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Spatial Distribution of *Mycobacterium leprae* Infection and Association with Unsafe Water and Sanitation in Eastern Minas Gerais, Brazil

By

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By

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Bachelor of Science
University of Arizona
2019

Thesis Committee Chair: Jessica Fairley, MD, 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 Global Epidemiology
2024

Abstract

Spatial Distribution of *Mycobacterium leprae* Infection and Association with Unsafe Water and Sanitation in Eastern Minas Gerais, Brazil
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Background: The mode of transmission of *M. leprae*, the causative agent of Hansen's Disease (HD), remains uncertain due to the long incubation period of HD and the inability to culture *M. leprae* in the lab. Our research investigates the role of household-level water, sanitation, and hygiene (WASH) factors on HD transmission.

Methods: We conducted a cross-sectional study of 1,315 participants living in four municipalities of a HD-endemic area of Minas Gerais, Brazil. Individuals were tested for *M. leprae* infection determined via antibody testing against LID-1, a recombinant *M. leprae* protein. Data were collected on participants' household WASH factors and demographics through questionnaires. We created smooth relative risk surfaces to estimate the spatial distribution of relative risk of anti-LID-1 positivity and used logistic regressions to estimate associations between sets of exposures and anti-LID-1 positivity.

Results: Among the study population, the highest anti-LID-1 positivity rates were found in the municipality of Mantena (12.26%). These results are consistent with patterns of reported HD cases, indicating that reported HD cases may act as reasonable representations of underlying *M. leprae* distribution. Analyzed household-level WASH factors of piped household drinking water (aOR = 2.06, 95% CI 0.86, 5.64), piped water for toilet (aOR = 0.60, 95% CI 0.30, 1.29), and shared household toilet facility (aOR = 0.56, 95% CI 0.29, 1.03) did not have a significant association with anti-LID-1 positivity on adjusted analysis. Residence in rural regions (cOR = 2.53, 95% CI 1.45, 4.28) and residence in Mantena (cOR = 2.77, 95% CI 1.59, 4.76) were found to be significantly associated with anti-LID-1 positivity in univariate analysis. In adjusted analysis, the estimated association with rural residence was mitigated (aOR = 1.68, 95% CI 0.73, 4.10).

Conclusions: Disease mapping of anti-LID-1 positivity showed that there is heterogeneity of *M. leprae* infection in the eastern region of Minas Gerais. Our analyses of residence in rural regions as an exposure indicates that there may be additional environmental or structural characteristics at the municipality level that are not inherently related to rural living that affect the risk of anti-LID-1 positivity.

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Acknowledgements

I would like to thank my advisors, Dr. Jessica Fairley and Dr. Lance Waller, for their continued support and mentorship in navigating the thesis process, and Dr. Julie Clennon for geocoding participants' addresses for use in this analysis. I would also like to thank Dr. Michael Kramer and Dr. Lauren Christiansen-Lindquist for their excellent methods courses that provided foundational skills and knowledge I used throughout my thesis. Finally, I would like to thank my friends and family for their invaluable support while writing this thesis and over the past two years at RSPH.

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CHAPTER I: LITERATURE REVIEW

Literature Review

Leprosy (Hansen's Disease)

Leprosy, also known as Hansen's Disease, is a chronic infection predominantly caused by *Mycobacterium leprae* (*M. leprae)* and less commonly *Mycobacterium lepromatosis* (*M. lepromatosis*)[1]. While the term 'leprosy' is still often used in the present day, there has been a shift towards using the term 'Hansen's Disease' instead due to the significant stigma associated with 'leprosy'. In Brazil in particular, 'Hansen's Disease' is used exclusively, with the term 'leprosy' prohibited by law[2]. For this reason, this thesis will exclusively use the term 'Hansen's Disease' or 'HD' when describing this disease.

Clinical Characteristics of Hansen's Disease

Hansen's Disease has a wide range of clinical manifestations. The majority of people exposed to *M. leprae* do not develop symptomatic infection, with an estimated 95% of the population being resistant to disease[3]. In reality, many people in this 'resistant' population may in fact have latent infections. *M. leprae* also has a long and variable incubation period, ranging from 1 to 20 years from the time of infection to the time of symptom onset[4]. The different clinical manifestations of HD are a result of differences in immune response and have implications for ongoing transmission.

There are also different classification schema for subclasses of HD. In the *Madrid classification*, there are four main clinical classifications: indeterminate (I), tuberculoid leprosy (TT), borderline leprosy (BB), and lepromatous leprosy (LL). In the *Ridley-Jopling classification*, borderline disease can be further subdivided into borderline tuberculoid (BT), mid-borderline (BB), and borderline lepromatous (BL). In comparison, the World Health Organization simplifies classification in order to streamline treatment decisions. Cases with less than 5 lesions are described as paucibacillary (PB) and are characterized by a Th1 immune response. Typically PB HD includes tuberculoid and borderline tuberculoid. PB HD results in fewer skin lesions and little or no neurological damage, and is generally considered not to be contagious. In contrast, multibacillary (MB) disease includes 5 or more lesions and the clinical categories of some BT, BB, BL and LL. MB is characterized by a Th2 immune response, with patients typically presenting with higher bacterial loads and more skin lesions. MB HD also often results in more severe neurological damage[5,6] A systematic review of HD studies including over 10,000 HD patients across eight countries found a wide range of proportions of MB, ranging from 14% to 89% MB, with a pooled proportion of 69%[7]. Within the four studies from Brazil, the pooled MB proportion was 73%[7] Another study utilizing over 500,000 reported cases in Brazil from 2001 to 2013 found the annual MB proportion to range from 51.3% to 65.1%, with higher proportions of MB generally found in more recent years[8]. However, the generally higher proportions of MB could be a result of under recognition of PB or over diagnosis of MB, rather than a true difference.

Global Epidemiology of Hansen's Disease

Due to the long incubation period associated with Hansen's Disease, reported numbers are likely an underestimate of the true worldwide burden of HD. HD is predominantly found in low- and middle-income countries in the Global South and is designated a Neglected Tropical Disease (NTD) but does occur globally in over 120 countries. Over 200,000 incident cases are reported per year; the vast majority (>10,000 each) are in Brazil, India, and Indonesia[5,9].

Transmission Routes of Hansen's Disease

The nature of *M. leprae* makes identifying the specific route of transmission very difficult, and it has never been definitively proven. Unlike many other human pathogens, *M. leprae* cannot be cultured in a lab, making it difficult to identify viable bacteria in potential reservoirs like soil or water. Epidemiologic clues to transmission are limited due to the extremely long incubation period, which often makes it impossible to identify the source of infection. In addition to the droplet and zoonotic transmission routes, environmental transmission has also been proposed as a route of infection, with many studies in recent years investigating this route.

M. leprae infections in humans is traditionally considered to be transmitted person-to-person via droplets, and this information is typically reported on informational health websites, such as the World Health Organization's Leprosy Fact Sheet[9]. Other sources, including the US Centers for Disease Control and Prevention, also include zoonotic transmission from the nine-banded armadillo, although noting this as a very rare transmission route[10]. However, in practice the observed transmission routes of HD are significantly more complicated and, in some cases, contentious and difficult to ascertain.

Person-to-Person Transmission

In support of person-to-person droplet transmission, *M. leprae* has been found in the nose of HD patients and their household contacts which suggests a role for asymptomatic transmission, although this is in conflict with the predominant understanding that only symptomatic multibacillary infections are contagious[11–13]. Being a household contact of someone with HD has also been shown to be one of the most important risk factors for developing HD[14], although proponents of the environmental route of transmission may use such associations as an indication of a shared, but unknown, household environmental exposure. It should be noted however that many HD patients do not report contact with an infected person, suggesting an alternate zoonotic or environmental transmission route[15]. Furthermore, it was more common for patients to not be associated with an identified index case in areas of high HD transmission, as compared to areas of low HD transmission, further suggesting an environmental reservoir in high transmission areas[15]. An alternative hypothesis to support this phenomenon posits that asymptomatic cases, potentially common in high transmission areas, are the true source case or cases, although again this is in conflict with the current understanding of which types of HD are contagious[15].

Zoonotic Transmission

The nine-banded armadillo is considered to be the only other major reservoir of *M. leprae* besides humans, although recent students have investigated other potential reservoirs such as red squirrels in the British Isles[15,16]. Hansen's Disease has also been observed in wild chimpanzees with *M. leprae* strains notably different than those common in humans in those countries[17]. Zoonotic transmission has been strongly supported through molecular evidence showing that strains of *M. leprae* found in armadillos and local human HD patients were nearly identical[18]. Epidemiological evidence is more nuanced. A systematic review regarding the association between HD and contact with armadillos found that studies varied in their definition of contact, ranging from eating armadillo meat, direct contact, and indirect contact[19]. Many of the studies had small sample sizes or did not control for confounding variables, and recall bias is likely in the case-control studies[19]. Nevertheless, direct contact was consistently found to have an association with HD[19]. Many HD patients did not have close contact or household contact with a known HD patient, which lends further support to alternate transmission routes[20]. However, the long incubation period of HD and potential role of subclinical cases in transmission indicates that self-report of contact with a known HD patient may be unreliable. Furthermore, some HD patients report no contact with armadillos or another HD patient, suggesting an environmental transmission component. One hypothesis combining zoonotic and environmental transmission is that an environmental reservoir such as water or soil acts as an intermediary between armadillos and humans, explaining how people may become infected even while not reporting direct contact with armadillos. This is supported by a case report from Georgia where a woman was diagnosed with HD without having direct contact with an infected person or armadillos. However, she frequently spent time in a garden where armadillos lived and were buried, suggesting that *M. leprae* may have been introduced to the soil where the woman subsequently came in contact with the pathogen[21].

