Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Jessica Stephens Date

Spatial Associations of Leprosy and Schistosomiasis and Potential effects of this co-endemic helminth on the transmission of leprosy in Minas Gerais, Brazil

By

Jessica Stephens
Master of Public Health

Epidemiology

_____

Uriel Kitron, MPH, PhD
Committee Chair

_____

Jessica Fairley, MPH, MD
Committee Member

_____

Julie Clennon, MSc, PhD
Committee Member

Spatial Associations of Leprosy and Schistosomiasis and Potential effects of this co-endemic helminth on the transmission of leprosy in Minas Gerais, Brazil

By

Jessica Stephens

Bachelors of Science
University of Washington
2013

Thesis Committee Chair: Uriel Kitron, PhD, MPH

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2017

Abstract

Spatial Associations of Leprosy and Schistosomiasis and Potential effects of this co-endemic helminth on the transmission of leprosy in Minas Gerais, Brazil

By Jessica Stephens

Background Brazil has the second highest prevalence of leprosy (Hansen's Disease, HD), but factors contributing to transmission remain unclear. Pilot data from Minas Gerais, Brazil suggest a potential spatial association between HD and schistosomiasis, an important helminth infection. Studies have also shown a predisposition to the more infectious multibacillary leprosy (MB) in those co-infected with helminths, supporting biological plausibility for the increase in HD transmission in areas with co-endemic helminths.

Methods An ecological study using public health surveillance and census data was conducted to investigate whether the occurrence of HD -and specifically MB disease- is associated with the presence of schistosomiasis in a community in 41 municipalities of the state of Minas Gerais, Brazil, 2011 to 2015. Multivariate logistic regression and spatial statistics (K-function, bivariate K-function, bivariate local indicator of spatial autocorrelation [LISA], Kulldorff's spatial scan) were applied to the data.

Results The average annual incidence was high for HD at 35.3 per 100,000. *Schistosoma mansoni* average annual incidence was 26 per 100,000. Fifteen high-high clusters of local bivariate autocorrelation for HD and schistosomiasis were identified, while 11 clusters were detected for MB and schistosomiasis. However, there was no overlap between significant most likely clusters of HD or MB disease with schistosomiasis. While living in a census tract with reported schistosomiasis was not associated with MB disease on an individual level, census level multivariate analysis found the risk of MB presence was over 1.5 time greater in tracts with reported schistosomiasis than in tracts without, adjusted for population density, household density, and household income (aOR=1.66, 95% CI 1.01, 2.71).

Conclusion This study provides a novel means to study HD transmission using spatial analysis to analyze co-occurrence of schistosomiasis which may affect HD transmission in an area with clusters of hyperendemic HD. Bivariate LISA clusters depict areas with substantial co-occurrence. Furthermore, census tract multivariate analysis indicate that the association of schistosomiasis with MB disease warrants more detailed analysis through co-infection studies. These findings not only suggest that helminth infections are associated with HD transmission, but they also can guide control programs in co-endemic areas to decrease the burden of each infection.

Spatial Associations of Leprosy and Schistosomiasis and Potential effects of this co-endemic helminth on the transmission of leprosy in Minas Gerais, Brazil

By

Jessica Stephens

Bachelors of Science
University of Washington
2013

Thesis Committee Chair: Uriel Kitron, MPH, PhD

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2017

Table of Contents

CHAPTER I: LITERATURE REVIEW

Although it is recognized as an ancient disease, the length of time which humans have known about leprosy has not translated into a concrete understanding of its transmission. The physical manifestation of leprosy described in ancient texts is not consistent with modern day diagnosis and understanding of the disease, and has contributed to the social stigma perpetuated today. Nearly 50 years after the official renaming of leprosy to Hansen's disease the pejorative context behind "lepra" (leprosy in Portuguese) still exists (1).

Hansen's disease (HD) is recognized as a neglected tropical disease (NTD) under the World Health Organization's (2). Since the 1980s and the introduction of multidrug therapy (MDT) to treat HD, significant improvements in treatment and outlook for patients has led to global elimination and eradication goals (3). Despite this improvement, progress has slowed in global elimination and new goals for HD control and elimination have been issued by the WHO. The 2016-2020 Global Leprosy Strategy has three aims: to strengthen government ownership, coordination and partnership; to stop HD and its complications; and to stop discrimination and promote inclusion. (4).

Since the introduction of multi drug treatment (MDT) for HD in the 1980's, infections have been drastically reduced, the worldwide prevalence decreasing from 5.4 million cases to a few hundred thousand (5). In 2015, 211,973 new cases were diagnosed globally with *Mycobacterium leprae* infection, or HD (6). In 1991, the WHO called for the global elimination of HD by 2000, defined as one case per 100,000. In 2012, sixteen

countries still reported at least 1,000 new cases (7). Of these countries, Brazil has the second highest prevalence of HD in the world, with the second highest burden of disease after India {8}.

In Brazil, incidence of HD has decreased between 2006 and 2012 from 44,436 new cases to 33,303. In 2012, the incidence of HD was 29,311 (9). However, the annual years of healthy life lost per 100,000 people from HD have increased by 37.7% between 1990 and 2013, an average of 1.6% a year (10). While global incidence is decreasing, areas of high endemicity still struggle to control infections. This increase in disability indicates that new measures are necessary to reach elimination within Brazil.

Hansen's disease, a chronic bacterial infection, can cause a chronic infection in humans mainly affecting peripheral nerves and skin. Presence of *M. lepra* bacilli in the skin produces the dermatological manifestations of the disease, presented as many types of rashes and legions, one common manifestation being hypopigmentation of the skin known as macules. The concurrent nerve infection is the main cause of disability and deformation due to sensory loss, stereotypically associated with HD (3). Various dermatologic and nerve manifestations seem to be more related to host response as opposed to variable virulence of the bacilli. *M. leprae* variability and virulence is limited, and cannot explain the diverse clinical forms of HD, as seen below.

Diagnosing Hansen's disease can be very complex without widely available or reliable modern methods. Diagnoses can be made on a spectrum diagnosis characterized

within five categories from the least to the most severe, tuberculoid (TT), borderline tuberculoid (BT), borderline borderline (BB), borderline lepromatous (BL), and lepromatous (LL). The least to most severe spectrum also coincides with the level of immune response (3). However, HD is also widely classified under the WHO scheme as either the paucibacillary (PB) or multibacillary (MB) clinical manifestations.Visible symptoms (skin patches or macules) and the presence or absence of bacilli from baciolloscopy (slit-skin smears from elbows, earlobes and/or knees) are used to classify clinical manifestations, if smear available (3, 11). Practitioners use this classification to determine the treatment model. MB is recognized as the more infectious form of HD and is characterized by five or more skin patches. Unfortunately, determining clinical characteristic solely based on the number of legions can result in over- or under-diagnosis of HD, generating more questions about its variability and virulence (3).

While cases of HD have decreased over the last 20 years, other obstacles remain to reduce the transmission of *M. leprae*, including a long incubation rate, stigma, and suboptimal public health measures (3). Increasing our understanding of factors associated with HD's transmission, infection and host immunology is important in order to contribute to the ongoing control of HD.

Researchers believe that Hansen's disease spreads through droplets, similar to *Mycobacterium* tuberculosis. *M. leprae* infection is thought to spread by the respiratory route after researchers isolated bacilli from nasal swabs, yet these means of transmission are uncertain (3). However, 95% of the world's population are not genetically susceptible

to HD (12). Due to this and visible clustering in family units, questions regarding genetic susceptibility have abounded. Since the availability of whole genome scans, research have been able to address this question and have found multiple loci for HD susceptibility in different communities. However, the current two-step model for the development of HD proposes that *M. leprae* is first established in genetically predisposed persons, and the subsequent clinical manifestation (MB vs PB) of disease is influenced by other host factors and environmental factors (13).

Demographic, socio-economic and environmental factors such as poverty (undernutrition), crowding and soil moisture have a demonstrated role in transmission of HD (3): the prevalence of HD is higher in areas with high levels of poverty and crowding (14). Further determination in the role of environmental predictors is increasingly important as new reservoirs of infection are identified, such as soil (15). Distinguishing the role of these factors in the differentiation of HD into MB, the more infectious form, or PB may create more opportunities in the control and spread of Hansen's disease.

Host factors including age and sex demographics are commonly included in studies on clinical manifestations of HD. The percentage of children among all incident cases of HD is an important epidemiological indicator, as it reflects active disease transmission in the community (16, 17). Studying this age group also controls for mobility, as young people had less time in which to move, allowing for the identification of locations with recent transmission (14). A 2:1 ratio male to female is commonly observed in the

diagnosis of HD patients (18). A high male to female ratio of MB is also supported by multiple studies (19 - 23).

One possible socio-demographic factor in HD transmission may be the number of people living in a household. In a study in Indonesia, clustering of seropositive HD patients indicated that close contact is important for transmission of HD. This study also showed that seroprevalence is higher among people living less than 75 meters from seropositive patients, and that household with more than seven people showed a significantly higher incidence of HD than those with four or fewer (14). The effect of crowding in hyperendemic areas of Brazil has also been studied. In Para State, a study found that more than half of those affected by HD lived in homes in which two or more people shared a bedroom (24). Acknowledging the possibility of confounders, Bakker hypothesized that clustering can also be caused by family members with high genetic susceptibility living together or underlying socioeconomic status, but describes a homogeneity of poverty levels in the study area in Indonesia.

Nutrition, another factor associated with poverty, has also been examined as a factor related to HD. A recent period of food shortage and not poverty *per se* was identified as the only socio-economic factor significantly associated with clinical manifestation of HD (25). In a case control study in India, the nutritional status of HD patients was compared finding undernutrition more common in the people with HD than the control group. This points to a potential role of nutrition in HD acquisition, but, notably, Rao noted the possible opposite manifestations where undernutrition is the

results of HD related stigma, disability and/or depression (26). Another study in Para, Brazil, also found that the numbers of reports of ‘starvation’ (having experienced full days with no meals) were higher in the hyperendemic region than the average for the general population of the region and five times higher than that for the national population of Brazil (24).

Non-human sources of *M. leprae* have also been pro[posed, motivated by observations of HD in individuals with no apparent history of exposure to known cases and the observations of clustering in particular areas such as near water source (27). Matsuoaka *et al* strongly suggested that non-human sources such as soil and water may be responsible for continued HD transmission (28), and this is supported by indirect evidence of hospital and community outbreak of mycobacterial infections cause by contaminated water supply systems (29). Although the role of soil and water is yet to be demonstrated experimentally.,t as early as 1987 *M. leprae* has been isolated from soil samples (15). More recently, multiple studies detected viable *M. leprae* in soil and water samples in India (30-32). One study found viable *M. leprae* RNA in 25.4% of soil samples surrounding HD patient’s homes and in 24.2% of water outlets (30). Whether these cells actually replicate rather than just persist for a matter of day or even weeks is not known (27). In Ceara, Brazil, bathing in open water within 10 years prior to the study was associated with an increased risk of HD (3, 33). Truman makes the point that these environments may reflect conditions were *M. leprae* can persist outside the human body or, conversely, the apparent clustering of HD in particular environments may simply reflect that certain environments are associated with certain social groups, health

conditions or behaviors which predispose to *M. leprae* transmission or the manifestation of HD (27).

While helpful in describing transmission pattern of HD, demographic, socio-economic and environmental factors cannot explain the differences in clinical forms of HD, which are attributed largely to the host's immune response. The *M. leprae* infections presenting as MB disease, such as lepromatous leprosy, more commonly exhibit a Th2 immune (Type 2 Helper T) response during which the innate Th1 response, associated with PB infection, is downregulated (34). Examining possible factors influencing the hosts shift towards the Th2 immune response may help delineate risk factors for the infectious from of HD.

