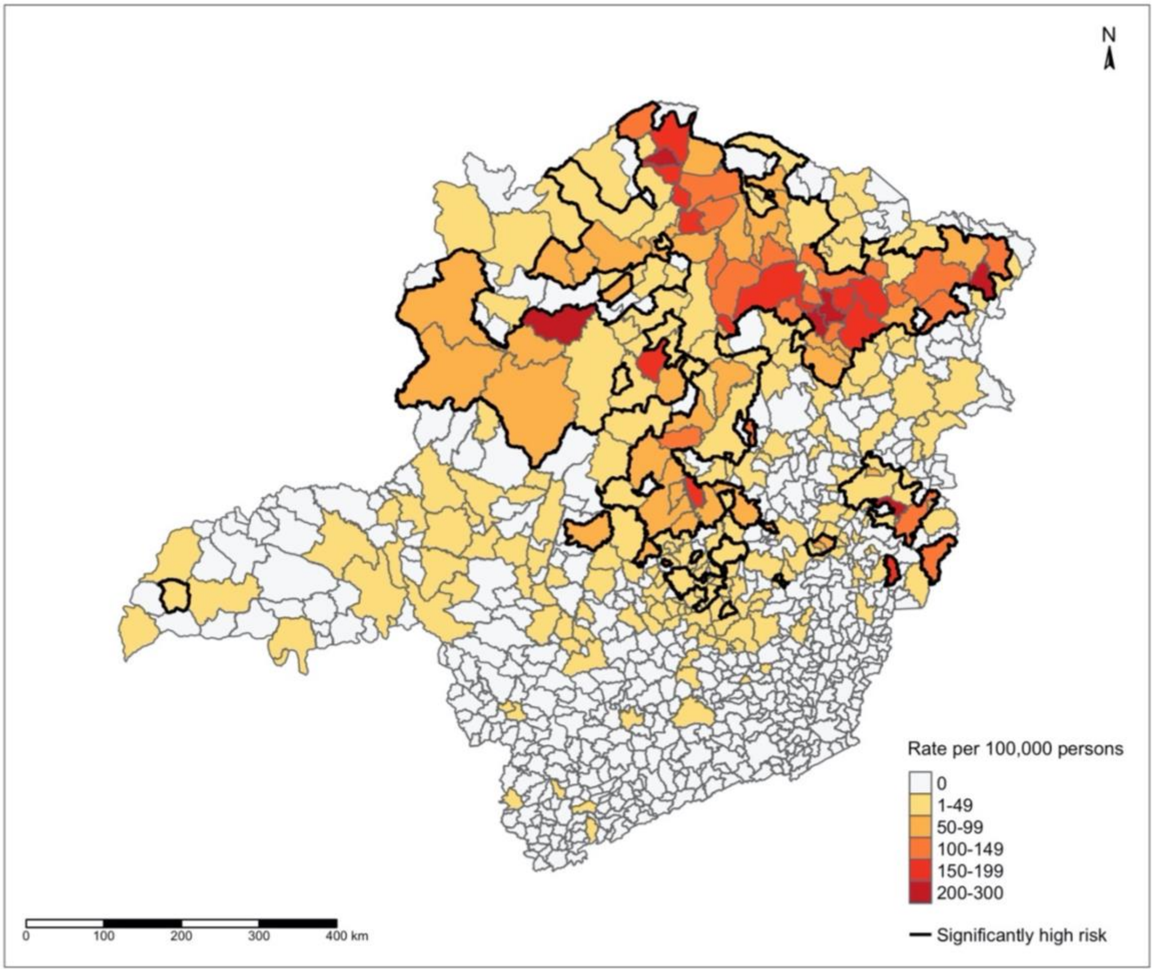
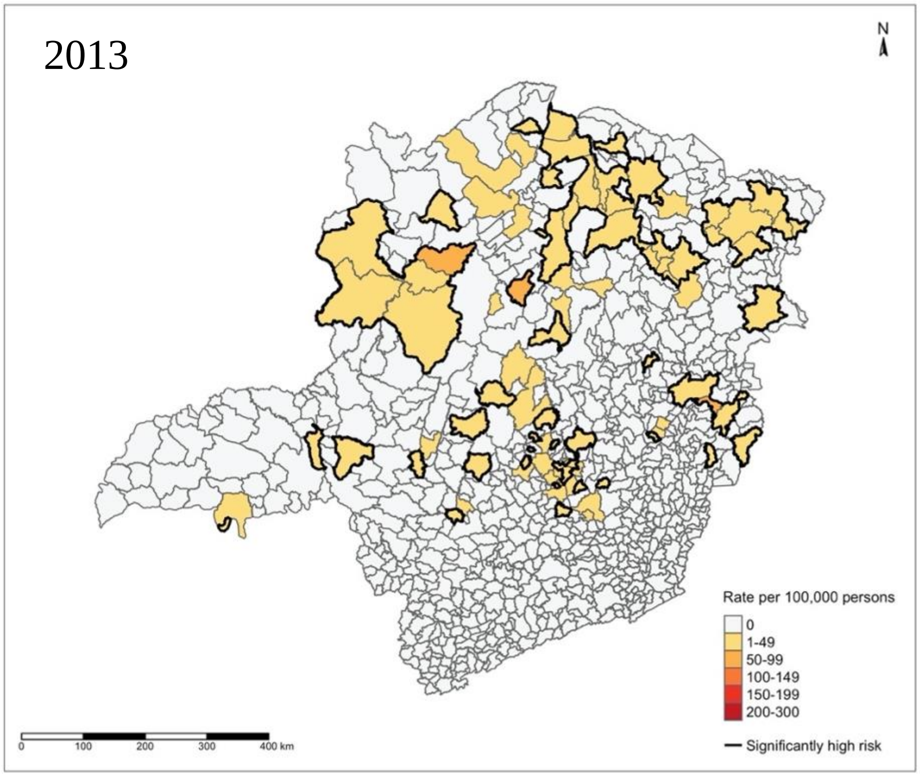