Environmental Transmission

The high risk of HD from household contacts with the absence of evidence for person-to-person transmission from casual contact and the evidence of indirect contact with armadillos (i.e., living or working in an armadillo habitat) as a HD risk factor both suggest the potential role of an environmental reservoir in HD transmission. Multiple studies have shown the presence of *M. leprae* DNA in soil and water near the homes of HD patients. However, this is not conclusive evidence as DNA can persist in the environment long after an organism's death, and the observed DNA could simply be from patients shedding the pathogen into the environment[1]. More compelling is RNA evidence, which unlike DNA degrades very quickly upon organism death and is therefore considered indicative of living *M. leprae*. More convincing still is laboratory evidence supporting *M. leprae* viability outside of living cells, which can be verified through mouse footpad (MFP) assay, considered the gold standard for viability. *M. leprae* has been shown to survive for 46 days in wet soil, 60 days in room temperature saline, 7 days after exposure to direct sunlight for 3 hours per day, and 60 days at 4 degrees Celsius[22]. Furthermore, *M. leprae* has been shown to survive for up to 8 months encysted in *Acanthamoeba* amoebas, which could serve as an intermediate host[23]. With this in mind, the question is not so much one of viability, but rather one of feasibility as a transmission route.

With that context, several studies have been conducted outside of lab environments in an effort to determine if environmental reservoirs play a role in *M. leprae* transmission. *M. leprae* RNA has been found in water and soil samples in Brazil and India in multiple environmental studies, and there has consistently been an association between water sources with *M. leprae* RNA and households with HD patients[24–29]. Furthermore, poor water, sanitation, and hygiene (WASH) factors including open defection and poor access to soap have been positively associated with HD, which lends epidemiological support to the hypothesis of transmission of *M. leprae* from the environment to people[30]. These studies suggest that environmental reservoirs may play a significant role in HD transmission, either as a supplement to the traditionally understood droplet transmission or even entirely replacing it. However, the nature of this role is not well understood, and skepticism remains about if it is a transmission factor at all. Although 16S RNA is generally considered robust evidence of viable bacteria, RNA is able to survive for a short period outside of a living cell, and studies have shown that 16S rRNA transcripts can be detected in dead *M. leprae*. Alternative targets, *hsp18* and *esxA* mRNA transcripts, have been proposed, as they show a stronger correlation with living bacteria using MFP as reference[31].

CHAPTER II: MANUSCRIPT

Introduction

Epidemiology of Hansen's Disease in Brazil

Hansen's Disease remains a significant public health problem in Brazil, accounting for 13.8% of worldwide incident HD cases in 2019, second only after India[32]. Within the country, there are pockets of hyperendemicity with rates higher than 40 cases per 100,000 population[33]. However, these reported numbers likely represent an underestimation of true cases, due to the high rate of undiagnosed infections and long incubation period.

With such a high burden of disease and so little known about transmission, the geographic distribution of HD in Brazil has been extensively studied. Many studies conduct descriptive analyses on an ecological scale using municipalities as the unit of analysis, in states including Maranhão, Pernambuco, and Minas Gerais[32,34,35]. Distribution has also been evaluated on the federative unit and regional scale, which found HD cases concentrated in the North, Northeast, and Midwest regions[36,37]. While this does provide helpful information about regions with unusually high levels of HD, it obscures differences in disease burden within municipalities. In this study, we use individuals as the unit of analysis to elucidate more nuanced intra-municipality differences. Furthermore, many studies rely on reported HD cases and do not utilize active case investigation[32,34–37]. This likely underestimates the true number of infections due to the high rate of latent infections.

As discussed previously, much remains unknown about the transmission of HD. In this study, we investigated the relationship between environmental exposures and HD. Because of the long incubation period, this study used an anti-LID-1 antibody test to quantify *M. leprae* exposure. This test, which detects antibodies against the *M. leprae* LID-1 protein, has the benefit of detecting pre-symptomatic infections. However, the antibody test also has the potential to overestimate the number of cases, as it cannot differentiate between individuals who were exposed to *M. leprae* and completely cleared the infection from those with latent infections that will go on to develop disease in the future[38]. This approach allows for a more effective analysis of factors associated with infection, and also has significant clinical benefits that may allow some infected individuals to get treatment earlier.

Environmental and WASH exposures were identified based on previous studies and included both individual and household level exposures. These factors considered the previously described research proposing environmental exposure as a component of HD transmission.

Methods

Study Population

Minas Gerais is a state in the Southeast Region of Brazil. It has a high rate of Hansen's Disease, averaging 8.71 cases per 100,000 people in the period between 2004 and 2019[32]. Participants for this study were recruited from four municipalities within Minas Gerais: Governador Valadares, Inhapim, Mantena, and Teófilo Otoni, as indicated in Figure 1. Reported incident HD cases in this subregion were mapped using Hansen's Disease data obtained from Brazil's Information System for Notifiable Diseases (SINAN). Population data were obtained from Brazilian Institute of Geography and Statistics (IBGE). Municipality incident rates were calculated by dividing summed incident HD cases for each municipality from 2015 to 2021 by summed populations over the same time period.

Data Collection

Participants were randomly recruited from neighborhoods in the indicated municipalities. Neighborhoods were divided into smaller subunits, and one family member per household was invited to participate in the study. A sampling strategy was developed with the community health workers, ensuring households chosen for recruitment were evenly spaced throughout the neighborhood. Families self-selected which member would participate but were told at the time of recruitment that the study was a general infectious disease study, avoiding emphasis on Hansen's Disease, in an attempt to minimize selection bias.

Participants completed a one-time cross-sectional survey including demographic questions and questions about individual and household WASH exposures. Fingerstick blood draws were performed on all participants to make dried blood spots that were then tested at the U.S. Centers for Disease Control and Prevention using a multiplex-beaded assay that tested for anti-LID-1 reactivity. As previously described, anti-LID-1 reactivity is indicative of infection with *M. leprae* and is the primary outcome for this analysis.

Descriptive Summary

Demographic data were collected on age, sex, race, number of household members, employment status, occupation, household income, education, rural versus urban residence, and BCG vaccination (tuberculosis vaccine) history.

To evaluate household-level WASH and environmental exposures, participants were asked their household's main source of drinking water, main source of water used for cooking, main source of water used for bathing and handwashing, soap availability for hand washing, type of toilet facility used most often, whether the toilet facility is shared with other households and if so how many, frequency of changing bed linens, number of individuals per room, and whether there were pets or animals in the home or on the property. Participants were also asked if they had ever lived in a house with a sand or mud floor, and if they have lived in a house with a sand or mud floor in the past 10 years.

Data were also collected on the following individual-level WASH and environmental exposures: hand washing before meals, treating water before drinking, washing fruits and vegetables before cooking and serving, sharing bed or sleeping space with others, frequency and type of contact with natural water sources from work or recreation, whether they worked in an agricultural field in the past 10 years, whether they worked in a forest in the past 10 years, whether they hunted in the past 10 years and how frequently, and if they have ever had contact with an armadillo and if so what type of contact.

Due to the large number of categories with very few participants, select environmental exposures were dichotomized for analysis. For participants' main source of drinking water, main source of water used for cooking, and main source of water used for bathing and handwashing, piped water to household and piped water to yard were considered to be a piped source of water. Public tap/standpipe, borehole, protected dug well, unprotected dug well, protected spring, unprotected spring, rainwater collection, bottled water, cart with small tank/drum, tanker-truck, and surface water were considered to not be a piped source of water. Answers of other, refused to answer, and don't know were not included in the dichotomized variables. For type of toilet facility used most often, participants were considered to have a piped water system as the primary household toilet facility if they selected the response option of piped water system. Septic tank, cesspit, open-air, don’t know location, pit latrine with slab, pit latrine without slab, double well with slab, double well without slab, other composting toilets, bucket, container-based sanitation, suspended toilet, and no facilities were all considered not to be a piped water system. Answers of other, refused to answer, and don’t know were not included in the dichotomized analysis.

Race was dichotomized to ‘white’ and ‘not white’, with white as the reference group. ‘Not white’ included respondents who self-identified as Black, indigenous, mixed, and other. Answers of refused to answer and don’t know were not included in the dichotomized variable. Household monthly salary was dichotomized to less than one times the monthly minimum wage versus higher.

Logistic Regression: WASH Characteristics

The set of exposures considered for regression analysis was limited to household-level WASH variables: participants’ household’s main source of drinking water, main source of water used for cooking, main source of water used for bathing and handwashing, type of toilet facility used most often, and whether the toilet facility is shared with other households. In order to assess baseline estimates of associations and set the stage for more detailed analyses involving those exposures directly related to water and sanitation, we considered these separately and together, exploring which variables indicated associations with each other and with the outcome.

Demographic variables of age, sex, race, rural versus urban residence, municipality, and household income were included in the model as potential confounders of interest for the environmental associations. The outcome of interest was anti-LID-1 positivity. As noted above, risk factors for HD can be notoriously difficult to identify and measure, so our strategy attempts to provide clearer insight into potential environmental exposures for ongoing risk assessments in the area.