Chronic helminth infections can cause immunomodulation, or altering of the immune response (35). While PB infections are characterized by a strong Th1 response, MB HD notably lacks a Th1 (cell-mediated) response and instead expresses Th2 associated cytokines and inflammatory markers, especially in lepromatous disease (36). Helminth infections are characterized by a shift to the Th2 immune response (37), possibly providing a mechanistic explanation for immune deregulation that can shift disease manifestations towards lepromatous HD, or at least MB disease (3). Two studies by Diniz in Brazil pointed to the possibility that helminth infections cause a shift in HD host immune response, causing *M. leprae* to proliferate, thus increasing the risk of transmission (38, 39). Studies specifically examining the relationship between HD and schistosomiasis are rare; however, associations between other helminth infections and

HD have been studied. Helminth infections, including onchocerciasis, have been associated with the more transmissible, lepromatous, end of the HD disease spectrum (40). Whether helminths co-infections have an effect on the manifestation of HD is not clear, but the immunological profile of both infections provides biological plausibility to an effect of schistosomiasis coinfection on *M. leprae* transmission.

Examining the co-occurrence of Hansen's disease and schistosomiasis follow s growing interest in coinfections synergism. Since the early 2000's, attention to coinfections has been on the rise for reasons of public health and societal impacts of disease synergism studies (41, 42). Fenn *et al* characterized the co-occurrence of illnesses at the community level in Ghana, examining diarrhea in children under five (43). In the past 15 years, a push towards understanding parasitic coinfections in Sub-Saharan Africa has improved knowledge on helminth infections, such as *S. mansoni* and hookworm, and *Plasmodium falciparum* (41, 44, 45). In HD research, an excess number of deaths were found in a HIV positive co-infected group compared to HIV negative patients (RR 4.8). However, the evidence presented showed no risk of developing HD or developing a multibacillary diagnosis rather than paucibacillary (46).

Developing models around co-infections can lead to relevant planning for regional disease control efforts that simultaneously target multiple infections through large scale integrated control methods (41). Nakagawa *et al* describes more far reaching impacts beyond immediate health impact of multi-disease, multi-sectoral approaches contributing to sustainable development, raising educational attainment, increasing productivity and

reducing health inequities (42).Using co-occurrence to estimate proportions co-infected would utilize available surveillance data and address recent interest in the effects of schistosomiasis on HD manifestation.

Spatial analysis of georeferenced data, managed using Geospatial Information Systems (GIS), can contribute to epidemiological assessment of risk factors for MB HD. The WHO recommends using GIS to direct HD control strategies (47), using it as a tool to analyze and visualize trends, dependencies and inter-relationships. Najafabadi calls it a common platform for the convergence of multi-disease surveillance activities, making it a good option to describe the relationship between HD and schistosomiasis cases (48).

Brooker recognized the importance of cataloging the geographical perspective of infectious disease epidemiology and described progress towards mapping helminth infections in sub-Saharan Africa in 2000 (44). Prior to these efforts to map *S. mansoni*, hookworm and *P. falciparum* parasites, little was known about the spatial distributions of parasitic infections other than malaria, trypanosomiasis and onchocerciasis (41, 45). Other parasitic infections have recently been mapped in areas of overlapping infections for Loa loa, onchocerciasis and lymphatic filariasis (49). HIV and TB has also been the target of recent application ofspatial tools to study co-infections and geographic overlap. (50, a 51, 52). Vector borne diseases, such asdengue, are extensively mapped (53). However, only recently have studies been conducted to map dengue and chikungunya co-occurrence (54).

GIS has been applied in studies ofh HD and schistosomiasis in Brazil and in other endemic areas, but studies on the co-occurrence are very rare. Spatial and temporal studies of HD in endemic areas in Brazil using surveillance data collected by Information System for Notifiable Diseases [Sistema Nacional de Agravos de Notificação] (SINAN) have been conducted by Nicchio *et al* in 2016, Cabral-Miranda *et al* in 2014 (spatial regression) and Alencar *et al* in 2012 (cluster analysis) (14, 56, 57). Spatio-temporal studies of case clustering in the Amazonas region were also conducted through household contacts and active detection in schools (24). In 2010, Guimaraes *et al* used GIS in Minas Gerais to study schistosomiasis, specifically the risk for the presence of the vector, *Biomphalaria glabrata.* Guimaraes established a regression for the presence or absence of *Biomphalaria spp*. in four regions of Minas Gerais state using environmental, social, biological and remote-sensing variables (58). Pilot data from collaborators in a four municipalities in Minas Gerais, Brazil suggest a potential association between HD and schistosomiasis. This study found a relative risk of 6.8 (CI=1.46, 31.64) of leprosy in neighborhoods with schistosomiasis, independent of population density and purchasing power per capita (55). These findings provided an impetus for the current study, in attempt to extrapolate these findings at a larger scale.

CHAPTER II: MANUSCRIPT

A. Abstract

Title: Spatial Associations of Leprosy and Schistosomiasis and Potential effects of a co-endemic helminth on the transmission of leprosy in Minas Gerais, Brazil

Author: Jessica L Stephens

Background Brazil has the second highest prevalence of leprosy (Hansen's Disease, HD), but factors contributing to transmission remain unclear. Pilot data from Minas Gerais, Brazil suggest a potential spatial association between HD and schistosomiasis, an important helminth infection. Studies have also shown a predisposition to the more infectious multibacillary leprosy (MB) in those co-infected with helminths, supporting biological plausibility for the increase in HD transmission in areas with co-endemic helminths.

Methods An ecological study using public health surveillance and census data was conducted to investigate whether the occurrence of HD -and specifically MB disease- is associated with the presence of schistosomiasis in a community in 41 municipalities of the state of Minas Gerais, Brazil, 2011 to 2015. Multivariate logistic regression and spatial statistics (K-function, bivariate K-function, bivariate local indicator of spatial autocorrelation [LISA], Kulldorff's spatial scan) were applied to the data.

Results The average annual incidence was high for HD at 35.3 per 100,000. *Schistosoma mansoni* average annual incidence was 26 per 100,000. Fifteen high-high clusters of local bivariate autocorrelation for HD and schistosomiasis were identified, while 11 clusters were detected for MB and schistosomiasis. However, there was no overlap between significant most likely clusters of HD or MB disease with schistosomiasis. While living in a census tract with reported schistosomiasis was not associated with MB disease on an individual level, census level multivariate analysis found the risk of MB presence was over 1.5 time greater in tracts with reported schistosomiasis than in tracts without, adjusted for population density, household density, and household income (aOR=1.66, 95% CI 1.01, 2.71).

Conclusion This study provides a novel means to study HD transmission using spatial analysis to analyze co-occurrence of schistosomiasis which may affect HD transmission in an area with clusters of hyperendemic HD. Bivariate LISA clusters depict areas with substantial co-occurrence. Furthermore, census tract multivariate analysis indicate that the association of schistosomiasis with MB disease warrants more detailed analysis through co-infection studies. These findings not only suggest that helminth infections are associated with HD transmission, but they also can guide control programs in co-endemic areas to decrease the burden of each infection.

B. Introduction

Brazil has the second highest prevalence of *M. leprae,* leprosy (Hansen's disease), infection in the world, sharing the largest burden of disease with India. While cases of Hansen's disease (HD) have decreased over the last 20 years, other obstacles remain to reduce the transmission of *M. leprae*, including a long incubation rate, stigma, and suboptimal public health measures. *M. leprae* infection is thought to spread by the respiratory route, yet means of transmission remain uncertain (10).

Pathogen variability and virulence are limited, and cannot explain the diverse clinical forms of HD (40); therefore, other factors need to be considered when trying to explain patterns of transmission and host immunology. The prevalence of HD is higher in areas with high levels of poverty and crowding, suggesting that factors associated with poor socioeconomic conditions may be conducive to infection (14). Due to overlapping factors related to poverty, helminth infections, which are also associated with low socio-economic status (SES), are commonly endemic in areas where HD is prevalent. These diseases share many of the same geographic areas including in many endemic areas in Brazil (55). Whether helminths co-infections have an effect on the manifestation of HD is not clear.

Spatial data from a pilot study in four municipalities in Minas Gerais, Brazil suggest a potential association between HD and schistosomiasis, an important helminth infection. This study found a relative risk of 6.8 (CI=1.46, 31.64) of leprosy in neighborhoods with schistosomiasis, independent of population density and purchasing power per capita (55).

Other helminth infections, including onchocerciasis, have been associated with the more transmissible, lepromatous, end of the HD disease spectrum (40). Co-infection studies in Brazil have identified a possible shift in HD host immune responses due to helminth infections, so that *M. leprae* proliferates, thus increasing the risk of transmission (38, 39). The *M. leprae* infections presenting as lepromatous leprosy more commonly exhibit a Th2 immune response, during which the innate Th1 response is downregulated. Helminth infections are also characterized by a shift to the Th2 immune response, possibly providing a mechanistic explanation for immune deregulation that can make patients more susceptible to the more infectious form of HD (multibacillary disease, MB), and thus sustain continuous source of transmissible *M. leprae* infection in the community (3).

The current two-step model for the development of HD proposes that *M. leprae* is first established in genetically predisposed persons and the subsequent clinical manifestation (MB vs PB, paucibacillary) of disease is influenced by other host factors and environmental factors (13). Here we address the second step of disease dynamic in MB manifestation, including socio-economic factors recognized in HD dynamics and the novel predictor: schistosomiasis. This preliminary study on schistosomiasis and MB diseases uses co-occurrence data as an estimate of co-infections due to the availability of unidentified surveillance data for each disease, respectively. Spatial and non-spatial method are used for descriptive, spatial, and regression analyses at two spatial scales - for individual cases and for aggregated cases on the census tract level.

Geographic Information Systems (GIS) have been used to study both HD and schistosomiasis in Brazil and in other endemic areas, but studies on their co-occurrence are limited. Spatial and temporal studies of HD in endemic areas in Brazil using surveillance data collected by Information System for Notifiable Diseases [Sistema Nacional de Agravos de Notificação] (SINAN) have applied spatio-temporal cluster analysis and spatial regression (14, 24, 56, 57). GIS and spatial analysis have been used to study schistosomiasis in Brazil, using environmental, social, biological and remote-sensing variables to model and control for risk (58).

Here we applied spatial analyses to identify geographical patterns of HD, MB diseases, and schistosomiasis and to determine areas of high incidence of both neglected tropical diseases (NTDs) to facilitate planning of public health interventions. We present descriptive spatial analysis of raw average annual incidence, a spatial Bayesian approach to incidence smoothing, and local and global spatial statistics. Spatial and non-spatial results are compared and critiqued at individual and census tract levels of analysis.

C. Methods

Ethical considerations

This study was approved by the Institute of Health Sciences Research Ethics Committee from the Universidade Vale do Rio Doce (CAAE: 56700816.3.0000.5157 CEP) and the Emory University Institutional Review Board (IRB: IRB00087575). All data acquired were anonymized.

Study Area

This study was conducted in the Brazilian southeastern state of Minas Gerais, Brazil's. second most populous state, fourth largest by area and with the third highest gross domestic product. The capital is Belo Horizonte, the fourth largest city in Brazil. Governador Valadares (GV) is the ninth largest city in Minas Gerais (MG) and is the economic center of the Rio Doce valley, 324 km from Belo Horizonte (59). The climate n Governador Valadares is tropical, with an average temperature of 24.2 °C and average annual rainfall of 1,109 mm, e (60). The area is endemic for both HD and schistosomiasis.