Figure 6. Municipalities Considered Signficiant High Risk Areas for Visceral Leishmaniasis in Minas Gerais, Brazil, 2013 & 2017.



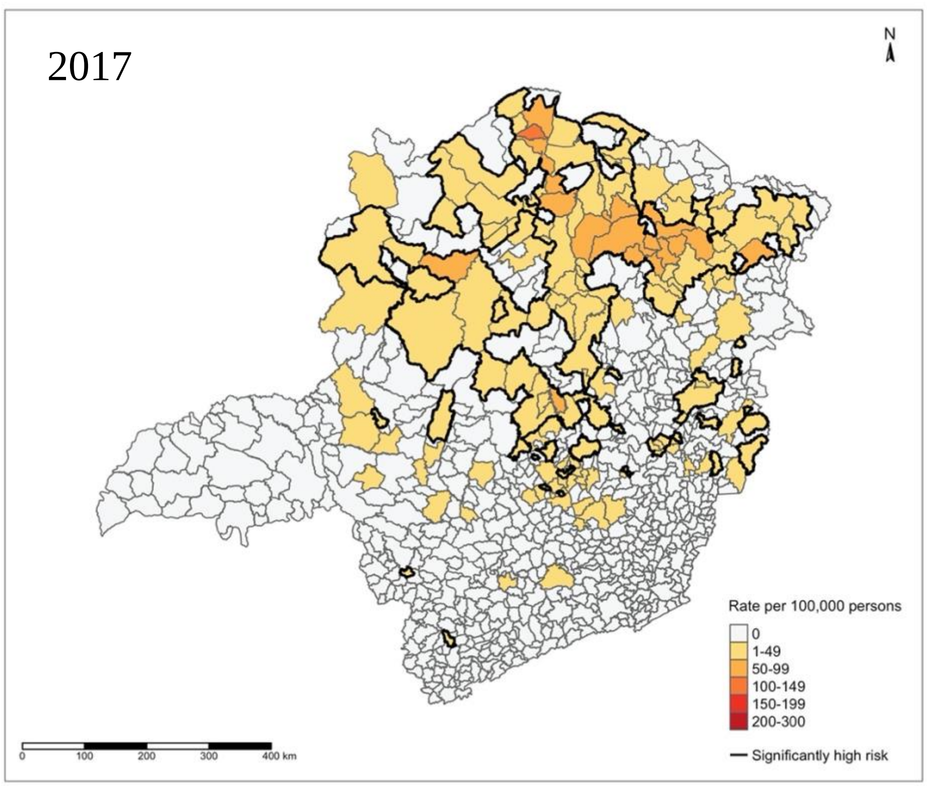


Figure 7. Local Spatial Autocorrelation of Visceral Leishmaniasis Incidence, Minas Gerais, Brazil, 2012-2018.