The resulting full multivariate model is as follows:

$$\begin{aligned} logit(P(LIDPOS = 1) &= \alpha + \beta_1 WATER\_SOURCE + \beta_2 COOKING\_WATER\_SOURCE \\ &+ \beta_3 HANDWASH\_WATER\_SOURCE + \beta_4 TOILET\_TYPE + \beta_5 TOILET\_SHARED \\ &+ \gamma_1 AGE + \gamma_2 SEX + \gamma_3 RACE + \gamma_4 MUNIC + \gamma_5 RURAL + \gamma_6 INCOME \end{aligned}$$

Where LIDPOS = anti-LID-1 positive, WATER_SOURCE = if the main source of household drinking water is piped, COOKING_WATER_SOURCE = if the main source of household cooking water is piped, HANDWASH_WATER_SOURCE = if the main source of water for bathing and handwashing is piped, TOILET_TYPE = if the primary household toilet facility was a piped water system, TOILET_SHARED = if the household toilet facility was shared with other households, AGE = age, SEX = RURAL = rural (versus urban), AGE = age, SEX = male, and RACE = white, MUNIC = municipality, RURAL = rural, INCOME = less than one minimum wage, salary, α = intercept, β = exposure of interest, γ = potential confounder.

Due to the potential correlations between environmental and household exposures, we also assessed covariates for multicollinearity, resulting in the below model:

$$\begin{aligned} logit(P(LIDPOS = 1) &= \alpha + \beta_1 WATER\_SOURCE + \beta_2 TOILET\_TYPE + \beta_3 TOILET\_SHARED + \gamma_1 AGE \\ &+ \gamma_2 SEX + \gamma_3 RACE + \gamma_4 RURAL + \gamma_5 INCOME \end{aligned}$$

Potential confounding was assessed for all 3 remaining exposures of interest using a 10% threshold difference. Race was dropped as a potential confounder, resulting in the final reduced model below:

$$\begin{aligned} logit(P(LIDPOS = 1) &= \alpha + \beta_1 WATER\_SOURCE + \beta_2 TOILET\_TYPE + \beta_3 TOILET\_SHARED + \gamma_1 AGE \\ &+ \gamma_2 SEX + \gamma_3 RURAL + \gamma_4 INCOME \end{aligned}$$

Logistic Regression: Rural Residence

In our analyses, municipality of residence was considered as a potential confounder, and contact with armadillos was considered as a potential effect modifier, resulting in the following model:

$$logit(P(LIDPOS = 1) = \alpha + \beta_1 RURAL + \gamma_1 MUNIC + \gamma_2 ARMADILLO + \delta_1 MUNIC \times ARMADILLO$$

Where LIDPOS = anti-LID-1 positive, RURAL = rural, MUNIC = municipality, ARMADILLO = any contact with armadillos, α = intercept, β = exposure of interest, γ = potential confounder, δ = interaction term.

This model was assessed for multicollinearity, and the interaction term was removed. All other variables were retained for confounding assessment. Potential confounding was assessed using a 10% threshold difference and armadillo contact was dropped as a potential confounder, resulting in the final reduced model below:

$$logit(P(LIDPOS = 1) = \alpha + \beta_1 RURAL + \gamma_1 MUNIC$$

Disease Mapping

Addresses were collected from participants at the time of the survey. Participants' addresses were geocoded automatically using an ESRI database, and those that could not be automatically matched were manually geocoded. Addresses that could not be geocoded or fell outside the geographic region of interest were excluded from further analysis, as described in Figure 2. From the initial data set of 1,315 participants, 3 were excluded from all descriptive summaries due to not having anti-LID-1 results (n = 2) or reporting residence in a municipality outside Minas Gerais (n = 1). For regression and spatial analysis, participants were further excluded if they could not be geocoded based on the provided address (n = 261) or if their geocoded address location did not fall within the area of interest (n = 33). The area of interest was defined as south of the northern edge of Teófilo Otoni, east of the western edge of Governador Valadares, north of the southern edge of Inhapim, and east of the western edge of Mantena. Municipality borders were obtained from IBGE.

To determine if bias was introduced through the elimination of points that could not be geocoded or were geocoded outside the region of interest, we described the percent anti-LID-1 positivity by municipality based on the total dataset of valid points (n = 1,312) and based on the set of points used for subsequent analysis (n = 1,018).

All data and maps were converted to EPSG 3857 for mapping. Anti-LID-1 results and selected household WASH characteristics - piped household water for drinking, piped household toilet, and whether the household toilet was shared - were mapped. Each characteristic was mapped on a regional scale showing all four municipalities and on a neighborhood map of Governador Valadares in order to better visualize the large amount of data in this municipality.

Spatial Trends

Two spatial epidemiology tools commonly used to assess clustering are relative risk surfaces and spatial scan statistics. Each strategy uses different metrics to identify areas of high risk and are useful exploratory tools to inform downstream analysis. However, these tools have limited application in the context of the four geographically separated municipalities in this cross-sectional survey. Our data include small numbers of densely concentrated point locations within each municipality limiting spatial power and potential insight provided by the spatial scan statistic.

To create smooth relative risk surfaces, we compared smooth kernel estimates of the spatial distribution of geocoded point locations of households with anti-LID-1 positive participants to that of households with anti- LID-1 negative participants. Due to the disconnected nature of the data, separate risk surfaces were calculated for each of the four municipalities. Relative risk surfaces were calculated using both fixed and adaptive bandwidths.

All analyses and maps were completed using R version 4.3.1 in RStudio version 2023.06.1. Maps were created using the tmap package. Relative risk surfaces were created using the sparr package.

Results

Descriptive summary

Demographic characteristics of participants are described in Table 1. Most participants self-described their residence as urban (n = 1,090, 83%), and 63% of participants lived in the municipality of Governador Valadares (n = 821). Distribution of age and proportions of sex, race, household monthly salary below minimum wage, and highest level of education were similar between anti-LID-1 positive and anti-LID-1 negative participants. Notable demographic differences were observed based on geography motivating our detailed spatial analyses below. For example, 27% (n = 25) of anti-LID-1 positive participants reported rural residence compared to only 16% (n = 195) of anti-LID-1 negative participants, and 28% (n = 26) of anti-LID-1 positive participants lived in Mantena compared to 15% (n = 186) of anti-LID-1 negative participants.

Household environmental and WASH characteristics of participants are described in Table 2. The majority of participants had piped water as their main source of household drinking water (n = 1,071, 85%), cooking water (n = 1,053, 84%), and bathing and handwashing water (n = 1,101, 88%). At the household level, WASH characteristics of interest included having a piped water system as the primary household toilet facility (78% among anti-LID-1 positive participants versus 85% among anti-LID-1 negative participants), whether the toilet facility was shared with other households (24% versus 32%), whether there were pets or animals in the home or on the property (75% vs 60%), and whether the participant had lived in a home with a sand or mud floor in the past 10 years (45% vs 51%).

Individual environmental and WASH characteristics are described in Table 3. Almost two thirds of participants (n = 814, 64%) reported treated their water prior to drinking, but this low percentage may be because many participants have safe drinking water that does not require treatment rather than because unsafe water is not being treated. The only individual WASH characteristic notably different between anti-LID-1 positive and negative participants is how fruits and vegetables are washed.

In Table 4, we show the percent of anti-LID-1 positivity by municipality for the full dataset and for the dataset used for analysis after data were removed due to inability to geocode the participant's address or due to the geocoded address falling outside the study region Although the percent of data points from each region that remain included varies from 54.4% to 90.8%, the difference is not differential; total percent of data included, percent of positives included, and percent of negatives included are reasonably consistent within each municipality.

Incidence of HD in Governador Valadares, Mantena, Teófilo Otoni and Inhapim and surrounding municipalities based on reported cases from SINAN is shown in Figure 3 Among the four municipalities included in our study, reported HD cases follow similar trends to our study data of anti-LID-1 positivity, as shown in Figure 4; Mantena has the highest rates and Inhapim has the lowest. Our data are further subdivided into neighborhoods within Governador Valadares, due to the high number of participants from this municipality. The highest rates of anti-LID-1 positivity were found in the municipality of Mantena (12.26%). Figure 5 shows anti-LID-1 results for all mapped data points.

Logistic Regression: WASH Characteristics

In the multicollinearity assessment for the regression examining household WASH characteristics, whether the main source of water for bathing and handwashing was piped had a variance inflation factor (VIF) of 4.2 and was removed from the model. Multicollinearity was assessed again and whether the main source of household drinking water was piped and whether the main source of household cooking water was piped had VIFs of 3.6 and 3.3 respectively. Whether the main source of household cooking water was piped was removed from the model. Multicollinearity was assessed again and municipality of residence had a VIF of 3.7 and was removed from the model. All retained variables had a VIF below 1.5.

Results for full and reduced multivariate models, as well as univariate regression, are shown in Table 5. In the reduced model, none of the three household WASH exposures of interest – piped household drinking water, piped toilet facility, and whether the household toilet facility was shared – were significantly associated with the outcome in our data ($p < 0.05$). Geographic distributions of these characteristics are shown in Figure 6. As with anti-LID-1 positivity, some of these characteristics also show spatial heterogeneity. Lack of piped household drinking water is concentrated in Mantena and Inhapim, and not sharing a toilet household toilet facility appears noticeably more common in Mantena as compared to the other three municipalities. At the municipality level, whether the primary household toilet facility is piped appears to be reasonably spatially homogenous.