Data Sources and Methods

In Brazil, Hansen's Disease and schistosomiasis (*Shistosoma mansoni* infection) are included in SINAN. Hansen's is a compulsory notifiable disease; thus all patients detected through surveillance are registered with clinical, demographic, and address data. Notification of schistosomiasis is recommended by the Schistosomiasis Control Program [Programa de Controle da Esquistossomose], but not compulsory (61). Residential addresses, demographic and epidemiological variables (age, gender and operational classification) were collected from SINAN. Patient recorded addresses were geocoded using Google Earth Pro version 7.1.7 using two algorithms with varying specificity.

Other census level demographic variables believed to be associated with HD were obtained from the 2010 census, available through the Brazilian Institute of Geography

and Statistics (Instituto Brasileiro de Geografia e Estatıstica 2011, IBGE) (62). These variables included population per census tract (residents in permanent private households), household density (average number of residents in private households) and income (nominal average monthly income of persons responsible for permanent private households). Household density was categorized as high (above 3) or low (3 or less) split. Income and population density were categorized as tertiles.

By joining information from SINAN and IBGE it was possible to conduct a spatial analysis combined with non-spatial regression models of HD and MB for individual cases and cases aggregated to census tracts.

Spatial Data Analysis

Spatial analysis was conducted using individual georeferenced patient location and cases aggregated to the census tract. At the georeferenced point level, we present a spatial analysis of HD, MB disease and schistosomiasis patient presence. At the aggregated level, we present a spatial analysis of the average annual incidence of HD cases per 100,000, the average annual incidence of MB disease cases per 100,000, and the average annual incidence of schistosomiasis cases per 10,000. All maps were produced in ArcGIS 10.4 (ESRI, Redlands, CA, USA) using the spatial reference SIRGAS 2000 UTM Zone 24S.

For georeferenced point level data, Ripley's K-function was used to assess global spatial clustering considering a range of distance 50 m to 5,000 m or 17,500 m (univariate or

bivariate, respectively) with distance lags of 500 m (with edge correction) (63). This range was chosen to include a fine scale because of the increased risk of infections for household contacts, neighbors and neighbors of neighbors of HD patients, with the risk inversely decreasing with increasing distances (14, 35, 64). Under the hypothesis of complete spatial randomness, statistical significance was evaluated by comparing the observed values with expected values with 999 Monte Carlo permutations. ArcGIS was used to calculate Ripley's K-function for individual outcomes (HD, MB disease and schistosomiasis, respectively) and Point Analysis, Spatial Statistics and Geographic Exegesis (PASSaGE v2) was used to assess dichotomous data using bivariate K-function (MB disease vs PB disease) (65).

Kulldorff's spatial scan statistics were also applied to detect the most likely high-risk clusters of individual cases considering a distribution of controls (Bernoulli model). For the pure spatial analysis, this statistical technique uses a flexible elliptical geographic scanning window to included different sets of neighbors, ranging from the minimum distance between points to half the width of the study area. This method was used to test the null hypothesis of constant risk between points (66). StatScan version 9.4.4 (Harvard Medical School, Boston, USA) was used to perform Kulldorff's spatial scan statistics.

For aggregated data, to minimize effect of small numbers on statistical instability, we performed spatial empiric Bayesian smoothing to estimate smoothed incidence between contiguous areas (using a queen spatial weigh matrix) (56). Additionally, Anselin's local indicator of spatial association (LISA) was applied to characterize areas with statistically

significant ($p<0.05$) spatial autocorrelations for univariate (all HD incidence, MB incidence alone, schistosomiasis incidence) and bivariate associations (all HD incidence with schistosomiasis, MB incidence alone with schistosomiasis) (67). Finally, Kulldorff's spatial scan statistics were again applied to detect the most likely high-risk clusters of cases per census tract considering the rest of the population as controls to identify adjacent census tracts least consistent with the hypothesis of constant risk. The following software were used for these spatial analyses: Geoda 1.6 (GeoDa Center for Geospatial Analysis and Computation, Tempe, AZ, USA) to calculate spatial weight matrix, spatially empirical Bayes incidence per census tract and LISA; and StatScan to perform Kulldorff's spatial scan statistics.

Logistic Regression Data Analysis

Non-spatial analysis was performed by means of logistic regression using SAS 9.4 (Cary, NC). Models were created at the point level for MB as the outcome and aggregated level for both HD and MB (presence/absence) as the outcome, respectively. Census tract level variables obtained from the 2010 census (average household density, population density and average household income) were assigned to individual cases based on census tract of residence. To assess collinearity, variables were included in a logistic regression for the respective outcome with schistosomiasis presence as the main predictor and the other independent variables (5 for point level model, 3 for aggregated level model). Variables found to be collinear were left out of the model. Effect modification was tested by adding all possible interaction terms for significant variables to the model and repeating the analysis each time. All non-collinear terms were controlled for in the final models if their

removal significantly decreased precision. The final model Odds Ratios (ORs) were calculated, but, because of the number of events (rare disease assumption), these were comparable to relative risks (RRs).

D. Results

Study population

The data included reported cases of HD (n=1,078) and Schistosomiasis (n=783) from January 1, 2011 to December 23, 2015 for the region surrounding Governador Valadares (21,100 km²). This region reports data to Governador Valadares, and comprises 41 municipalities and 1,272 census tracts (Figure 1). The population of the study area in 2010 was 610,618 people. In the study area, the annual incidence of Hansen's disease is 35.3 per 100,000 (7.3% in children 15 years old and under, 55.3% MB disease). Schistosomiasis annual incidence is 2.6 per 10,000.

Within the study area, 755 cases of HD (83.0% of cases with street addresses) and 313 cases of schistosomiasis (95.7% of cases with street addresses) were mapped through geocoding. Most cases not geocoded were due to missing street addresses (58.2% schistosomiasis, 16.7% HD). A flowsheet of inclusion is shown in Figure S1. Georeferenced data were compared to the original dataset and there was no significant difference between descriptive and clinical statistics for the two, and the percent representation of cases by municipality was representative (**Figure S2, S3**).

MB cases represent 388 of the total HD cases georeferenced (51.4%, Table 1). Of total HD cases 51.3%were male; average age was 47.1 years (8.6% ≤15 years). From census records of tracts with HD, the average monthly income was $974.8 Brazilian Reales, average household density was 3.2 people per household, and average population density was 5,492 people per square kilometer. The average incidence of schistosomiasis in the census tracts containing HD is 6.82 per 10,000, with an incidence of 2.66 per 10,000 in the entire study area.

Individual Level Bivariate and Multivariate Analysis

The unadjusted risk of MB disease is 2.83 times higher in men than in women ($p<0.001$) (Table 2). All age categories are also statistically significantly associated to MB diseases ($p<0.01$, respectively), each with a higher risk of MB diseases than children ≤ 15 years, and those aged 31-45 years with the highest risk of MB diseases (OR=11.80, $p<0.001$). More children have PB infections than MB (58 and 7, respectively). 11.3% of MB cases and 8.2% of PB cases occur in census tracts with reported schistosomiasis. No census variable showed a bivariate association with MB disease.

In the multivariate logistic regression model for individual MB cases, schistosomiasis present in the census level was not significant at the 5% significance level (aOR=1.58, $p>0.05$), adjusting for all other variables (Table 3). Age and sex were associated with MB diseases ($p<0.05$), adjusting for all other variables. With all other variables in the model, men had 3.01 times the risk of MB diseases than women ($p<0.001$). All age categories were also statistically significantly associated to MB diseases ($p<0.01$), each with a

higher risk of MB diseases than children ≤15 years old, controlling for other variables in the model.

Individual Level Spatial Analysis

Ripley's k-function for global clustering of individual cases, comparing observed pattern to an expected Poisson distribution, identified significant clustering of each disease starting from 500 meters to the maximum distance tested. Randomness was indicated in the conditional association between MB and PB disease (Figure S4). Kulldorff's spatial scan analysis to identify the most likely clusters of cases given the presence of controls resulted in no local clustering for all HD cases, MB disease, or schistosomiasis (Table 4).

Census Tract Description

In the raw data, HD was reported in 355 tracts (27.9%), MB diseases are present in 247 tract (19.4%), and Schistosomiasis is present in 107 census tracts (8.4%) (Figure 2A. 1-3.). Taking into account their closest neighbor with local Bayesian analysis, HD was extrapolated to 723 tract and MB disease to 633 tracts. Hyperendemic HD (defined as >40 incident cases / 100,000) existed in 210 tracts, with a maximum annual incidence of 500 cases per 100,000 people. The distribution of raw average annual incidence is depicted in Figure 1A. 1-3. Additionally, 210 tracts were hyperendemic for HD in the SEB analysis, while the maximum average annual incidence shrunk to 202.9 cases per 100,000 people (Figure 1B. 1-3).

Census Tract Spatial Analysis

LISA's detected significant associations ($p<0.05$) between census tracts with high incidence rates to their neighbors (high-high). Visualized in Figure 2C. 1-3., 15, 40, and 42, LISA clusters were identified for all HD cases, MB cases, and schistosomiasis, respectively (Table 5). Kulldorff's spatial scan statistics also indicated the most likely clusters of HD, MB diseases, and schistosomiasis (Figure 1D. 1-3 and Table 5). One most likely cluster of HD was identified in GV. The most likely cluster of MB disease ($p<0.001$) was loosely knit, containing 10 census tracts, spread from the center of the study areas in GV towards the northeast of the area. The three most likely clusters for schistosomiasis ($p<0.001$, respectively) were tight clusters; the most likely cluster consisted of one tract in Pecanha, the second most likely cluster consisted of one tract in Sao Joao Evangelista, and the third consisted of three tracts in Conselheiro Pena. Some similarities in LISA and Kulldorff clustering were identified for MB diseases and schistosomiasis. The most likely high cluster for MB overlapped in two tracts in GV, one tracts in Cuparaque, and one tract in Mantena. The 3rd most likely cluster of schistosomiasis overlapped, and all three tracts were high-high LISA clusters.

Schistosomiasis is present in 37 tracts with HD and 28 tracts with MB disease, representing 2.9% and 2.2% of the study area, respectively, but 10.4% of HD present and 11.3% of MB present census tracts, respectively. Bivariate LISA detected significant associations ($p<0.05$) between the census tracts with high incidence rates of HD and schistosomiasis (high-high). For MB disease and schistosomiasis, eleven areas of high-high clusters were identified surrounding six towns. For all cases of HD, fifteen high-high clusters were identified surrounding seven towns (Figure 3, Table 5).

Census Tract Multivariate Analysis

With the census tract as the unit of comparison, the outcomes of HD cases present and MB disease present were each modelled using logistic regression, with schistosomiasis (present in the census tract), household density (when significant or variable presence improved precision), population density, and average monthly income as covariates. Controlling for all covariates, schistosomiasis presence was not statistically significantly associated with HD presence at the 5% significance level HD (aOR = 1.56, CI= 0.98, 2.48) (Table 6). For HD, medium and high population density, as well as average and high average monthly income, were significantly associated ($p<0.005$, respectively), controlling for other covariates. However, controlling for all covariates, schistosomiasis presence was statistically significantly associated with MB presence; census tracts with schistosomiasis cases were more than 1.5 times more likely to have MB cases than tracts without schistosomiasis cases (aOR=1.66, CI=1.011, 2.71) (Table 7). Medium (aOR=4.44, CI=3.05, 6.45) and high (aOR=5.15, CI=3.55, 7.48) population density and average (aOR=1.68, CI=1.18, 2.40) and high (aOR=2.77, CI=1.71, 4.49) e monthly income were again significantly associated with MB presence.