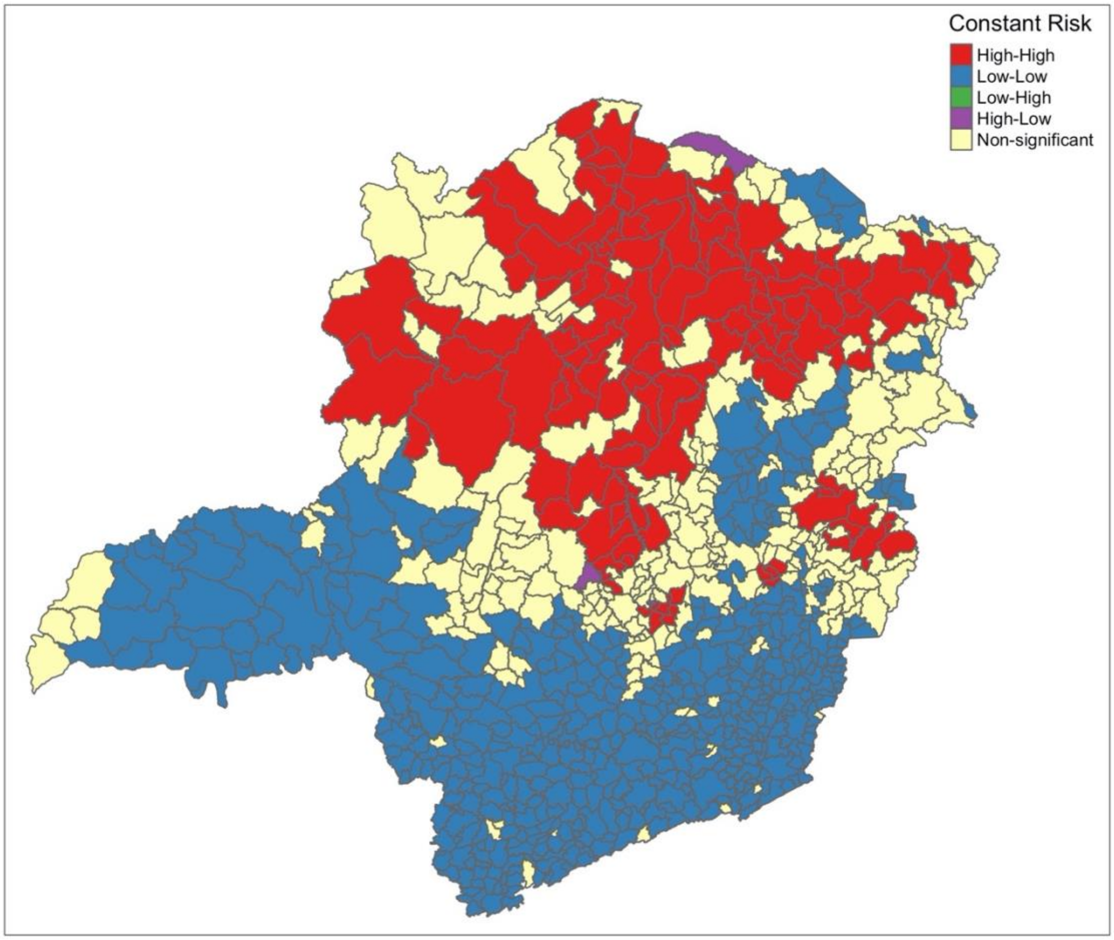
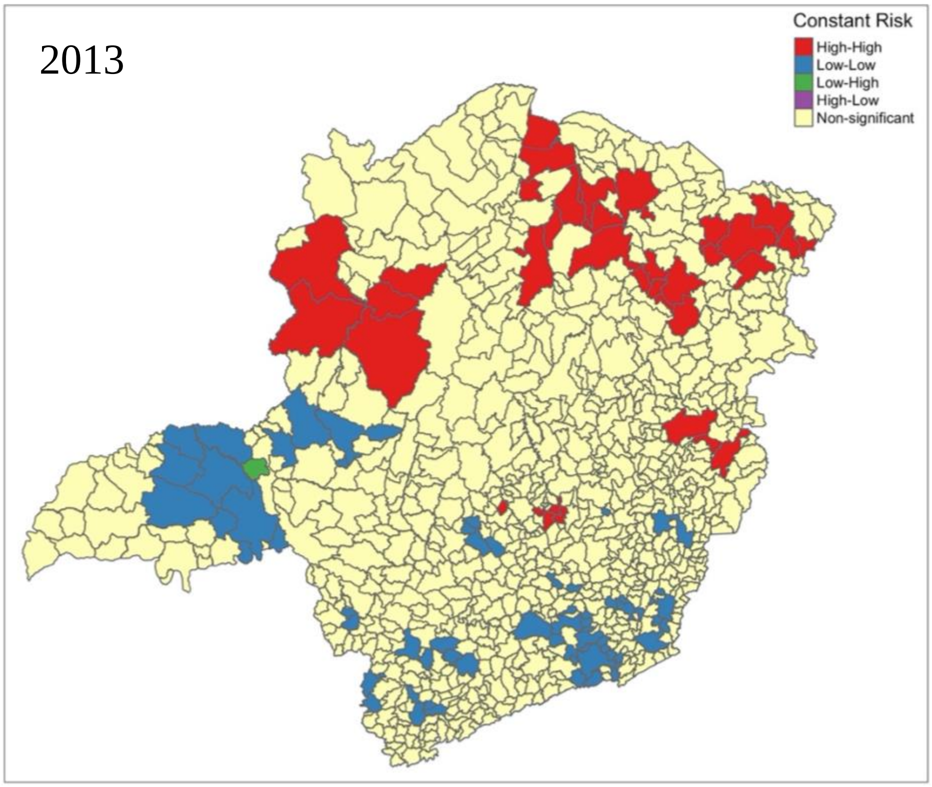


Figure 8. Local Spatial Autocorrelation of Visceral Leishmaniasis Incidence, Minas Gerais, Brazil, 2013 & 2017.