Logistic Regression: Rural Residence

Due to the difference in proportion of anti-LID-1 positive participants who reported rural residence as compared to anti-LID-1 negative participants, we further examined rural residence as an exposure. As described in Table 6, rural residence was closely linked with municipality. In Governador Valadares, 0% of participants reported rural residence, compared to 41% in Inhapim, 66% in Mantena, and 1.9% in Teófilo Otoni. Results for full and reduced multivariate models examining rural residence as an exposure, as well as univariate regression, are shown in Table 7. Although rural residence was strongly associated with anti-LID-positivity in univariate analysis (cOR = 2.53, $p < 0.001$), this association was reduced and no longer significant when adjusted for municipality (aOR = 1.68, 95% CI 0.73, 4.10).

Spatial Patterns in M. leprae Distribution

The relative risk surfaces for Governador Valadares neighborhoods comparing the observed patterns for anti-LID-1 positives versus negatives are shown in Figure 7. Two different iterations of relative risk surfaces are shown. The first uses all 651 data points from households that were able to be geocoded and reported residence in the municipality of Governador Valadares. This has the benefit of including all data and minimizing any bias that would result from truncating the included data. However, several points notably outside the urban center introduce wide variation to the relative risk surfaces, minimizing interpretability. To account for this, we also developed a second risk surface that only included data points with coordinates within the bounds of the central Governador Valadares neighborhoods, which excluded 5 points. Relative risk surfaces for the other three municipalities and for the entire region are shown in Figure 8 and Figure 9, respectively. For all of the municipalities, we again display risk surfaces calculated with all data from participants that reported residence in that municipality, and risk surfaces created by limiting the data to those whose geocoded addresses were within the municipality bounds.

In Governador Valadares, there is a region of high relative risk (increased local relative risk of positivity) in the southwest corner of the urban area indicated by the neighborhood boundaries, which differs significantly ($p < 0.05$) from a null hypothesis of constant local relative risk on both maps. On the map that incorporates all Governador Valadares data, there is also significantly low relative risk (decreased local relative risk of positivity) in the eastern portion of the map, outside of the central urban neighborhoods. When we examined relative risk patterns at the highest level with all of the data points included in the spatial analysis, only Mantena showed significant high relative risk.

Discussion

Although associations between poor WASH and Hansen's Disease have been previously observed, minimal research has been done on the topic and HD is not typically considered a WASH-related disease[30]. We hypothesized that anti-LID-1 positivity would be associated with poor WASH, as inadequate WASH infrastructure would result in exposure to *M. leprae* in the environment. Previous research has found associations between open defecation and lack of access to soap to be associated with HD[30]. This analysis focused on the household WASH exposures of piped household drinking water, piped sanitation for the primary household toilet facility, and sharing the primary household toilet facility.

None of the three exposures of interest had a significant ($p < 0.05$) association with anti-LID-1 positivity in our data. Having piped water as the primary source of household drinking had point estimates indicating a positive association with anti-LID-1 positivity in the adjusted multivariate model, in contrast to our hypothesis. This also appears internally inconsistent, as piped sanitation for the primary household toilet type had point estimates suggesting a negative association with anti-LID-1 positivity. Given the strong connection between a household having piped drinking water and having piped sanitation, it is unexpected that point estimates would reflect diverging associations. Also surprising is the point estimate for having a shared household toilet facility, which suggests a negative association with anti-LID-1 positivity. Most households in this area of Brazil do not share facilities so this question was likely misinterpreted by either the interviewers or the participants.

Although we assessed our model for collinearity, it is possible that the close connection of these variables introduces uncertainty, and a combined WASH score or individual analysis may provide a more accurate estimation. Previous research also did not find a significant association between unimproved household water source and HD[30]. Given the scarcity of research in the area of WASH and HD, it too early to say if this represents a true lack of association. Comparisons are further limited by variation in both the exposure and the outcome. WASH comprises a range of individual and household factors, and separating the distinct exposures that may be most relevant is challenging. Furthermore, this is the first WASH-focused study to assess anti-LID-1 positivity as the outcome, which is notably different from HD. Although anti-LID-1 positivity has the advantage of providing higher accuracy of *M. leprae* transmission, it limits the ability to directly compare with existing literature.

We also examined the role of rural residence in anti-LID-1 positivity. Rural residence was found to be significantly ($p < 0.001$) associated with anti-LID-1 positivity in univariate analysis (cOR = 2.53). This is consistent with previous research that has identified rural residence to be associated with higher HD prevalence[39].

We wanted to investigate if there was a true association between anti-LID-1 positivity and rural residence or if this was obscuring the role of municipality of residence. When adjusted for municipality, the estimated association with rural residence was mitigated. This may indicate that the crude association of rural residence with anti-LID-1 positivity may not be due to factors inherent to rural living, but instead due to characteristics of the municipality. These characteristics could be structural, such as WASH infrastructure, or environmental, such as climate and elevation. The interpretability of this finding is limited due to the restricted number of municipalities in the data set and the limited variation of rural vs urban residence within each municipality, and further research involving a wider distribution of data points would clarify this potential association. Previous research has investigated intra-municipality differences in HD rates and associated risk factors[40], and multiple studies have mapped spatial trends of HD by municipality in Brazil[32,34–37]. Despite these studies typically identifying clusters of high risk, little research exists examining municipality-level risk factors that might help explain this heterogeneity.

Given the differences in anti-LID-1 positivity observed between municipalities, we were interested in further exploring its spatial distribution. The mapped data as shown in Figure 5 provide a useful context for understanding where to apply relative risk surfaces and spatial scan statistics for relevant information. In Mantena, Teófilo Otoni, and Inhapim, the majority of data are collected from concentrated areas within each municipality, so using relative risk surfaces and scan statistics on the municipality level do not have strong data support across these municipalities. Therefore regions of high and low relative risk as displayed in Figure 8 cannot be meaningfully interpreted, as they predominantly reflect data distribution. In contrast, there is wide coverage of data throughout Governador Valadares neighborhoods, allowing for a more meaningful examination. It would be possible to examine only the densely populated areas where data are clustered in the other three municipalities, as is done with the focus on the city of Governador Valadares rather than the whole municipality. However, this has the potential to introduce bias as the more isolated excluded points are more likely to be rural, which is known to have a strong association with anti-LID-1 positivity.

Even so, the regions of significantly high and low relative risk in Governador Valadares must be interpreted cautiously. As shown in Figure 5, these regions have very few participants each, indicating that these apparent areas of high and low risk are in areas of high uncertainty due to locally sparse data and each merit closer investigation of the individual households. Outside of these areas, relative risk was predominantly homogenous on a neighborhood scale within Governador Valadares. This finding indicates that within the city, geographic location is not as relevant to *M. leprae* infection as other risk factors. This pattern also suggests that there may be relevant exposures on a level larger than individual neighborhoods.

We also observed that relative risk of anti-LID-1 positivity was found to be heterogenous on a municipality level, with the highest relative risk in Mantena, as shown in Figure 9. This is consistent with this municipality's high prevalence of anti-LID-1 positivity as observed in our study and its high incidence of reported HD cases. It is important to note, however, that this surface only takes into consideration the data that was collected in these four municipalities, all of which are known to be areas with high rates of HD[32]. Although Mantena stands out when compared only to these regions, it is likely that other areas with underlying high relative risk could be revealed if a representative examination of anti-LID-1 positivity could be completed across the entire region, so these results must again be interpreted with caution as an initial step toward a more comprehensive assessment of the local geography of HD in this area.

A key limitation in this study is the geographical confines of the available data, which restricts the function of traditional spatial epidemiology tools used to identify clustering. Additionally, rural residences may be more challenging to accurately geocode and therefore be disproportionately excluded from the geocoded dataset, so the distribution of anti-LID-positivity as described in Figure 5 should be interpreted with this in mind. Future work could seek to reduce this bias via GPS locations, but this would require additional field work.

Despite these limitations, mapping the anti-LID-1 positivity in these regions provides useful insights into the distribution of *M. leprae* infection within this region of Minas Gerais. Previous spatial analyses of HD in the region have been limited to the use of reported HD cases from SINAN, which is known to have incomplete and inconsistent data[41]. Even if this was not a concern, HD cases will inherently be an underestimate of *M. leprae* infection. Using antibody testing is more labor-intensive to collect but provides a more accurate estimation of infection. This increased specificity is helpful to researchers seeking to understand the transmission routes of *M. leprae* and to healthcare providers, who can use this data to target active HD surveillance and intervene earlier in disease progression.

The role of poor WASH in *M. leprae* transmission is less clear from this analysis and may be limited by the restriction to household-level WASH factors. This relationship may be clarified through the incorporation of individual-WASH factors and the consolidation of household WASH factors to a combined WASH score in future studies.

The potential role of geographic location, as quantified by municipality, as a confounder in the relationship between anti-LID-1 positivity and rural residence merits additional attention. Ideally, future studies could consider collecting anti-LID-1 positivity status for residents throughout a wider region of Minas Gerais rather than the four municipalities in this study. However, the invasive nature of the antibody test makes such an investigation challenging. Reported HD cases, available for most Minas Gerais municipalities, could be used as a substitute to investigate this relationship.