Using Bayes smoothed incidence to determine, models were rerun for HD and MB diseases. Schistosomiasis was not significantly associated with presence of HD or MB using SEB analysis. Interestingly, high household density became a significant predictor for HD (aOR=0.67, CI=0.49, 0.92) but not for MB. Again, medium (aOR_{HD}=4.71, CI=3.18, 6.97; aOR_{MB}=4.62, CI=3.21, 6.67) and high (aOR_{HD}= 12.81, CI= 7.02, 17.47;

aOR_{MB}= 11.072, CI= 7.015, 17.47) population density and average (aOR_{HD}= 2.04, CI= 1.56, 2.66; aOR_{MB}= 2.25, CI= 1.71, 2.95) and high (aOR_{HD}= 10.98, CI= 5.48, 21.98; aOR_{MB}= 11.33, CI= 6.22, 20.64) average monthly income were significantly associated with both HD and MB presence.

E. Discussion

Our data point to many areas of hyperendemic HD and schistosomiasis throughout the study area. We used spatial analyses to identify geographical patterns of HD, MB disease and schistosomiasis. We identified bivariate clusters of MB disease and schistosomiasis in the same 6 areas where clusters of HD disease and schistosomiasis occur. One additional HD and schistosomiasis cluster was identified outside this area. At the aggregated level, the pattern of all cases reported from 2010 to 2015 surrounding GV appeared in heterogeneous, although not randomly distributed, geographical patterns. Well defined most likely clusters of high risk were identified by Kulldorff's spatial scan for HD and schistosomiasis and a loose spatial cluster of high risk was identified for MB. The loose MB cluster overlapped with the HD cluster and these clusters were centrally located and included one census tract in GV. The schistosomiasis most likely clusters were located in the northwest (2 clusters) and south (1 cluster) of the HD/MB clusters.

For a rare disease like HD, small variations in the number of cases result in dramatic changes in disease rates. A spatially empirical Bayes (SEB) smoothed rate has been used in HD studies to smooth the random variations in small areas, such as census tracts.

Smoothing may enhance the visualization of spatial patterns; in this study the distinction between regions with HD, MB disease, and schistosomiasis was more clearly defined. However, but the smoothed data also had many more clusters. SEB can provide an estimation of suspected under-registrations in a geographical area, addressing problems in underreporting of HD and schistosomiasis. In addition, SEB does not take into account differences between neighbors, such as geographic barriers or population fluctuations, and can inflate disease incidence towards local means where cases would not be seen (72). SEB smoothed rates have been used in HD studies but its use in spatial statistics should be used with caution (24, 68-71).

SEB and raw incidence models were compared with respect to above considerations. Counter to earlier studies, household density, was inversely associated with HD in the SEB model (24). Based on the above uncertainty in SEB for modeling and unexpected relationship for household density, raw incidence models for aggregated census level data were compared to assess trends in HD and MB disease. While schistosomiasis presence showed a positive trend with HD, the trend was even stronger and significant for MB disease. These results for MB disease support our hypothesis of a geospatial co-occurrence of the two NTDs, while controlling for socio-economic characteristics that could confound the association.

At the individual georeferenced level, residence in census tract with reported schistosomiasis was not statistically significantly associated with MB diseases but trends in this association were identified that warrant further investigation. Univariate analysis

showed more cases of MB disease than PB disease occur in census tracts where schistosomiasis is present. Also, the risk of MB was trending about 1.5 times higher in those living in a census tract with schistosomiasis present than living in a tract without schistosomiasis.

Other characteristics of the individual level analysis are in agreement with previous research on MB diseases. While half of the overall cases were male, men were more than 3 times as likely to have MB diseases among cases as women, adjusted for other covariates. In a study of four endemic countries for HD, including Brazil, male to female ratios of MB diseases were as high as to 2:1 (but only 1.14 in Rio de Janeiro State) (73).

Due to the long incubation period of HD, the percentage of children among all incident cases of HD is an important epidemiological indicator which can be used to predict active disease transmission in the community (16, 17).Young people have had less time to move, which controls for mobility and can allow researchers to identify places with the most recent transmission (14). In this study, 8.6% of all HD cases were in children 15 or younger. Identifying patterns where with these childhood cases occur would be a good use of public health resources because they would be part of areas theoretically accepted as containing active transmission.

At the individual level, we did not find the potential confounders, population density and average monthly income, to be associated with MB disease even though they are expected predictors of HD infection. Additionally, household density did not trend in the

expected direction (15, 24). Due to missing demographics data in SINAN, these variables were acquired from Census geographic averages and assigned to individual level data, a limitation which may explain these unexpected results at the individual level.. As well, while income is used as a predictor of poverty, other predictors related to diseases of poverty, such as nutrition, may be more strongly associated with MB dynamics (25). In this study, population density was not associated at the individual level it was consistently significantly associated for HD and MB diseases at the aggregated census tract level. This may mean that while population density may be a predictor in determining areas of risk for HD, it does not directly correspond to individual risk. In this study, scale and passive surveillance methods effect the associations and should be taken into account in its limitations (74).

Underreporting of schistosomiasis is likely and is a limitation to our study. Municipality epidemiologists stated that many schistosomiasis infections go unreported unless they exhibit grave clinical manifestations. This underrepresentation could reduce the observed correlation with HD. Over 50% of the cases from SINAN, a passive surveillance system, did not report an address and could not be mapped to census tracts. 50% of reported schistosomiasis cases and 30% of reported HD cases is a limitation to this study; however, the distribution of cases were not drastically different by municipality between the full datasets and georeferenced subsets. Underreporting of HD studies another limitation. While HD reporting is compulsory, this is only done once cases are identified. One study in India found that for every diagnosed cases of HD there are 6 undiagnosed (75).

Conclusions

This study identified census level difference in disease dynamics when schistosomiasis is present in a census tract with MB disease. Since this is the first geospatial and multivariate analysis of this association for a region of this size even limited findings will provide a base for future research studies. This study on co-occurrences of schistosomiasis and HD show evidence of a statistically significant association at the census tract level and trends towards significance at the individual level. Efforts should continue in GIS using improved surveillance techniques to address limitations caused by passive surveillance, possibly through active case recruitment. Environmental factors expected to be associated with HD, including soil moisture, should also be considered in further spatial analysis. Ideally, co-infection studies using similar methods described should be considered. One such study is planned following an ongoing case-control study in the region targeting HD, helminth infections, and undernutrition.

F. Tables

Table 1. *Descriptive Characteristics Of Incident Cases Of Hansen's Disease In Minas Gerais, 2011-2015 (N=755).*

Characteristic	**Mean (SD) or N (%)**
Dependent variable	
Operational Classification	
Multibacillary	388 (51.4)
Paucibacillary	367 (48.6)
Residence in Census Tracts with reported Schistosomiasis	74 (9.8)
Demographic Characteristics	
Sex	
Male	387 (51.3)
Female	368 (48.7)
Age (continuous)	47.1 (19.6)
Age (by 15 year category)	
0-15	65 (8.6)
16-30	89 (11.8)
31-45	117 (23.4)
46-60	218 (28.9)
61-75	155 (20.5)
76-93	51 (6.5)
Average Household Density, by census	3.2 (0.2)
Population Density (people/km^2)	5,492 (4,545.8)
Average Household Income (Reales), by census*	974.8 (617.0)

*Nominal average monthly income of persons responsible for permanent private households

Table 2. *Unadjusted Bivariate Association Of Study Variables For Risk Of Multibacillary Hansen's Disease In Minas Gerais, Brazil, 2011-2015.*

	Multibacillary (n=388)		**Paucibacillary (n=367)**		**Unadjusted Odds Ratio**	
Characteristics	**N**	**%**	**N**	**%**	**Ratio (95% CI)**	**p-value**
Residence in Census Tract with Schistosomiasis						
Yes	44	11.3	30	8.2	1.44 (0.88, 2.24)	>0.1
No	344	88.7	337	91.8	1	REF
Sex						
Male	237	61.1	131	35.7	2.83 (2.10, 3.80)	**<0.001***
Female	151	38.9	236	64.3	1	REF
Age						
0-15	7	1.8	58	15.8	1	REF
16-30	39	10.1	50	13.6	6.46 (2.09, 10.14)	**<0.001***
31-45	104	26.8	73	19.9	11.80 (4.03, 17.50)	**<0.001***
46-60	119	30.7	99	27.0	9.96 (3.45, 14.58)	**<0.001***
61-75	90	23.2	65	17.7	11.47 (3.89, 17.15)	**<0.001***
76-93	29	7.5	21	6.0	10.92 (3.02, 17.66)	**<0.001***
Population Density (people/km^2)						
<3000	139	35.8	122	33.2	1	REF
3000-6000	127	32.73	119	32.4	0.94 (0.67, 1.34)	>0.5
=>6000	122	31.44	126	34.3	0.86 (0.60, 1.21)	.3817
Average Household Density, by census						
<3	97	25.0	104	28.3	1	REF
=>3	291	75.0	263	71.7	1.19 (0.85, 1.64)	>0.1
Average Monthly Income, by census						
<600	99	25.5	99	27.0	1	REF
600-1200	205	52.4	186	50.7	1.11 (0.79, 1.57)	>0.5
=>1200	84	21.7	82	22.3	1.04 (0.69, 1.56)	>0.5

*p-value significant <0.05

Table 3. *Adjusted Odds Ratios And 95% Confidence Intervals for Schistosomiasis and Covariates with Multibacillary Hansen's Disease in Minas Gerais, Brazil, 2011-2015.*

Covariate	aOR (95% CI)	P-value
Residence in Census Tract with Schistosomiasis		
Yes	1.578 (0.92, 2.71)	>0.05
No	REF	-
Sex		
Male	3.007 (2.20, 4.10)	**<0.001***
Female	REF	-
Age		
0-15	REF	-
16-30	7.10 (2.85, 17.68)	**<0.001***
31-45	12.68 (5.37, 29.90)	**<0.001***
46-60	11.48 (4.90, 26.87)	**<0.001***
61-75	12.48 (5.24, 29.75)	**<0.001***
76-93	10.96 (4.05, 29.63)	**<0.001***
Population Density (people/km^2)		
<3000	REF	-
3000-6000	0.93 (0.63, 1.36)	>0.5
=>6000	0.88 (0.60, 1.30)	>0.5
Average Monthly Income, by census		
<600	REF	-
600-1200	0.98 (0.67, 1.44)	>0.5
=>1200	0.94 (0.59, 1.49)	>0.5

*p-value significant <0.05

Table 4. *Kuldorff's Spatial Scan Most Likely Clusters Of HD, MD Disease And Schistosomiasis.*

# of Clusters	Type of Infection (s)	Level	Number of Census Tracts	Population at risk	Observed Cases	Expected Cases	Relative risk	P-value
0	MB/PB Leprosy	Individual	-	-	-	-	-	-
0	Multibacillary Leprosy	Individual	-	-	-	-	-	-
0	Schistosomiasis	Individual	-	-	-	-	-	-
1	MB/PB Leprosy	Census	1	139	5	0.3	17.12	<0.05
1	Multibacillary Leprosy	Census	10	6,433	41	13.15	3.37	<0.001
1	Schistosomiasis	Census	1	234	14	1.10	13.31	<0.001
2	Schistosomiasis	Census	1	832	22	3.9	5.99	<0.001
3	Schistosomiasis	Census	3	1,592	25	7.46	3.55	<0.001

Table 5. *Spatial Autocorrelation Of HD, MD Disease And Schistosomiasis.*

LISA Clusters	High Cluster	Low Clusters
Univariate		
MB and PB Leprosy	15	3
MB Leprosy	40	3
Schistosomiasis	42	4
Bivariate		
HD/Schistosomiasis	15	3
MB/Schistosomiasis	11	2