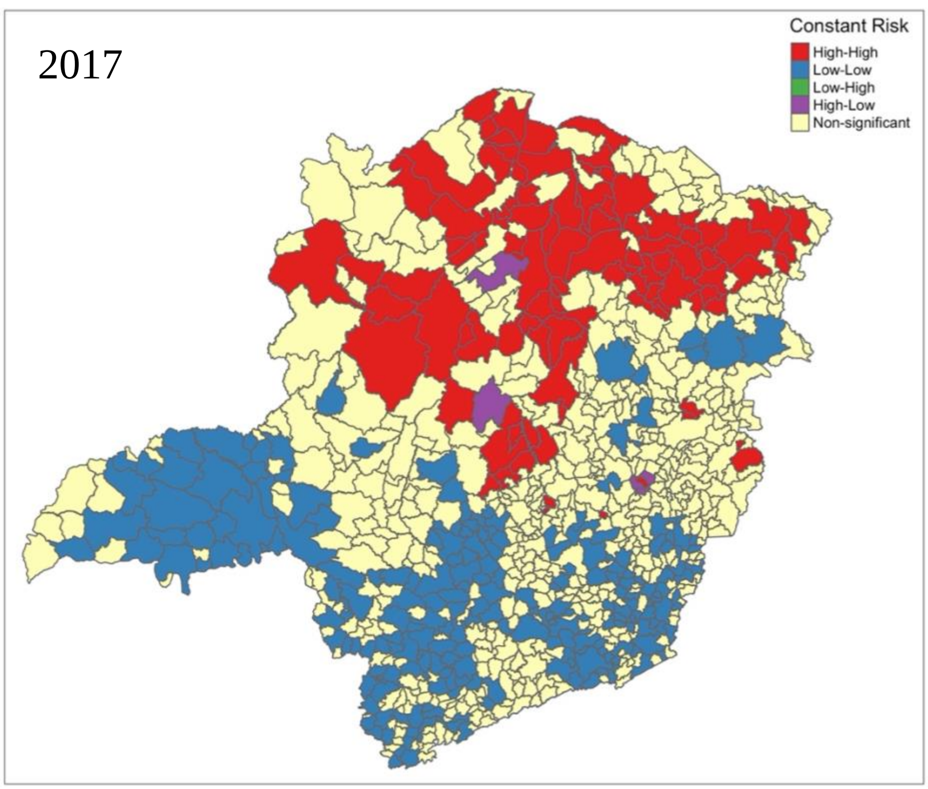
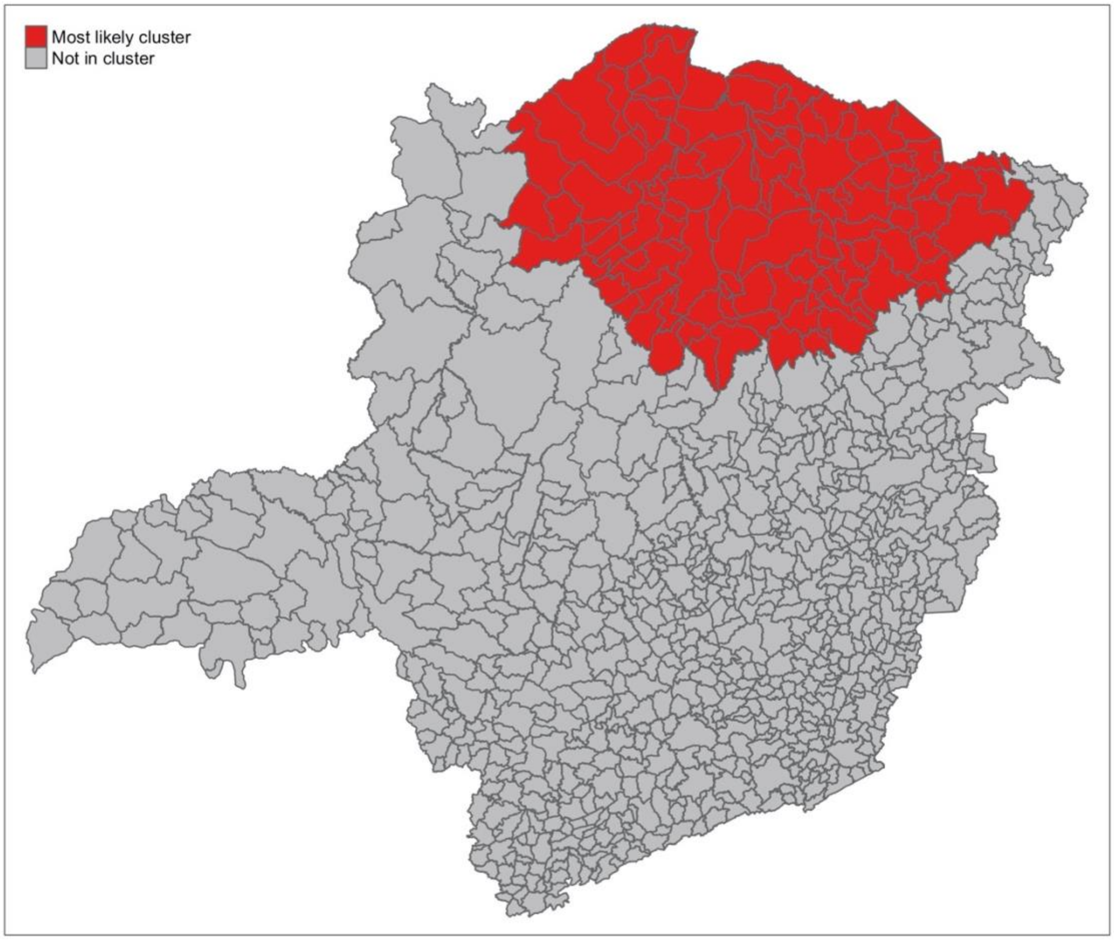