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Tables

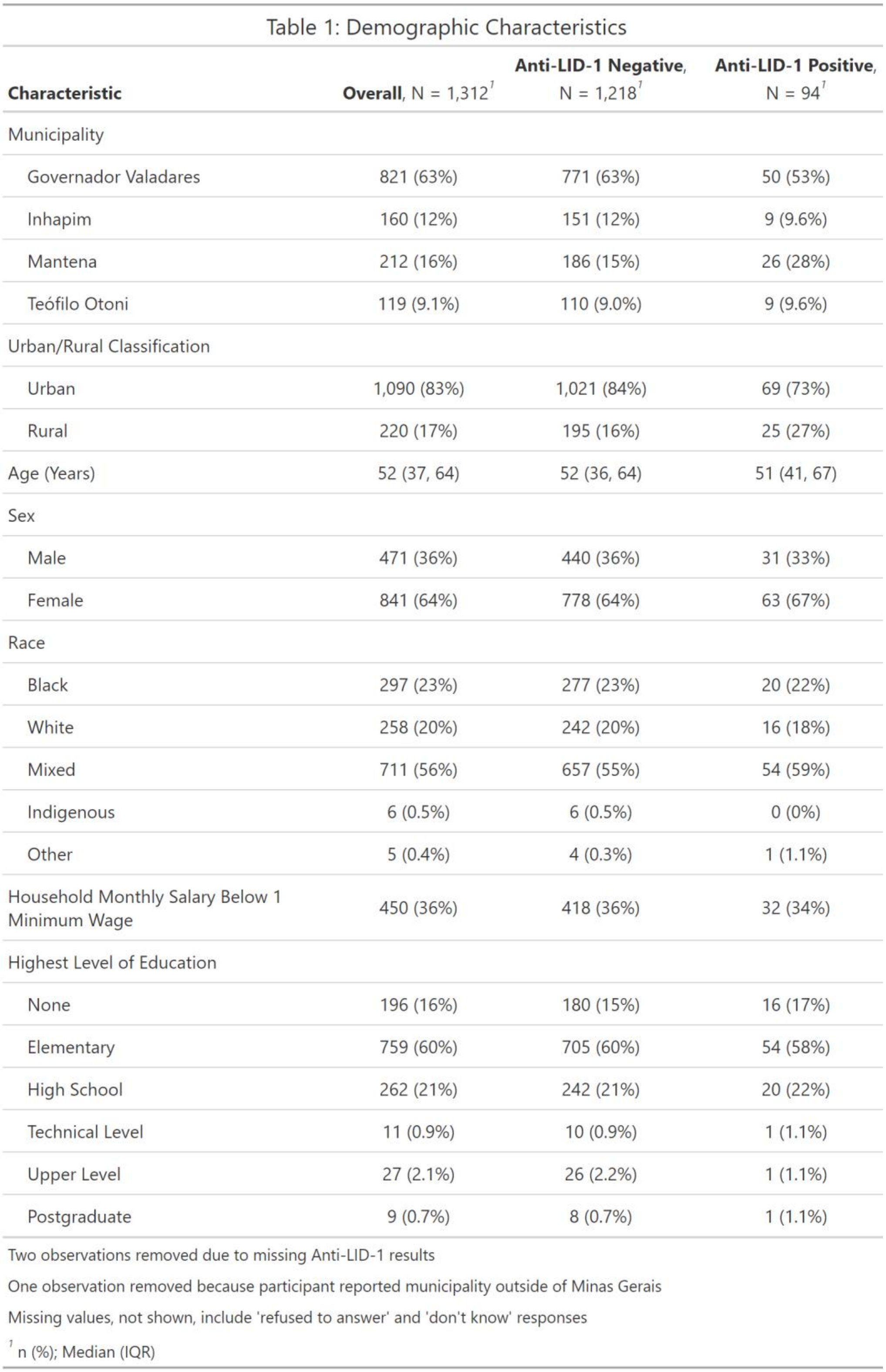


Table 1: Demographic Characteristics

Characteristic	**Overall**, N = 1,312[1]	**Anti-LID-1 Negative**, N = 1,218[1]	**Anti-LID-1 Positive**, N = 94[1]
Municipality			
Governador Valadares	821 (63%)	771 (63%)	50 (53%)
Inhapim	160 (12%)	151 (12%)	9 (9.6%)
Mantena	212 (16%)	186 (15%)	26 (28%)
Teófilo Otoni	119 (9.1%)	110 (9.0%)	9 (9.6%)
Urban/Rural Classification			
Urban	1,090 (83%)	1,021 (84%)	69 (73%)
Rural	220 (17%)	195 (16%)	25 (27%)
Age (Years)	52 (37, 64)	52 (36, 64)	51 (41, 67)
Sex			
Male	471 (36%)	440 (36%)	31 (33%)
Female	841 (64%)	778 (64%)	63 (67%)
Race			
Black	297 (23%)	277 (23%)	20 (22%)
White	258 (20%)	242 (20%)	16 (18%)
Mixed	711 (56%)	657 (55%)	54 (59%)
Indigenous	6 (0.5%)	6 (0.5%)	0 (0%)
Other	5 (0.4%)	4 (0.3%)	1 (1.1%)
Household Monthly Salary Below 1 Minimum Wage	450 (36%)	418 (36%)	32 (34%)
Highest Level of Education			
None	196 (16%)	180 (15%)	16 (17%)
Elementary	759 (60%)	705 (60%)	54 (58%)
High School	262 (21%)	242 (21%)	20 (22%)
Technical Level	11 (0.9%)	10 (0.9%)	1 (1.1%)
Upper Level	27 (2.1%)	26 (2.2%)	1 (1.1%)
Postgraduate	9 (0.7%)	8 (0.7%)	1 (1.1%)

Two observations removed due to missing Anti-LID-1 results

One observation removed because participant reported municipality outside of Minas Gerais

Missing values, not shown, include 'refused to answer' and 'don't know' responses

[1] n (%); Median (IQR)

Table 2: Household Environmental and WASH Characteristics

Characteristic	**Overall**, N = 1,312[1]	**Anti-LID-1 Negative**, N = 1,218[1]	**Anti-LID-1 Positive**, N = 94[1]
Piped Main Source of Household Drinking Water	1,071 (85%)	991 (85%)	80 (87%)
Piped Main Source of Cooking Water	1,053 (84%)	976 (84%)	77 (85%)
Piped Main Source of Water for Bathing and Handwashing	1,101 (88%)	1,018 (87%)	83 (90%)
Handwashing Soap Available at Household	1,172 (92%)	1,087 (92%)	85 (91%)
Piped Water System as Primary Household Toilet Facility	1,062 (84%)	990 (85%)	72 (78%)
Toilet Facility Shared with Other Households	404 (31%)	382 (32%)	22 (24%)
Number of Households Who Use Shared Facility	3.00 (2.00, 4.00)	3.00 (2.00, 4.00)	2.50 (2.00, 3.75)
Frequency Household Bed Linens are Changed			
Less than Biweekly	823 (66%)	765 (66%)	58 (64%)
Fortnightly or More Frequently	427 (34%)	394 (34%)	33 (36%)
Number of Individuals per Room			
0-3	1,133 (89%)	1,047 (89%)	86 (93%)
4+	140 (11%)	134 (11%)	6 (6.5%)
Pets or animals in Home or on Property	780 (62%)	710 (60%)	70 (75%)
Lived in a House with Sand or Mud Floor	394 (31%)	363 (31%)	31 (33%)
Lived in a House with Sand or Mud Floor in the Past 10 Years	198 (50%)	184 (51%)	14 (45%)

Missing values, not shown, include 'refused to answer' and 'don't know' responses

[1] n (%); Median (IQR)