Table 6. *Adjusted Odds Ratios And 95% Confidence Intervals For Schistosomiasis And Covariates With All Hansen's Disease At Aggregated Census Tract Level.*

Covariate	aOR (95% CI)	P-value
Raw Data		
Schistosomiasis Presence		
Yes	1.56 (0.98, 2.48)	>0.05
No	REF	-
Population Density (people/km^2)		
<3000	REF	-
3000-6000	4.95 (3.51, 6.97)	**<0.001***
=>6000	7.13 (5.04, 10.09)	**<0.001***
Average Monthly Income (Reales)		
<600	REF	-
600-1200	1.61 (1.18, 2.20)	**<0.01***
=>1200	2.94 (1.82, 4.46)	**<0.001***
SEB smoothed Data		
Schistosomiasis Presence		
Yes	0.996 (.74, 1.34)	>0.5
No	REF	-
Average Household Density, by census		
<3	Ref	-
=>3	0.67 (0.49, 0.92)	**<0.05***
Population Density (people/km^2)		
<3000	REF	-
3000-6000	4.71 (3.18, 6.97)	**<0.001***
=>6000	12.81 (7.46, 21.98)	**<0.001***
Average Monthly Income, by census		
<600	REF	-
600-1200	2.04 (1.561, 2.66)	**<0.001***
=>1200	10.98 (5.49, 21.98)	**<0.001***

*p-value significant <0.05

Table 7. *Adjusted Odds Ratios And 95% Confidence Intervals For Schistosomiasis And Covariates With Multibacillary Hansen's Disease At Aggregated Census Tract Level.*

Covariate	aOR (95% CI)	P-value
Raw Data		
Schistosomiasis Presence		
Yes	1.66 (1.01, 2.71)	<.**05***
No	REF	-
Average Household Density, by census		
<3	Ref	-
=>3	1.17 (0.82, 1.68)	>0.3
Population Density (people/km^2)		
<3000	REF	-
3000-6000	4.44 (3.05, 6.45)	**<0.001***
=>6000	5.15 (3.55, 7.48)	**<0.001***
Average Monthly Income, by census		
<600	REF	-
600-1200	1.68 (1.18, 2.40)	**<0.01***
=>1200	2.77 (1.71, 4.49)	**<0.001***
SEB smoothed Data		
Schistosomiasis Presence		
Yes	1.26 (0.94, 1.69)	>0.1
No	REF	-
Average Household Density, by census		
<3	Ref	-
=>3	0.85 (0.62, 1.17)	>0.3
Population Density (people/km^2)		
<3000	REF	-
3000-6000	4.62 (3.21, 6.67)	**<0.001***
=>6000	11.07 (7.02, 17.47)	**<0.001***
Average Monthly Income, by census		
<600	REF	-
600-1200	2.25 (1.71, 2.95)	**<0.001***
=>1200	11.33 (6.22, 20.64)	**<0.001***

*p-value significant <0.05

G. Figures

Figure 1. *Study Area: 41 Municipalities in Minas Gerais, Brazil.*

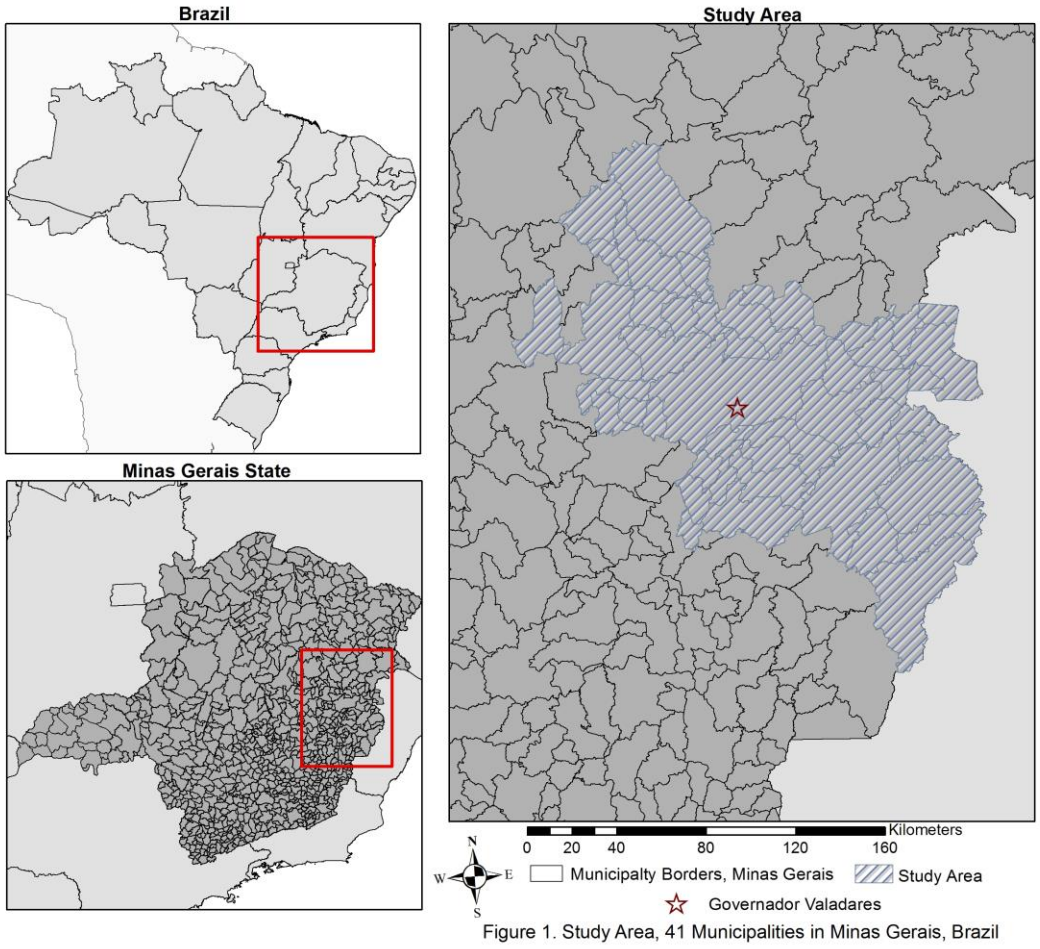


Figure 1. Study Area, 41 Municipalities in Minas Gerais, Brazil

Figure 2. *Univariate Spatial Analysis: Aggregated census tract level spatial analysis for all HD cases, MB disease and Schistosomiasis, respectively (1,2,3). Raw Annual Incidence (Column A), a Spatially Empirical Bayesian approach to smoothing (B), Local indicator of Spatial Autocorrelation (LISA, C), and Kulldorff's Spatial Scan Statistics most likely clusters (D) are presented.*

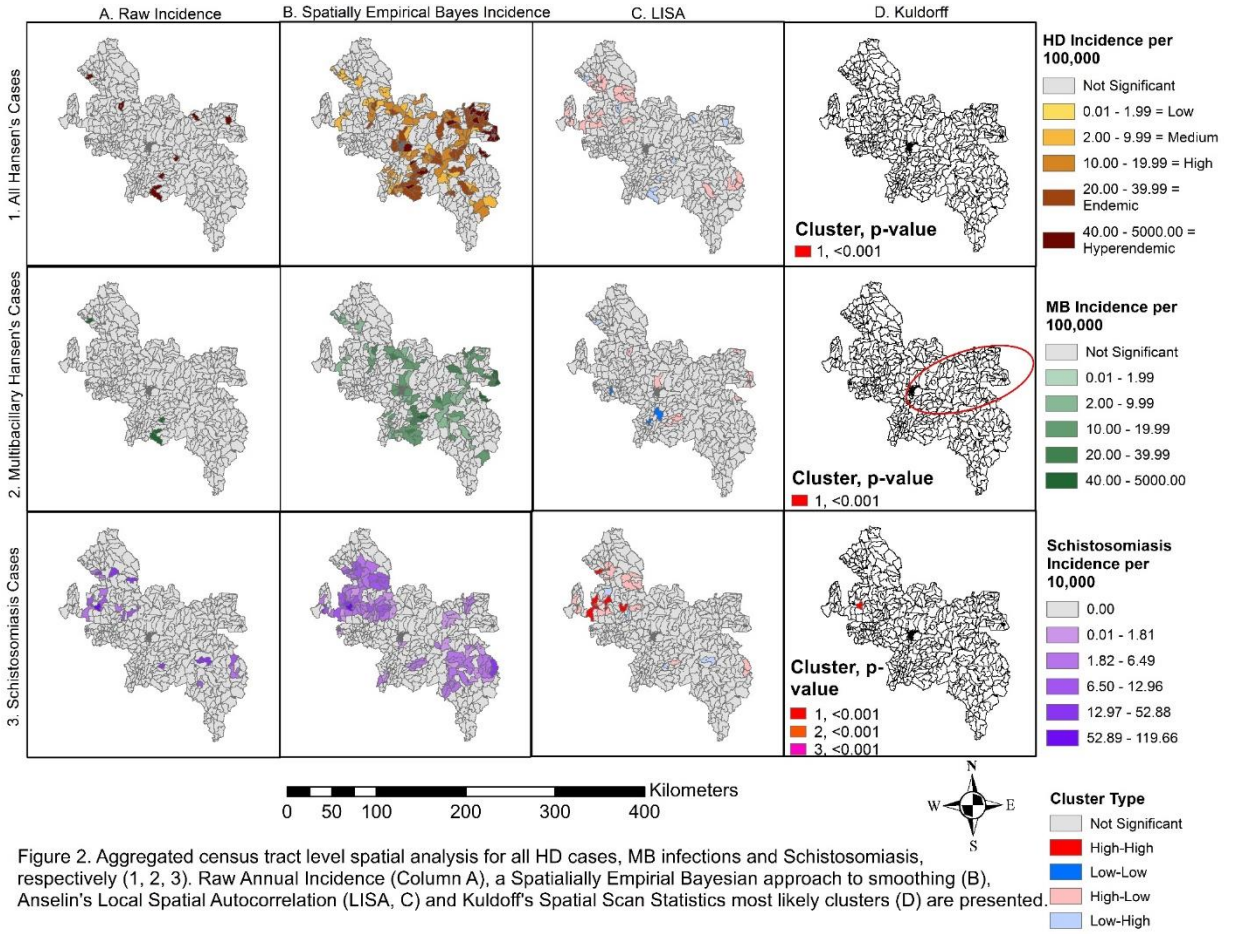
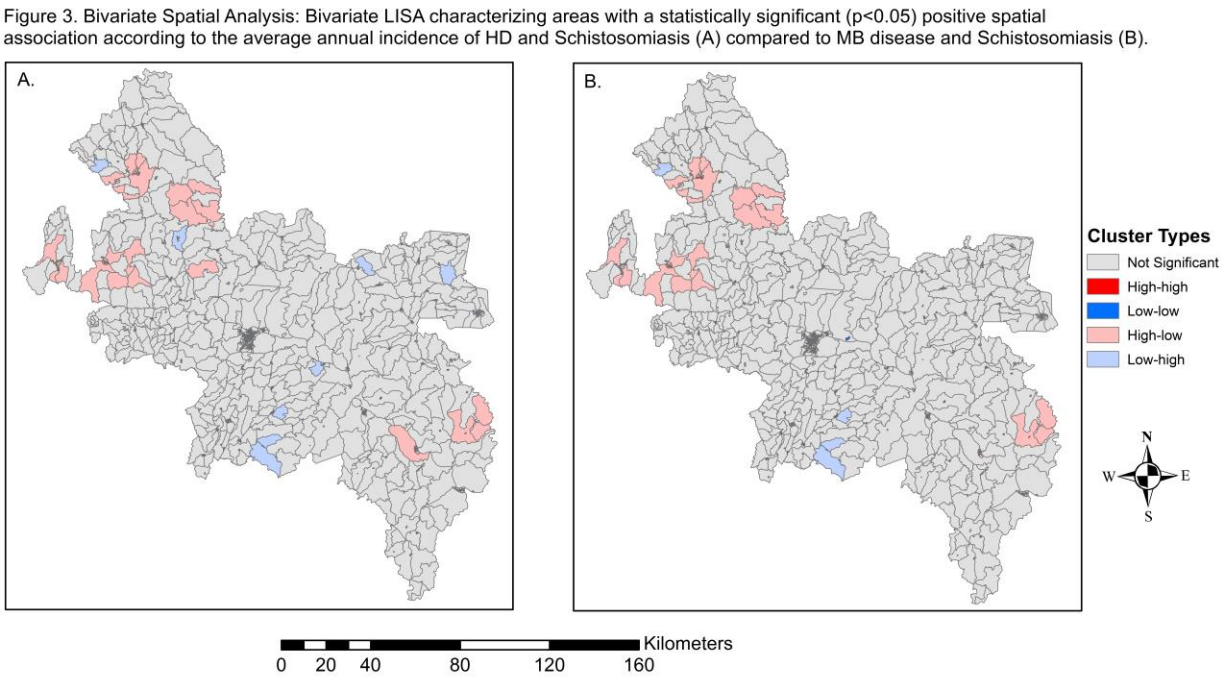