Figure 9. Locations of Most Likely Visceral Leishmaniasis Clustering, Minas Gerais, Brazil, 2012-2018.



CONCLUSIONS

SUMMARY

The aims of this study were to describe the incidence rates of visceral leishmaniasis by municipality in Minas Gerais from 2012-2018; examine environmental factors impacting visceral leishmaniasis in Minas Gerais during that time frame, such as temperature, rainfall, normalized vegetation indices, and urbanization; and review areas of potential high risk and clustering. Review of incidence rates informed the distribution of visceral leishmaniasis in Minas Gerais with the highest annual incidences and cumulative incidence being present in the northern region of the state or near uban municipalities. Statistical analyses indicated that environmental factors such as temperature, precipitation, and urbanization are potential drivers of visceral leishmaniasis incidence in the state during the study period. However, it appears these factors may vary by year as indicated by annual results.

PUBLIC HEALTH IMPLICATIONS

The results of this study enhance understanding of visceral leishmaniasis in Minas Gerais and environmental impacts on the disease. Spatial analyses indicated that clustering was present in the northern region, with municipalities there being significantly high risk. By understanding where disease burden is highest, public health officials can allocate resources to those municipalities in hopes to most effectively reduce overall incidence in the state. The areas identified as high risk in low incidence years such as 2013 should receive focus for control and prevention, with neighboring municipalities also having control and prevention plans in place for epidemic years, as observed in 2017. In addition, this study highlights a potential need for a more robust surveillance system or seroprevalence study in order to better ascertain true burden of disease.

FUTURE DIRECTIONS

This is the first known study to examine visceral leishmaniasis incidence at the municipality level in Minas Gerais and consider environmental risk factors. Therefore, data and understanding of visceral leishmaniasis and environmental drivers in the state are still inadequate. Studies are needed to better estimate the true burden of disease, through enhanced surveillance programs or seroprevelance studies. Reliance on passive surveillance can lead to underreporting, esoecially near underserved areas, so it is likely the incidence in Minas Gerais is higher than estimated here.

In addition, research is needed to further evaluate the relationship between environmental and social risk factors and visceral leishmaniasis in Minas Gerais. While the factors considered in this study accounted for 66% of the variance in the overall model, other environmental factors such as humidity and wind speed could have implications on visceral leishmaniasis. Factors related to the vector and canine leishmaniasis should be considered as vector presence and abundance is likely important in the distribution and intensity of visceral leishmaniasis and canine leishmaniasis burden has shown to precede human visceral leishmaniasis (8, 19, 42). Furthermore, Social factors such as access to health care and marginality should be considered in future research. The difference in incidence for reporting municipality versus resident municipality indicates that access to health care may vary across the state. Furthermore, São João das Missões, had the highest average annual incidence rate at 38.4 cases per 100,000 persons, but is considered one of the poorest municipalities in the state, primarily relying on agriculture.

The results observed in this study can help health officials in Minas Gerais better allocate resources to prevent and control visceral leishmaniasis by contributing to understanding of the distribution of disease in the state and what factors are potentially driving incidence. Future studies should expand to build upon this to evaluate other environmental and social factors and focus in on high incidence areas identified.

APPENDIX

Figure S1. Distribution of Visceral Leishmaniasis Cases by Reporting Municipality, Minas Gerais, Brazil, 2012-2018.