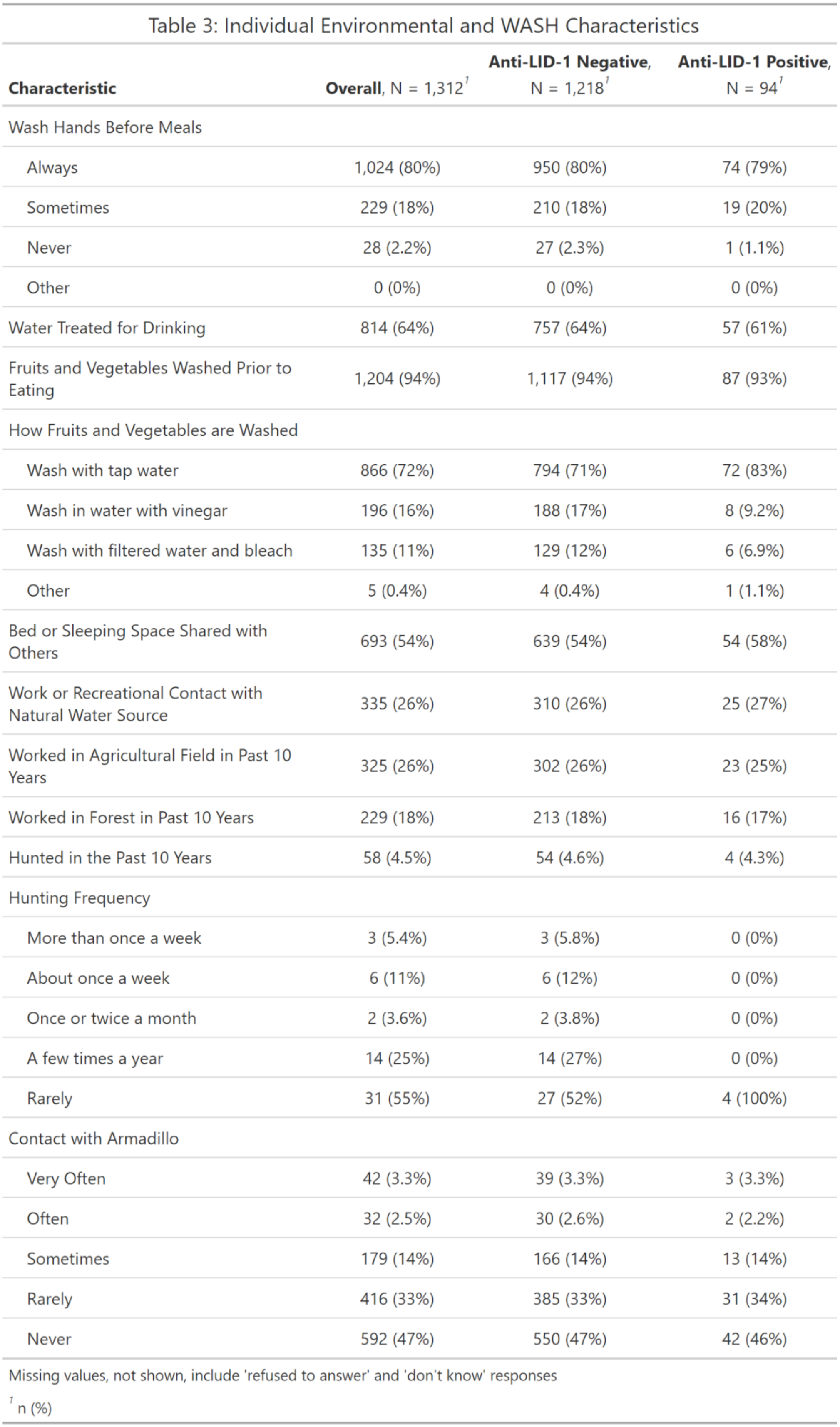


Table 3: Individual Environmental and WASH Characteristics

Characteristic	**Overall**, N = 1,312[1]	**Anti-LID-1 Negative**, N = 1,218[1]	**Anti-LID-1 Positive**, N = 94[1]
Wash Hands Before Meals			
Always	1,024 (80%)	950 (80%)	74 (79%)
Sometimes	229 (18%)	210 (18%)	19 (20%)
Never	28 (2.2%)	27 (2.3%)	1 (1.1%)
Other	0 (0%)	0 (0%)	0 (0%)
Water Treated for Drinking	814 (64%)	757 (64%)	57 (61%)
Fruits and Vegetables Washed Prior to Eating	1,204 (94%)	1,117 (94%)	87 (93%)
How Fruits and Vegetables are Washed			
Wash with tap water	866 (72%)	794 (71%)	72 (83%)
Wash in water with vinegar	196 (16%)	188 (17%)	8 (9.2%)
Wash with filtered water and bleach	135 (11%)	129 (12%)	6 (6.9%)
Other	5 (0.4%)	4 (0.4%)	1 (1.1%)
Bed or Sleeping Space Shared with Others	693 (54%)	639 (54%)	54 (58%)
Work or Recreational Contact with Natural Water Source	335 (26%)	310 (26%)	25 (27%)
Worked in Agricultural Field in Past 10 Years	325 (26%)	302 (26%)	23 (25%)
Worked in Forest in Past 10 Years	229 (18%)	213 (18%)	16 (17%)
Hunted in the Past 10 Years	58 (4.5%)	54 (4.6%)	4 (4.3%)
Hunting Frequency			
More than once a week	3 (5.4%)	3 (5.8%)	0 (0%)
About once a week	6 (11%)	6 (12%)	0 (0%)
Once or twice a month	2 (3.6%)	2 (3.8%)	0 (0%)
A few times a year	14 (25%)	14 (27%)	0 (0%)
Rarely	31 (55%)	27 (52%)	4 (100%)
Contact with Armadillo			
Very Often	42 (3.3%)	39 (3.3%)	3 (3.3%)
Often	32 (2.5%)	30 (2.6%)	2 (2.2%)
Sometimes	179 (14%)	166 (14%)	13 (14%)
Rarely	416 (33%)	385 (33%)	31 (34%)
Never	592 (47%)	550 (47%)	42 (46%)

Missing values, not shown, include 'refused to answer' and 'don't know' responses

[1] n (%)

Table 4: Distribution of Points Included in Spatial Analysis

Municipality	Total Number of Points	LID-1 Positivity (All)	Total Number of Included Points	LID-1 Positivity (Spatial Analysis Only)	Percent of Data Included	Percent of Positive LID-1 Included	Percent of Negative LID-1 Included
Governador Valadares	821	6.1%	651	5.5%	79.3%	72.0%	79.8%
Inhapim	160	5.6%	87	5.8%	54.4%	55.6%	54.3%
Mantena	212	12.3%	172	13.9%	81.1%	92.3%	79.6%
Teófilo Otoni	119	7.6%	108	7.4%	90.8%	88.9%	90.9%
Total	**1312**	**7.2%**	**1018**	**7.2%**	**77.6%**	**77.7%**	**77.6%**

Table 5: Household-Level WASH Logistic Regression

	Univariate				**Full Model**			**Reduced Model**		
Characteristic	**N**	cOR[1]	**95% CI**[2]	**p-value**	aOR[3]	**95% CI**[2]	**p-value**	aOR[4]	**95% CI**[2]	**p-value**
Piped Household Drinking Water	1,000	1.00	0.49, 2.31	>0.9	1.90	0.79, 5.21	0.2	2.06	0.86, 5.64	0.13
Piped Water for Toilet	994	0.55	0.31, 1.04	0.053	0.66	0.32, 1.44	0.3	0.60	0.30, 1.29	0.2
Shared Household Toilet Facility	1,011	0.52	0.28, 0.90	**0.027**	0.53	0.27, 0.98	0.053	0.56	0.29, 1.03	0.072
Age	1,018	1.01	1.00, 1.02	0.2						
Sex (Male)	1,018	0.87	0.51, 1.43	0.6						
Race (White)	1,011	0.83	0.42, 1.53	0.6						
Rural	1,017	2.53	1.45, 4.28	**<0.001**						
Household Salary Below 1 Minimum Wage	994	1.04	0.62, 1.70	0.9						

[1] Crude Odds Ratio

[2] CI = Confidence Interval

[3] Model adjusted for age, sex, race, rural residence, income

[4] Model adjusted for age, sex, rural residence, income

Table 6: Rural Residence by Municipality

	Overall, N = 1,018[1]	**Governador Valadares**, N = 651[1]	**Inhapim**, N = 87[1]	**Mantena**, N = 172[1]	**Teófilo Otoni**, N = 108[1]
Rural	151 (15%)	0 (0%)	35 (41%)	114 (66%)	2 (1.9%)
Urban	866 (85%)	651 (100%)	51 (59%)	58 (34%)	106 (98%)

[1] n (%)

Table 7: Regression for Rural Residence

	Univariate				**Full Model**			**Reduced Model**		
Characteristic	**N**	cOR[1]	**95% CI**[2]	**p-value**	aOR[3]	**95% CI**[2]	**p-value**	aOR[4]	**95% CI**[2]	**p-value**
Rural	1,017	2.53	1.45, 4.28	**<0.001**	1.69	0.74, 4.12	0.2	1.68	0.73, 4.10	0.2
Municipality	1,018									
Governador Valadares		—	—							
Inhapim		1.04	0.35, 2.51	>0.9						
Mantena		2.77	1.59, 4.76	**<0.001**						
Teófilo Otoni		1.37	0.58, 2.88	0.4						
Armadillo Contact	1,004	1.10	0.68, 1.79	0.7						

[1] Crude Odds Ratio

[2] CI = Confidence Interval

[3] Model adjusted armadillo contact and municipality

[4] Model adjusted for municipality

Figures

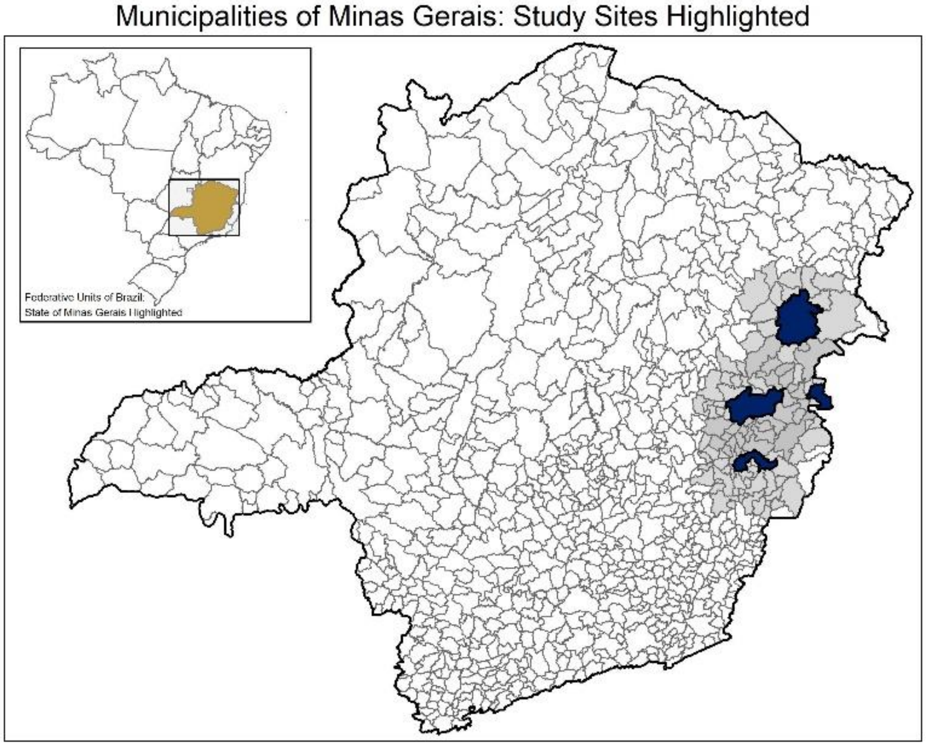

Municipalities of Minas Gerais: Study Sites Highlighted

Figure 1: Map of municipalities of Minas Gerais, with study sites of Governador Valadares, Inhapim, Mantena, and Teófilo Otoni highlighted in blue.