Figure 2. Aggregated census tract level spatial analysis for all HD cases, MB infections and Schistosomiasis, respectively (1, 2, 3). Raw Annual Incidence (Column A), a Spatialially Empirial Bayesian approach to smoothing (B), Anselin's Local Spatial Autocorrelation (LISA, C) and Kuldoff's Spatial Scan Statistics most likely clusters (D) are presented.

Figure 3. *Bivariate Spatial Analysis: Bivariate LISA characterizing areas with a statistically significant (p<0.05) positive spatial association with the average annual incidence of HD and schistosomiasis (A) compared to MB disease and schistosomiasis (B).*



CHAPTER III: CONCLUSION

Summary and Public Health Implications

This study provides a novel means to study HD transmission using spatial analysis and GIS to analyze co-occurrence of schistosomiasis which may affect HD transmission in an area with clusters of hyperendemic HD. Local spatial analysis provided a depiction of areas of high risk for co-occurrence. Furthermore, census tract multivariate analysis showing association of schistosomiasis with MB disease warrant more detailed analysis through co-infection studies. These findings not only suggest that helminth infections are associated with HD transmission, but that they can be used to help guide integrated control programs in co-endemic areas to decrease disease burden more effectively.

Our study has public health implications for the understanding and control of these two NTDs,. Theses preliminary findings of an association of schistosomiasis presence with MB disease can lead to further research on host susceptibility to MB disease. Our study also point to areas where efforts for HD and schistosomiasis control can be integrated (ie., locations that are affected by both). Spatial overlap of diseases, as found here, is one of the key elements for integration of vertical control programs (75, 76). While this study aims to address the biological and epidemiological significance of co-occurrence (which could be a marker of co-infections), the main operational issue for integrating programs is spatial co-occurrence (48). Census tracts identified for local bivariate clusters and the surrounding areas should be targeted for integrated control methods, reducing public health burden of multiples NTDs.

Possible Future Directions

This study is the first study to address spatial HD dynamics with another infection which may influence its transmission. Spatial analyses are used to identify geographical patterns of HD, MB diseases, and schistosomiasis and identify areas of high severity of both NTDs. Individual and aggregated level spatial statistics provide multiple levels of analysis, emphasizing the importance of spatial scale on results. Future studies using Kulldorff's spatial scan statistics should focus on different types of relationship or levels of outcomes (64). This study incorporated Bernoulli's models, with cases as the presence of HD, MB, or schistosomiasis, respectively, and controls as the rest of the population at risk. An ordinal model using recognized categories of endemicity may specify other most likely clusters, leading to more areas to target public health efforts.

Non-spatial analyses also test HD and schistosomiasis associations from multiple levels, controlling for covariates described as associated with HD in the literature. Trends towards a positive association of living in a census tract with schistosomiasis presence on MB individual cases and the significant positive association on MB presence in that tract, further analysis on this relationship would be beneficial. Future studies modelling the clinical forms of HD as outcomes, specifically lepromatous leprosy as this is a subtype of MB diseases with the strongest immune reaction similar to schistosomiasis (36, 37).

As data on biological plausibility of this interaction and patterns of co-occurrence advance, the next steps to examine this relationships would be to conduct co-infections spatial analyses. Geospatial studies on co-infections between multiple soil transmitted helminths, malaria, trypanosomiasis, and onchocerciasis have shown synergism and exacerbated disease outcomes

(41, 44, 45). Addressing the geographical association between schistosomiasis and MB through co-infections studies would strengthen the hypothesis that helminth infections cause a shift in HD host immune response (38, 39).

Since this is the first geospatial and multivariate analysis of this association for a region of this size, even limited findings can provide a base for future research, including an ongoing case-control study in the region targeting leprosy, helminth infections, undernutrition, and future geospatial studies involving active case finding. This study identifies seven cities (Itanhomi, Conselheiro Pena, Coroaci, Pecanha, Itabinha, Santa Maria do Suacui and Sao Jose da Saffra) which should be targeted for integrated NTD control for HD and schistosomiasis. It also recommends critical attention be brought to the cities with five highest HD incidence (GV, Mantena, Nova Belem, Sao Geraldo do Baixio and Itabirinha) to reduce the burden of HD disease in the region.

APPENDICES

Figure S1. Data Flow Chart

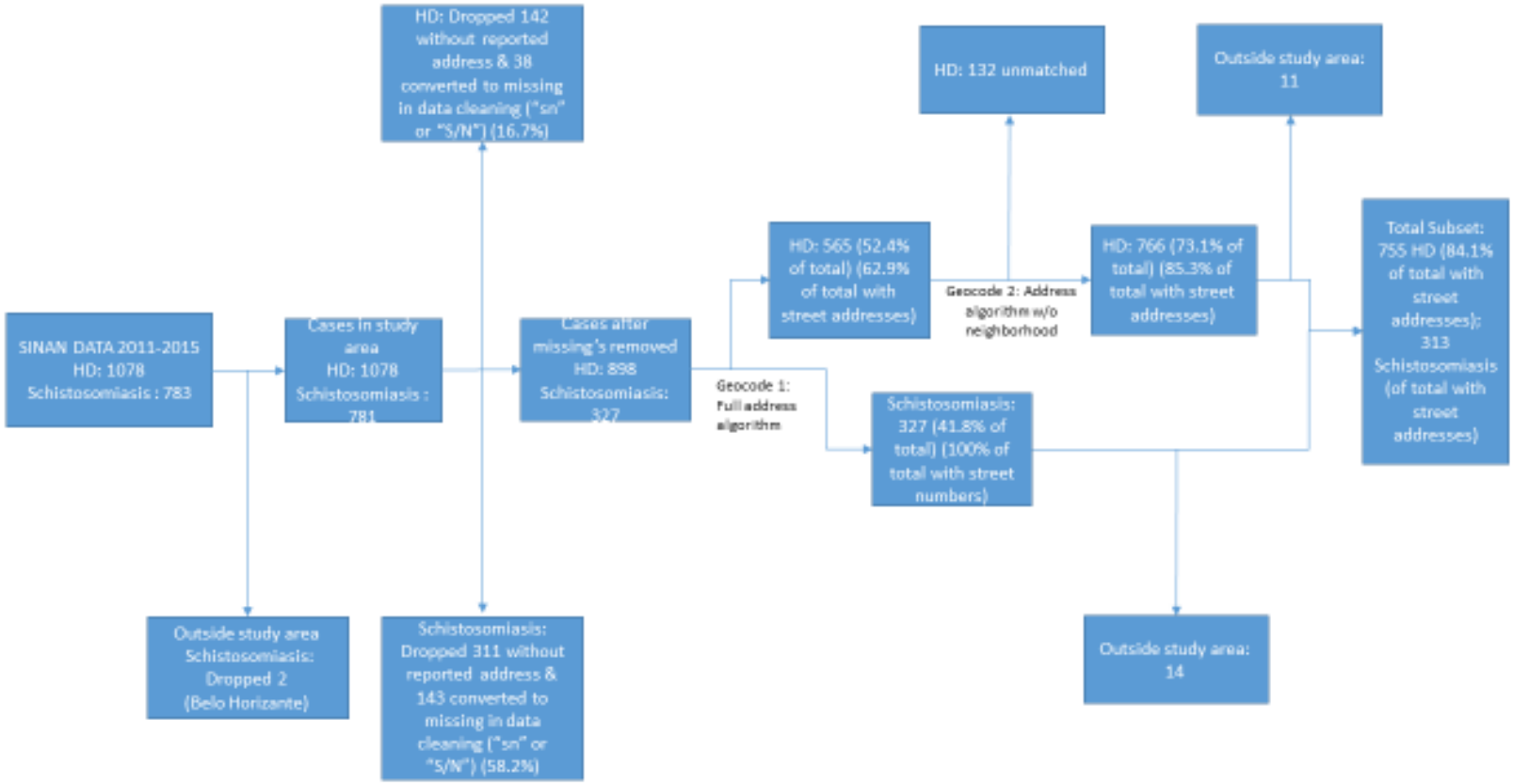


Figure S2. Distribution of HD Cases by Municipality, 2011-2015, Full vs Geocoded Data

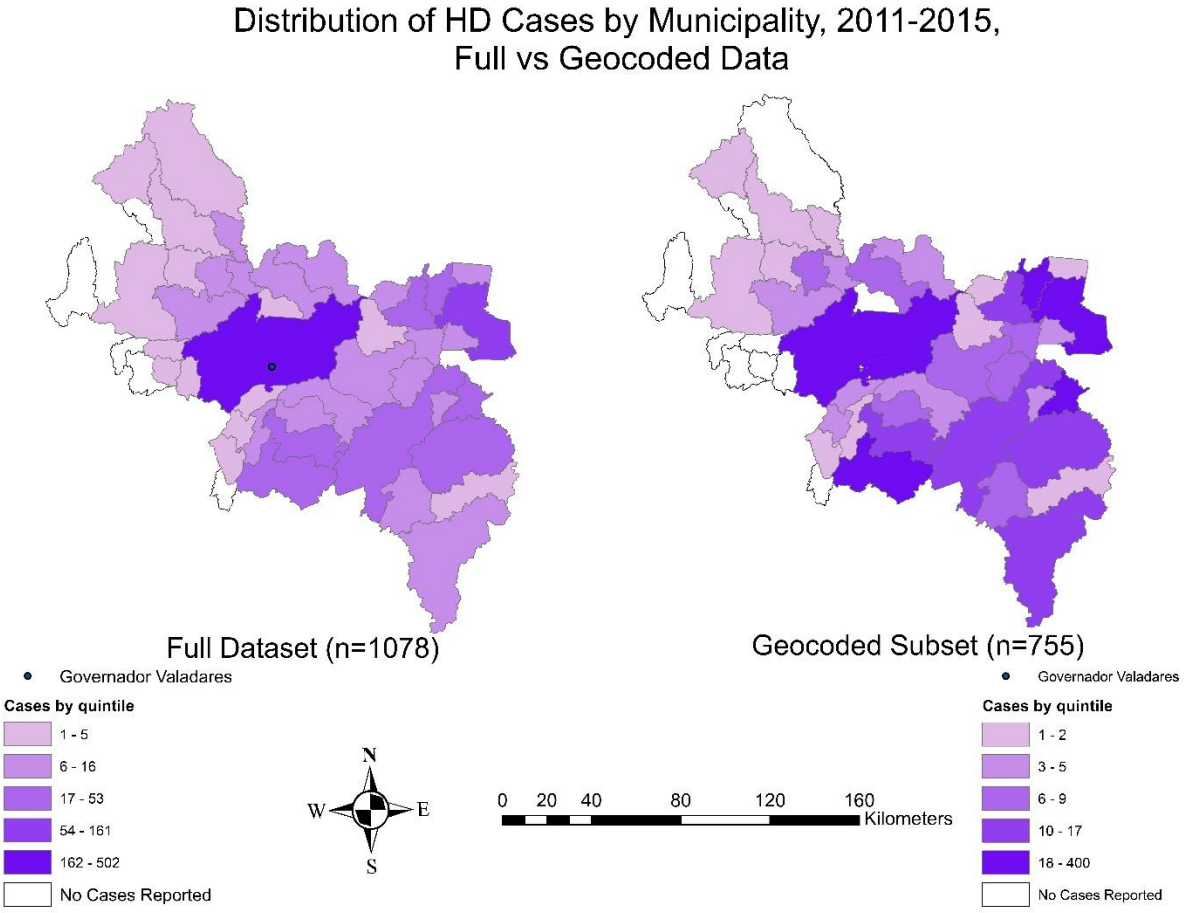


Figure S3. Distribution of Schistosomiasis Cases by Municipality, 2011-2015, Full vs Geocoded Data