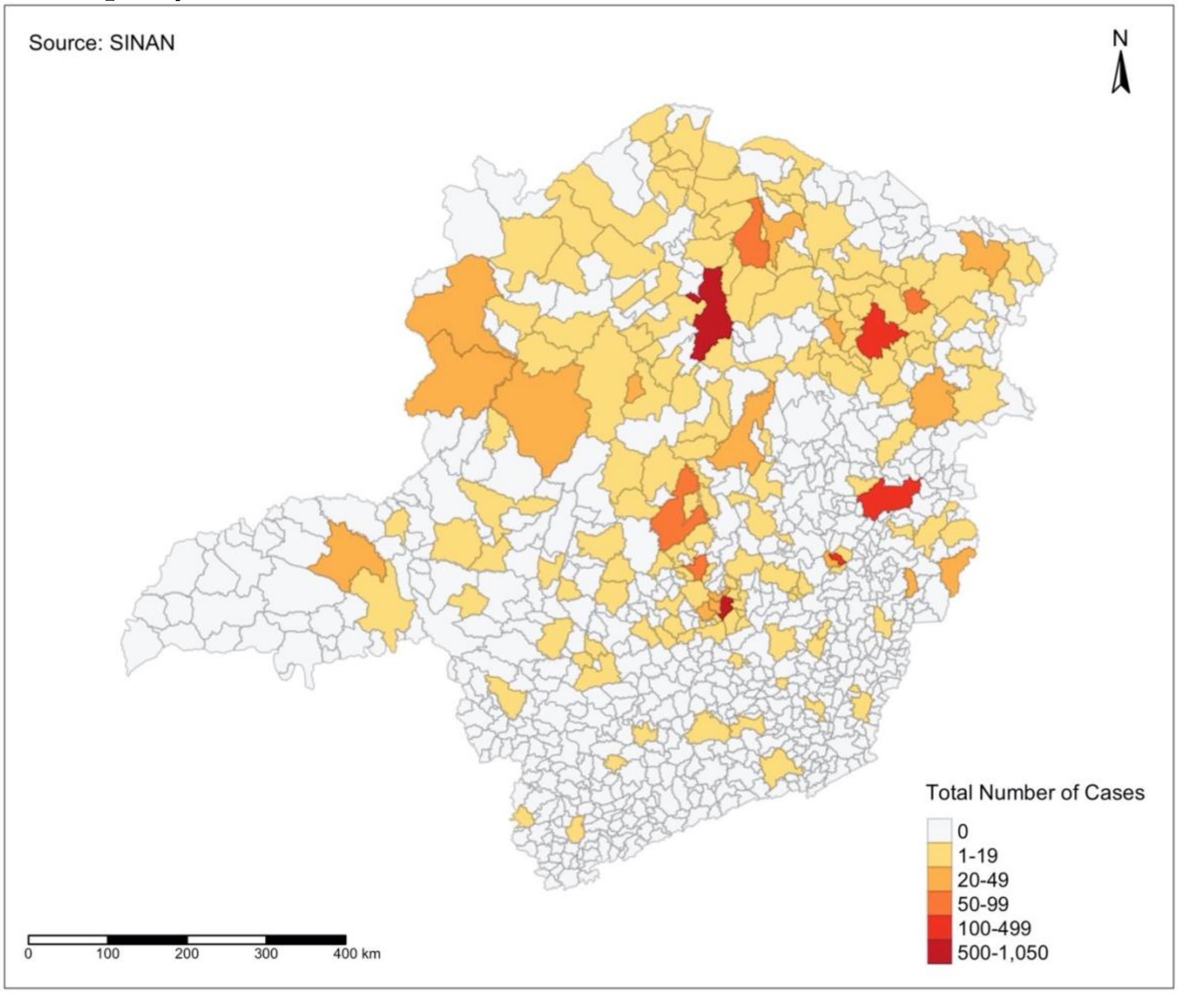


Figure S2. Cumulative Incidence of Visceral Leishmaniasis by Reporting Municipality in Minas Gerais, Brazil, 2012-2018.