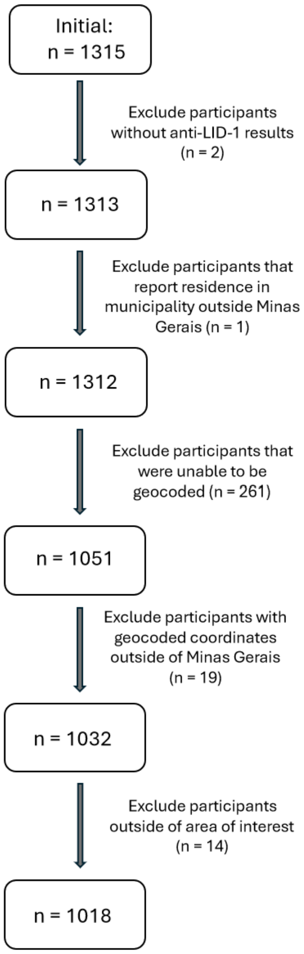


Figure 2: Description of data selection process for spatial analysis.

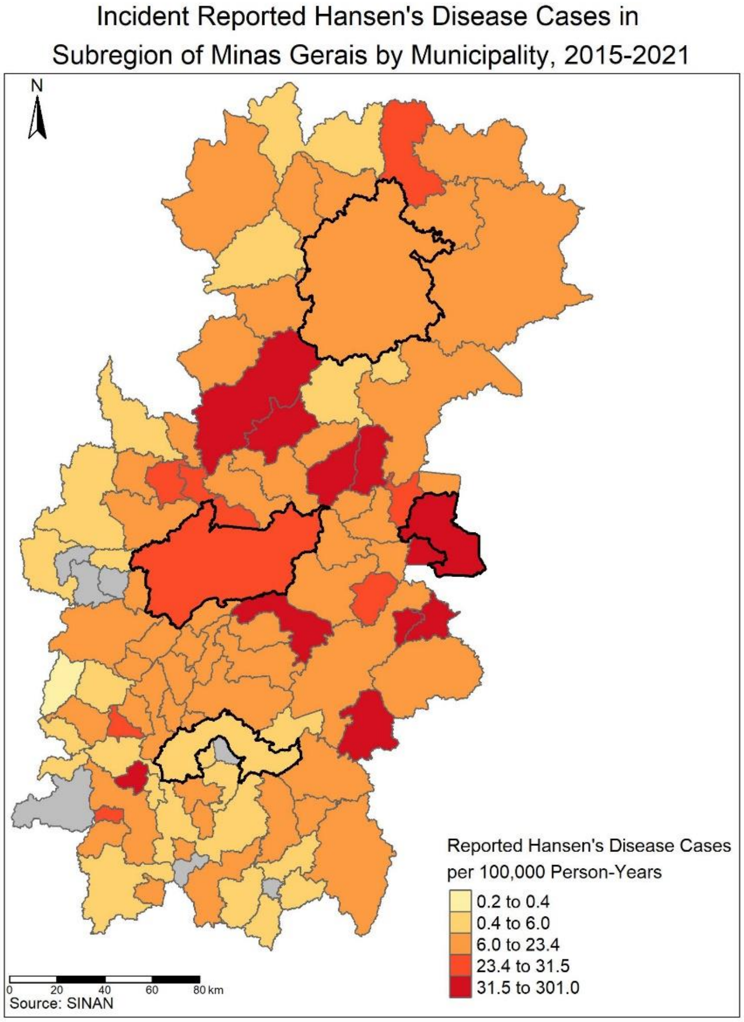


Figure 3: Incidence of Hansen’s Disease in subregion of Minas Gerais (indicated in gray on Figure 1) by municipality. Study site municipalities of Governador Valadares (center), Inhapim (south), Mantena (east), and Teófilo Otoni (north) indicated with bold outline.

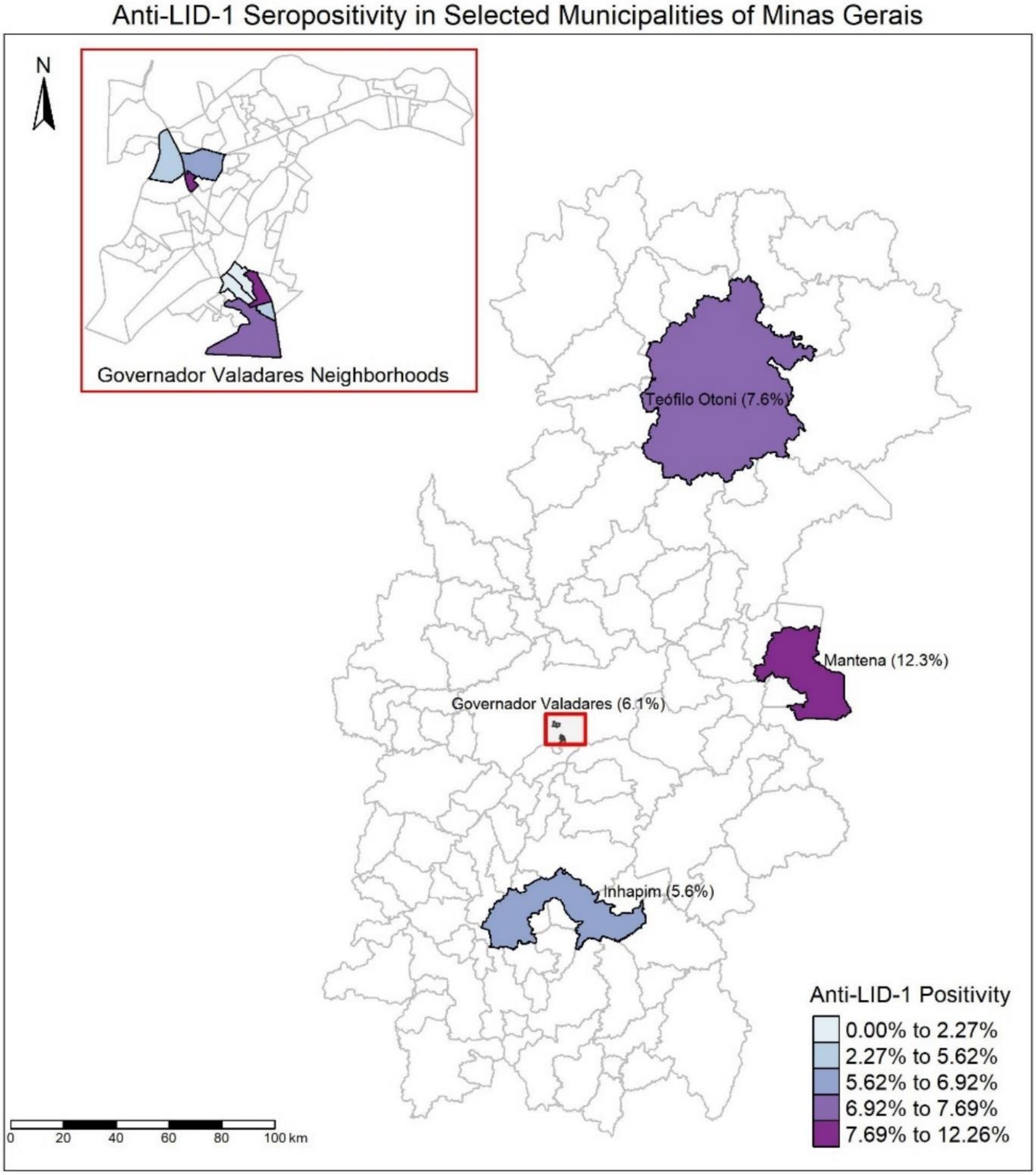
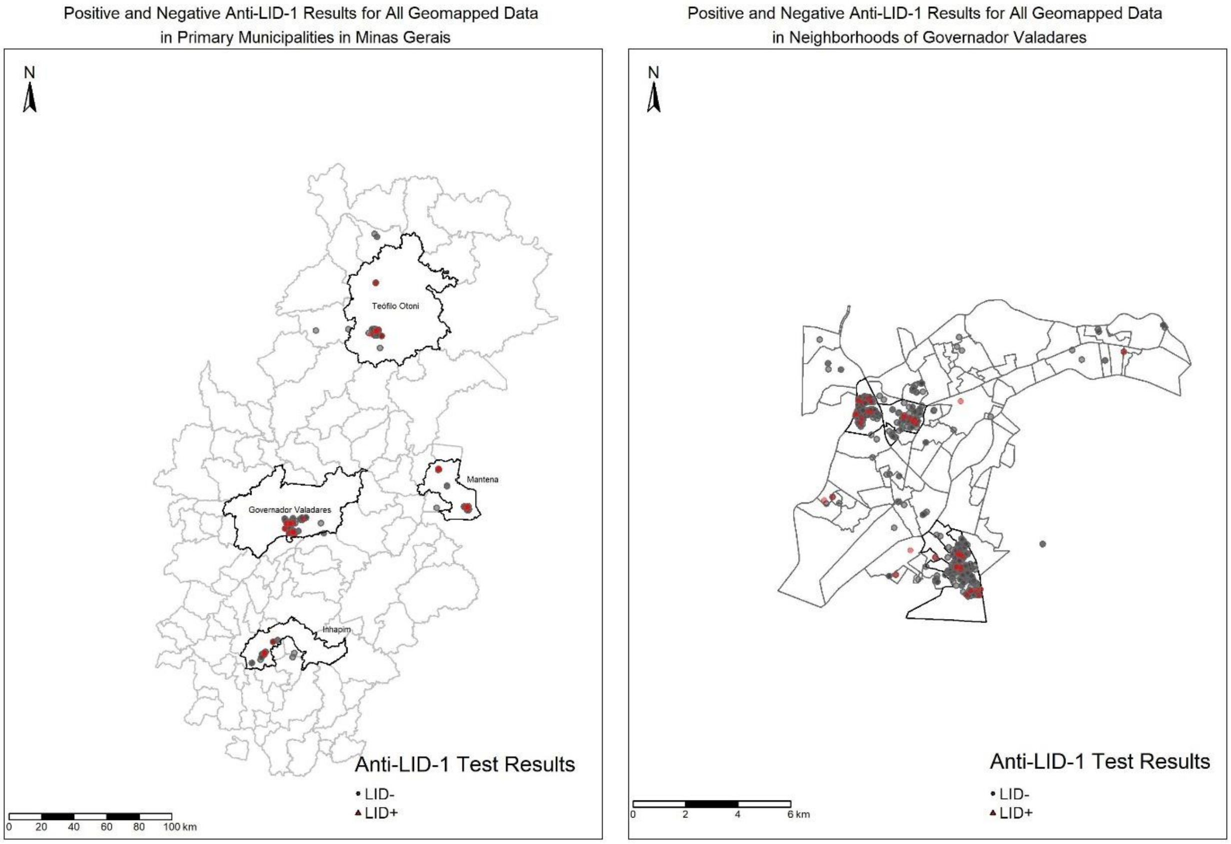