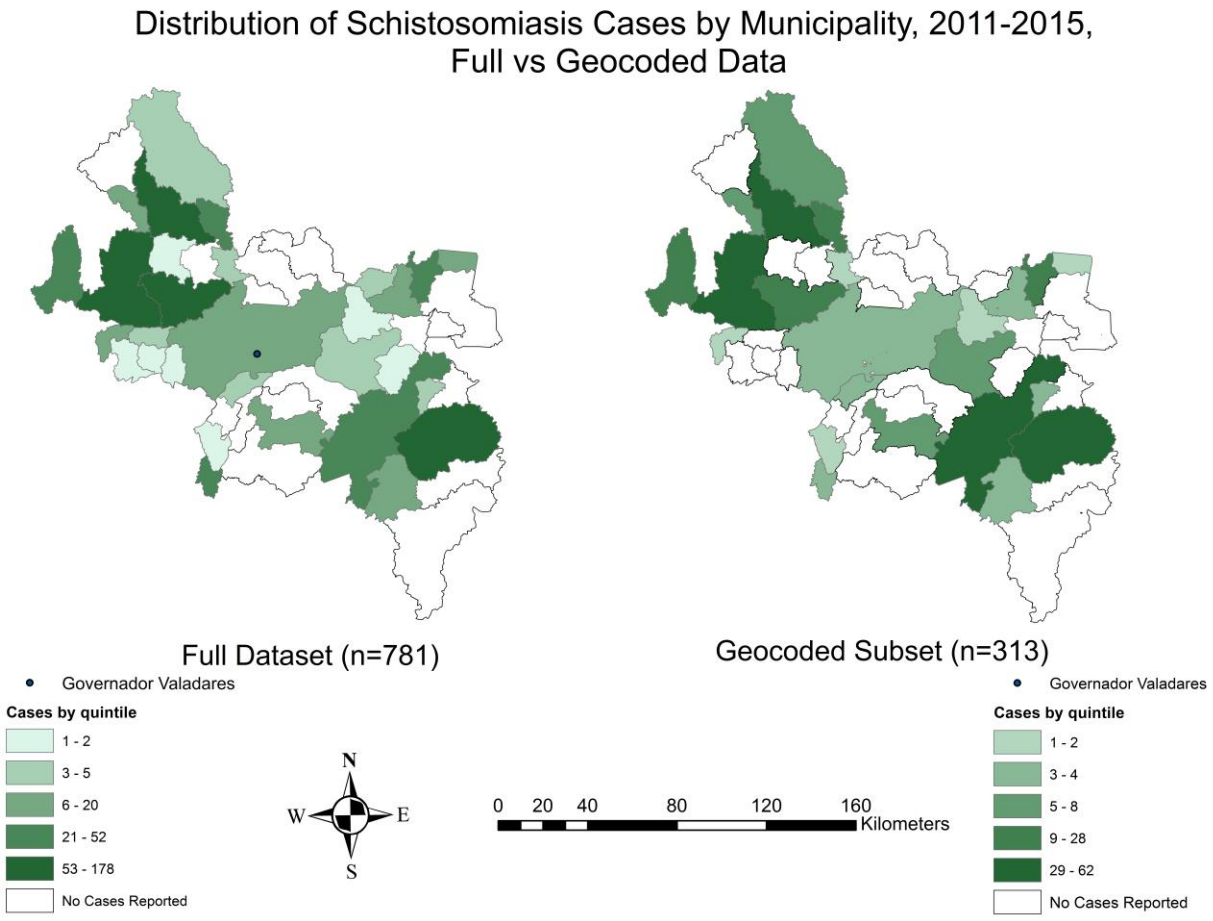
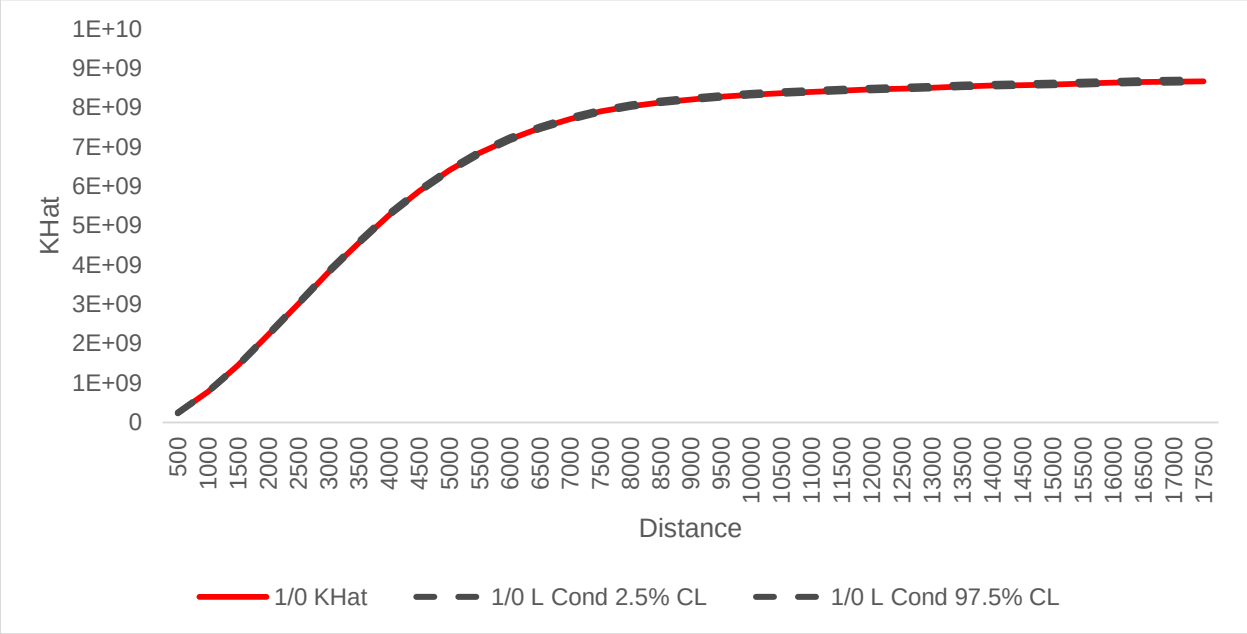


Figure S4. Bivariate K-function (MB disease vs PB disease)



REFERENCES

1. Nations, M.K., G.V. Lira, and A.M.F. Catrib, Stigma, deforming metaphors and patients' moral experience of multibacillary leprosy in Sobral, Ceará State, Brazil. Cadernos de Saúde Pública, 2009. 25: p. 1215-1224.
2. Holveck, J.C., et al., Prevention, control, and elimination of neglected diseases in the Americas: pathways to integrated, inter-programmatic, inter-sectoral action for health and development. BMC Public Health, 2007. 7(1): p. 6.
3. White, C. and C. Franco-Paredes, Leprosy in the 21st century. Clin Microbiol Rev, 2015. 28(1): p. 80-94.
4. World Health Organization (2016) "Global Leprosy Strategy 2016-2020: Accelerating towards a leprosy-free world." World Health Organization, Regional Office for South-East Asia. India: World Health Orgainzation. ISBN 978-92-9022-509-6. Assessed at http://www.wpro.who.int/leprosy/documents/globalleprosystrategy2016-2020.pdf
5. "Weekly Epidemiology Record." World Health Organization. 35(91): 405–420. Sept 2 2016. Assessed at http://apps.who.int/iris/bitstream/10665/249601/1/WER9135.pdf?ua=1
6. World Health Organization (2017). "Leprosy: Fact Sheet". World Health Organization, Media Centre. Assessed at http://www.who.int/mediacentre/factsheets/fs101/en/
7. "Weekly Epidemiology Record." World Health Organization. 35(88): 365–380. Aug 30, 2013, Assessed at http://www.who.int/wer/2013/wer8835.pdf?ua=1
8. "Global leprosy: update on the 2012 situation." *Weekly epidemiological record / Health Section of the Secretariat of the League of Nations.* **88**, 365 (Aug 30, 2013).

9. Brasil (2013) Saúde Brasil 2012: uma análise da situação de saúde e dos 40 anos do Programa Nacional de Imunizações; Ministério da Saúde, Secretaria de Vigilância em Saúde. Brasília: Ministério da Saúde. 536 p
10. Institute for Health Metrics and Evaluation. "Global Health Data Exchange". University of Washington. Assessed at ghdx.healthdata.org
11. Ridley, D.S., Histological classification and the immunological spectrum of leprosy. Bulletin of the World Health Organization, 1974. 51(5): p. 451-465.
12. Lockwood, D.N. and P.R. Saunderson, Nerve damage in leprosy: a continuing challenge to scientists, clinicians and service providers. Int Health, 2012. 4(2): p. 77-85.
13. Zhang, Fu-Ren, et al. "Genomewide association study of leprosy." New England Journal of Medicine 361.27 (2009): 2609-2618.
14. Cabral-Miranda, W., F. Chiaravalloti Neto, and L.V. Barrozo, Socio-economic and environmental effects influencing the development of leprosy in Bahia, north-eastern Brazil. Trop Med Int Health, 2014. 19(12): p. 1504-14.
15. Blake, L.A., et al., Environmental nonhuman sources of leprosy. Rev Infect Dis, 1987. 9(3): p. 562-77.
16. Dogra, S., et al., Childhood leprosy through the post-leprosy-elimination era: a retrospective analysis of epidemiological and clinical characteristics of disease over eleven years from a tertiary care hospital in North India. Lepr Rev, 2014. 85(4): p. 296-310.
17. Sasidharanpillai, S., et al., Childhood leprosy: a retrospective descriptive study from Government Medical College, Kozhikode, Kerala, India. Lepr Rev, 2014. 85(2): p. 100-10.

18. The World Health Organization. Transmission of Leprosy. Leprosy Elimination. Available at http://www.who.int/lep/transmission/en/. Accessed: April 15, 2016.

19. Peters, E. and A. Eshiet, Male-female (sex) differences in leprosy patients in south eastern Nigeria: females present late for diagnosis and treatment and have higher rates of deformity. Leprosy review, 2002. 73(3): p. 262-267.