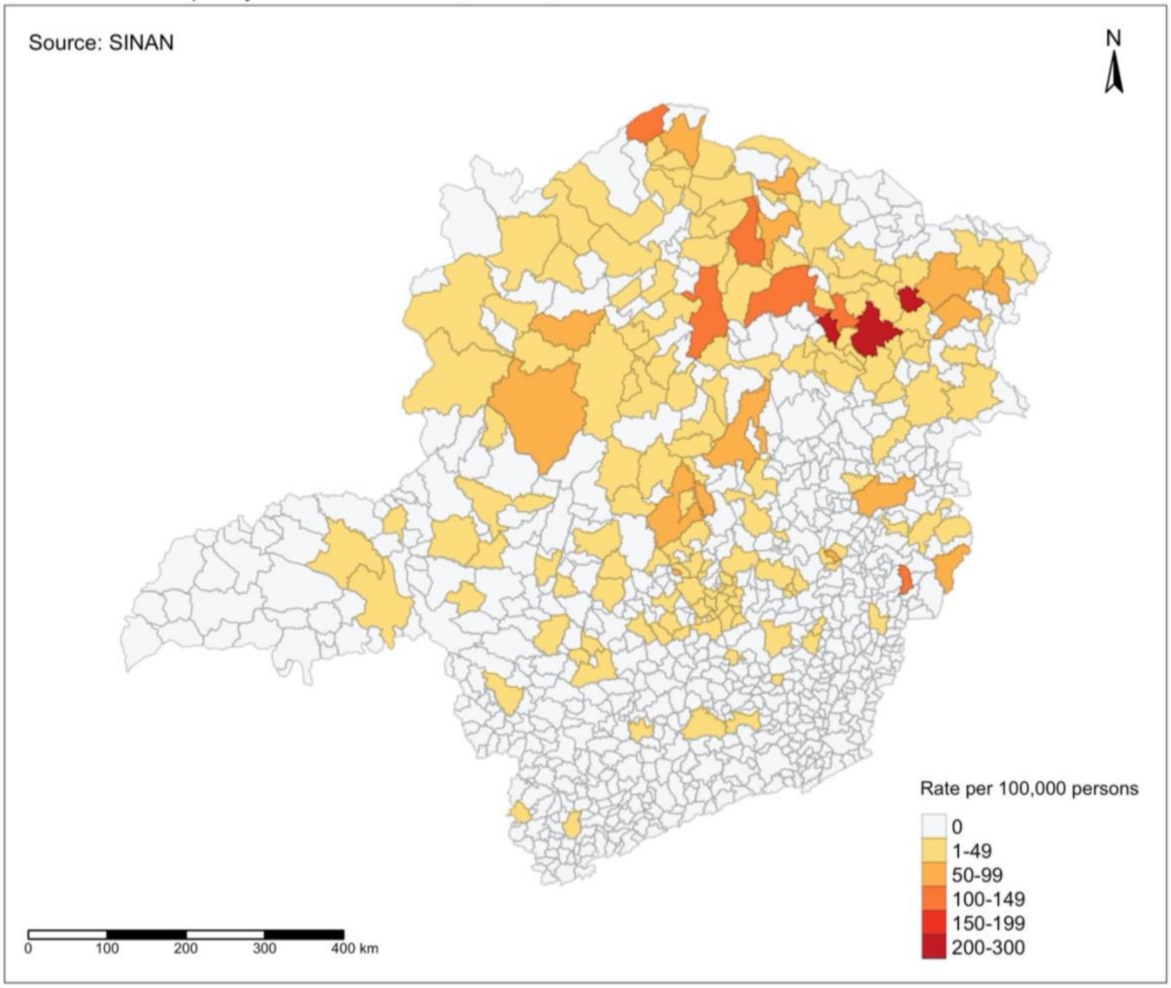


Figure S3. Annual Visceral Leishmaniasis Incidence by Reporting Municipality in Minas Gerais, Brazil, 2012-2018.

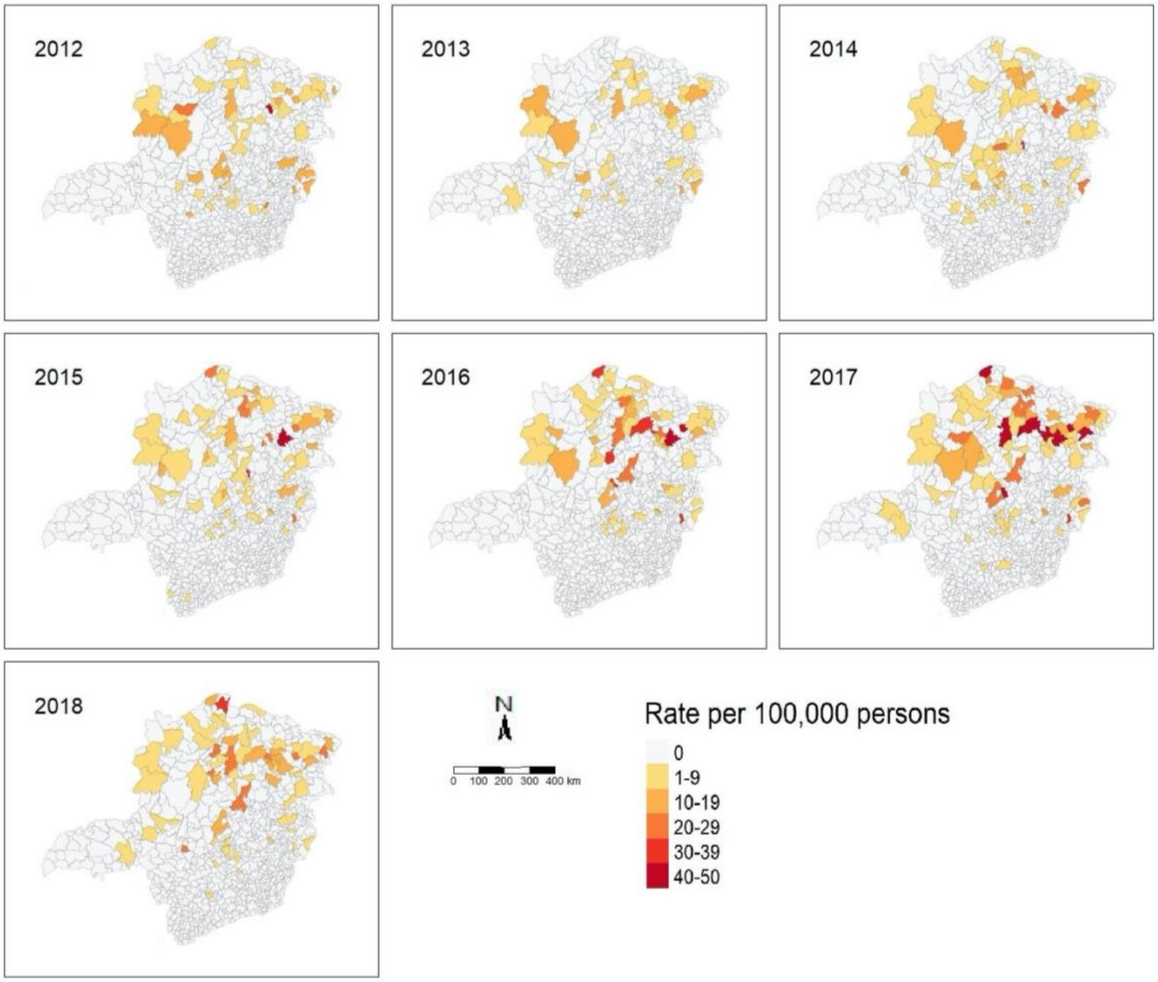


Figure S4. Aspatially Smoothed Bayes Annual Visceral Leishmaniasis Incidence by Resident Municipality in Minas Gerais, Brazil, 2012-2018.

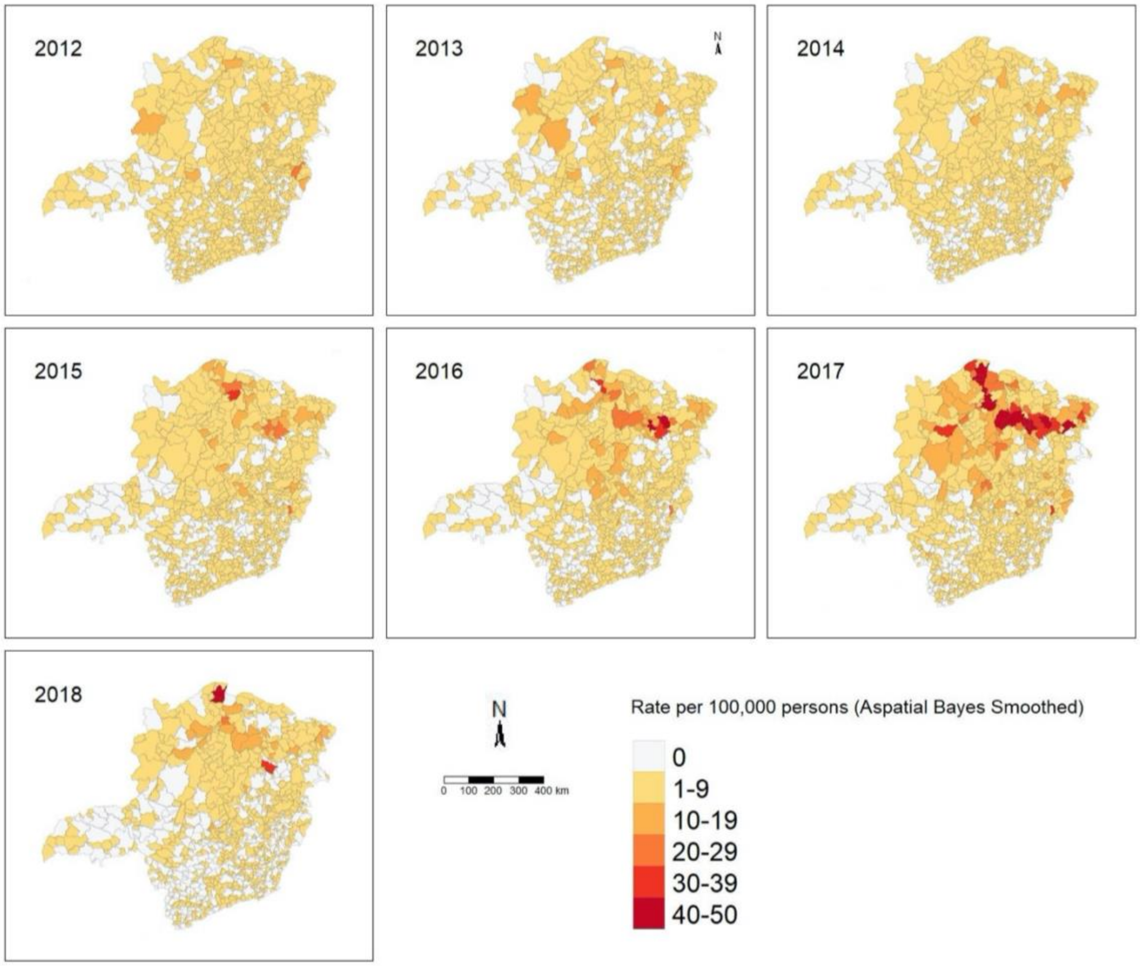


Figure S5. Spatially Smoothed Bayes Annual Visceral Leishmaniasis Incidence by Resident Municipality in Minas Gerais, Brazil, 2012-2018.