20. Fine, P.E., Leprosy: the epidemiology of a slow bacterium. Epidemiologic reviews, 1982. 4(1): p. 161-188.

21. Sk, N., The Epidemiology of Leprosy in: Hastings SRC. Leprosy. 1ª ed. New York: Churchill Livingstone, 1985: p. 15-30.

22. Richardus, J., et al., Case detection, gender and disability in leprosy in Bangladesh: a trend analysis. Leprosy review, 1999. 70(2): p. 160-173.

23. Schreuder, P.A., The occurrence of reactions and impairments in leprosy: Experience in the leprosy control program of three provinces in Northeastern Thailand, 1978-1995: III. Neural and other. International journal of leprosy and other mycobacterial diseases, 1998. 66(2): p. 170.

24. Pönnighaus, J., et al., Incidence rates of leprosy in Karonga District, northern Malawi: patterns by age, sex, BCG status and classification. International journal of leprosy and other mycobacterial diseases: official organ of the International Leprosy Association, 1994. 62(1): p. 10-23.

25. Barreto, J.G., et al., Spatial analysis spotlighting early childhood leprosy transmission in a hyperendemic municipality of the Brazilian Amazon region. PLoS Negl Trop Dis, 2014. 8(2): p. e2665.

26. Feenstra, S.G., et al., Recent food shortage is associated with leprosy disease in Bangladesh: a case-control study. PLoS Negl Trop Dis, 2011. 5(5): p. e1029.
27. Rao, P. and A. John, 3 Nutritional status of leprosy patients in India. Indian journal of leprosy, 2012. 84(1): p. 17.
28. Truman, R. and P. Fine, 'Environmental'sources of Mycobacterium leprae: issues and evidence. Leprosy review, 2010. 81(2): p. 89-95.
29. Matsuoka, M., Izumi, S., Budiawan, T., Nakata, N., Saeki, K., 1999. Mycobacterium leprae DNA in daily using water as a possible source of leprosy infection. Indian J. Lepr. 71, 61–67.
30. Conger, N.G., et al., Mycobacterium simae outbreak associated with a hospital water supply. Infect Control Hosp Epidemiol, 2004. 25(12): p. 1050-5.
31. Mohanty, P.S., et al., Viability of Mycobacterium leprae in the environment and its role in leprosy dissemination. Indian Journal of Dermatology, Venereology, and Leprology, 2016. 82(1): p. 23.
32. Turankar, R.P., et al., Presence of viable Mycobacterium leprae in environmental specimens around houses of leprosy patients. Indian J Med Microbiol, 2016. 34(3): p. 315-21.
33. Lavania, M., et al., Detection of viable Mycobacterium leprae in soil samples: insights into possible sources of transmission of leprosy. Infect Genet Evol, 2008. 8(5): p. 627-31.
34. Kerr-Pontes, L.R., et al., Socioeconomic, environmental, and behavioural risk factors for leprosy in North-east Brazil: results of a case–control study. International journal of epidemiology, 2006. 35(4): p. 994-1000.

35. Infante-Duarte, C. and T. Kamradt, Th1/Th2 balance in infection. Springer Seminars in Immunopathology, 1999. 21(3): p. 317-338.

36. van Riet, E., F.C. Hartgers, and M. Yazdanbakhsh, Chronic helminth infections induce immunomodulation: Consequences and mechanisms. Immunobiology, 2007. 212(6): p. 475-490.

37. Piris, A., A.Z. Lobo, and S.L. Moschella, Global dermatopathology: Hansen's disease–current concepts and challenges. Journal of cutaneous pathology, 2010. 37(s1): p. 125-136.

38. Geiger, S., Immuno-epidemiology of Schistosoma mansoni infections in endemic populations co-infected with soil-transmitted helminths: present knowledge, challenges, and the need for further studies. Acta tropica, 2008. 108(2): p. 118-123.

39. Diniz, L.M., et al., Short report: do intestinal nematodes increase the risk for multibacillary leprosy? Am J Trop Med Hyg, 2001. 65(6): p. 852-4.

40. Diniz, L.M., et al., Presence of intestinal helminths decreases T helper type 1 responses in tuberculoid leprosy patients and may increase the risk for multi-bacillary leprosy. Clin Exp Immunol, 2010. 161(1): p. 142-50.

41. Prost, A., M. Nebout, and A. Rougemont, Lepromatous leprosy and onchocerciasis. Br Med J, 1979. 1(6163): p. 589-90.

42. Brooker, Simon, et al. "An updated atlas of human helminth infections: the example of East Africa." *International journal of health geographics* 8.1 (2009): 42.

43. Nakagawa, Jun, et al. "Towards effective prevention and control of helminth neglected tropical diseases in the Western Pacific Region through multi-disease and multi-sectoral interventions." Acta tropica 141 (2015): 407-418.

44. Fenn, Bridget, Saul S. Morris, and Robert E. Black. "Comorbidity in childhood in northern Ghana: magnitude, associated factors, and impact on mortality." International Journal of Epidemiology 34.2 (2005): 368-375.

45. Brooker, S., and E. Michael. "The potential of geographical information systems and remote sensing in the epidemiology and control of human helminth infections." Advances in parasitology 47 (2000): 245-288.

46. Brooker, Simon, Archie CA Clements, and Don AP Bundy. "Global epidemiology, ecology and control of soil-transmitted helminth infections." Advances in parasitology 62 (2006): 221-261.

47. Gebre, Shibru, et al. "The effect of HIV status on the clinical picture of leprosy: a prospective study in Ethiopia." Leprosy review 71.3 (2000): 338-343.

48. http://www.who.int/lep/monitor/gis/en/ "Leprosy Elimination: Geographic Information Systems."

49. Najafabadi, A. T. "Applications of GIS in health sciences." Shiraz E-Medical Journal 10.4 (2009): 221-230.

50. Kelly-Hope, L.A., et al., Innovative tools for assessing risks for severe adverse events in areas of overlapping Loa loa and other filarial distributions: the application of micro-stratification mapping. Parasit Vectors, 2014. 7: p. 307.

51. Rodrigues, A.L., Jr., A. Ruffino-Netto, and E.A. de Castilho, [Spatial distribution of M. tuberculosis-HIV coinfection in Sao Paulo State, Brazil, 1991-2001]. Rev Saude Publica, 2006. 40(2): p. 265-70.

52. Goswami, N.D., et al., Geographic information system-based screening for TB, HIV, and syphilis (GIS-THIS): a cross-sectional study. PLoS One, 2012. 7(10): p. e46029.

53. Zhou, Y.B., et al., The geographic distribution patterns of HIV-, HCV- and co-infections among drug users in a national methadone maintenance treatment program in Southwest China. BMC Infect Dis, 2014. 14: p. 134.

54. Honório, Nildimar Alves, et al. "Spatial evaluation and modeling of dengue seroprevalence and vector density in Rio de Janeiro, Brazil." *PLoS Negl Trop Dis* 3.11 (2009): e545.

55. Zambrano, Lysien I., et al. "Estimating and mapping the incidence of dengue and chikungunya in Honduras during 2015 using Geographic Information Systems (GIS)." Journal of Infection and Public Health (2016).

56. Phillips, D.A., et al., A tale of two neglected tropical infections: using GIS to assess the spatial and temporal overlap of schistosomiasis and leprosy in a region of Minas Gerais, Brazil. Memórias do Instituto Oswaldo Cruz, 2017. 112(4): p. 275-280.

57. Alencar, C.H., et al., Clusters of leprosy transmission and of late diagnosis in a highly endemic area in Brazil: focus on different spatial analysis approaches. Trop Med Int Health, 2012. 17(4): p. 518-25.

58. Nicchio, M.V., et al., Spatial and temporal epidemiology of Mycobacterium leprae infection among leprosy patients and household contacts of an endemic region in Southeast Brazil. Acta Trop, 2016. 163: p. 38-45.

59. Guimaraes, R.J., et al., A geoprocessing approach for studying and controlling schistosomiasis in the state of Minas Gerais, Brazil. Mem Inst Oswaldo Cruz, 2010. 105(4): p. 524-31.

60. Census 2010. Base de informações do Censo Demográfico 2010: Resultados do Universo por setor censitário" Ministério de Planejamento, Orçamento e Gestão. Instituto

Brasileiro de Geografia e Estatística – IBGE. Centro de Documentação e Disseminação de Informações

61. Climate Minas Gerais: Temperature, Climate graph, Climate table for Minas Gerais - Climate-Data.org. https://en.climate-data.org/region/203/. Accessed 3.23.2017.
62. Martins, D.d.S., et al., Schistosomiasis in Southern Brazil 17 years after the confirmation of the first autochthonous case. Revista da Sociedade Brasileira de Medicina Tropical, 2015. 48(3): p. 354-357.
63. Instituto Brasileiro de Geografia e Estatıstica (2011) Base de informacß~oes do Censo Demografico 2010: Resultados do Universo por Setor Censitario. Rio de Janeiro: Centro de Documentacß~ao e Disseminacß~ao de Informacß~oes. 200p.41. Bakker, M.I., et al., Population survey to determine risk factors for Mycobacterium leprae transmission and infection. Int J Epidemiol, 2004. 33(6): p. 1329-36.
64. Ripley BD (1976) The second-order analysis of stationary point patterns. Journal of Applied Probability 13: 255–266.
65. Bakker M, Hatta M, Kwenang A, FaberW, van Beers S, et al. (2004) Population survey to determine risk factors for Mycobacterium leprae transmission and infection. Int J Epidemiol 33: 1329–133.
66. Rosenberg, M.S., and C.D. Anderson (2011) PASSaGE: Pattern Analysis, Spatial Statistics and Geographic Exegesis. Version 2. Methods in Ecology and Evolution 2(3):229-232
67. Kulldorff, M., SaTScanTM User Guide for version 9.3. 2014.
68. Waller, L.A. and C.A. Gotway, Applied spatial statistics for public health data. Vol. 368. 2004: John Wiley & Sons.

69. Imbiriba, E.N.B., et al., Social inequality, urban growth and leprosy in Manaus: a spatial approach. Revista de saude publica, 2009. 43(4): p. 656-665.

70. Duarte-Cunha, M., et al., Epidemiological aspects of leprosy: a spatial approach. Cadernos de Saúde Pública, 2012. 28(6): p. 1143-1155.

71. Sampaio, P.B., et al., Spatial analysis of new cases of leprosy in the State of Espírito Santo, Brazil, between 2004 and 2009. Revista da Sociedade Brasileira de Medicina Tropical, 2012. 45(3): p. 380-384.

72. Krishnamurthy, P., Hidden leprosy--who is hiding from whom? Leprosy Review, 2004. 75(4): p. 303.

73. Bailey, T., et al., Modeling of under-detection of cases in disease surveillance. Annals of epidemiology, 2005. 15(5): p. 335-343.

74. Varkevisser, C.M., et al., Gender and leprosy: case studies in Indonesia, Nigeria, Nepal and Brazil. Leprosy review, 2009. 80(1): p. 65-76.

75. Goodchild, M.F., Scale in GIS: An overview. Geomorphology, 2011. 130(1): p. 5-9.

76. Moet, Fake J., et al. "The prevalence of previously undiagnosed leprosy in the general population of northwest Bangladesh." PLoS Negl Trop Dis 2.2 (2008): e198.

77. Hotez, P., et al., Recent progress in integrated neglected tropical disease control. Trends in parasitology, 2007. 23(11): p. 511-514.

78. Clements, A.C., et al., Spatial co-distribution of neglected tropical diseases in the east African great lakes region: revisiting the justification for integrated control. Tropical medicine & international health, 2010. 15(2): p. 198-207.