Figure 4: Anti-LID-1 seropositivity by municipality.



Positive and Negative Anti-LID-1 Results for All Geomapped Data in Primary Municipalities in Minas Gerais

Positive and Negative Anti-LID-1 Results for All Geomapped Data in Neighborhoods of Governador Valadares

Figure 5: Positive (red) and negative (grey) anti-LID-1 results, shown at municipality level (left) and at neighborhood level in Governador Valadares (right).

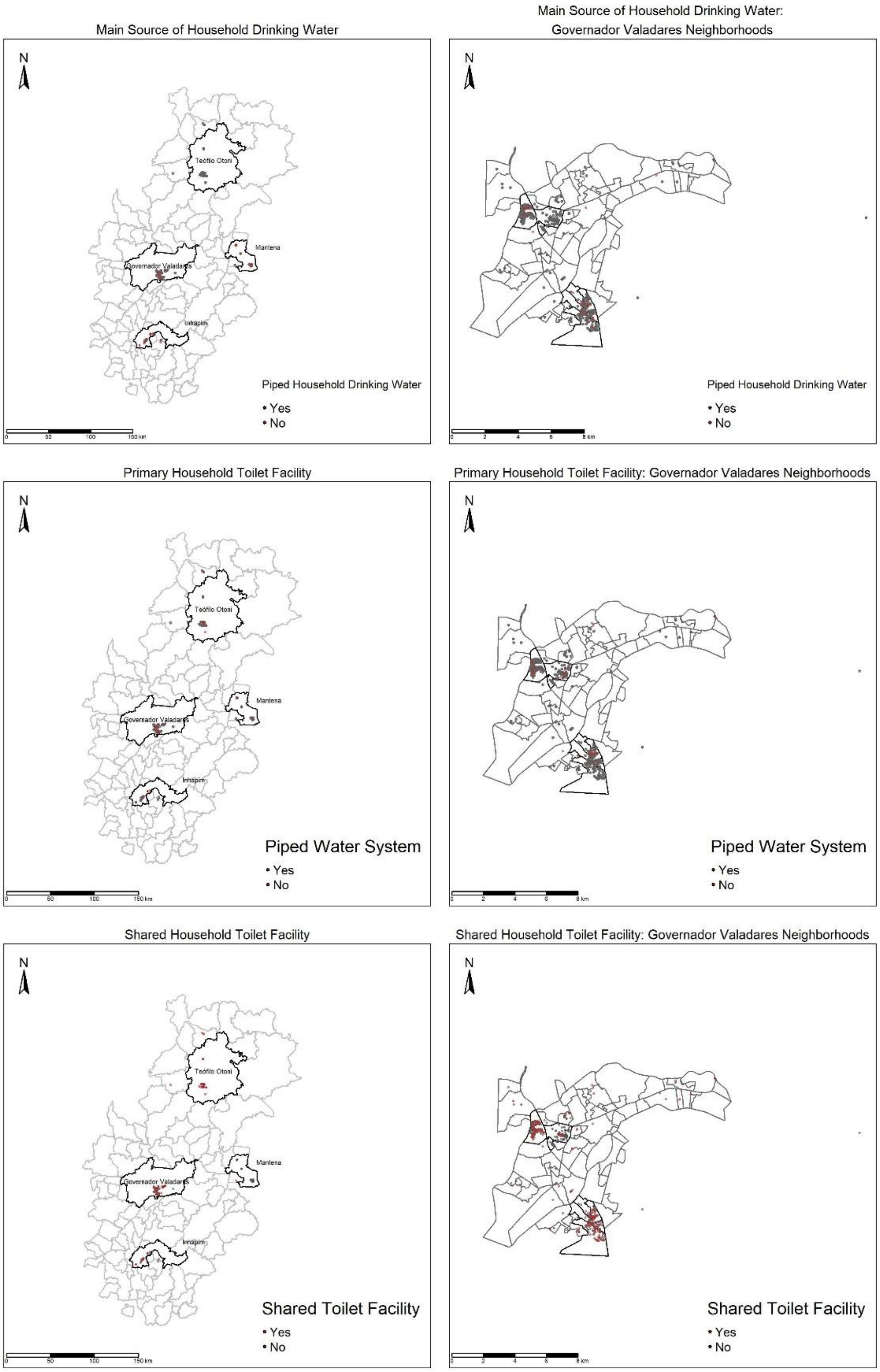


Figure 6: Distribution of Selected Environmental Exposures: Piped main source of household drinking water, piped primary household toilet facility, and shared primary household toilet facility

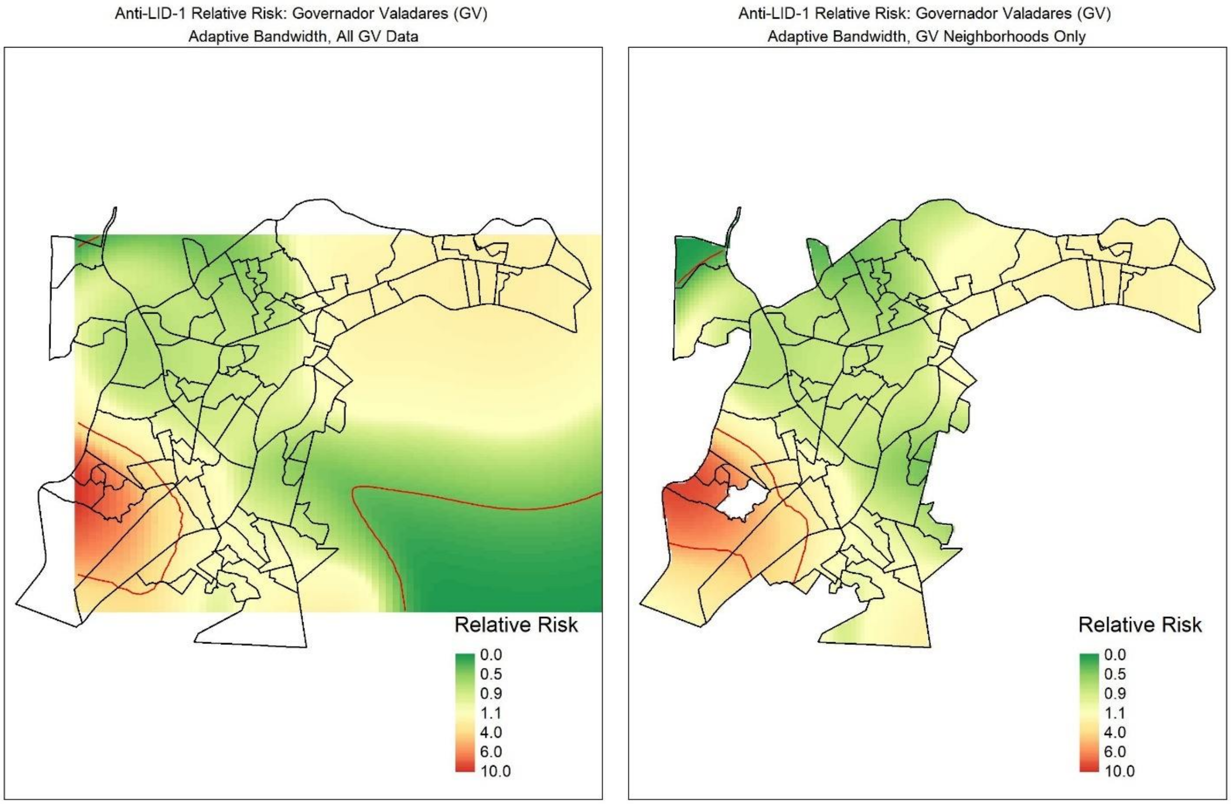
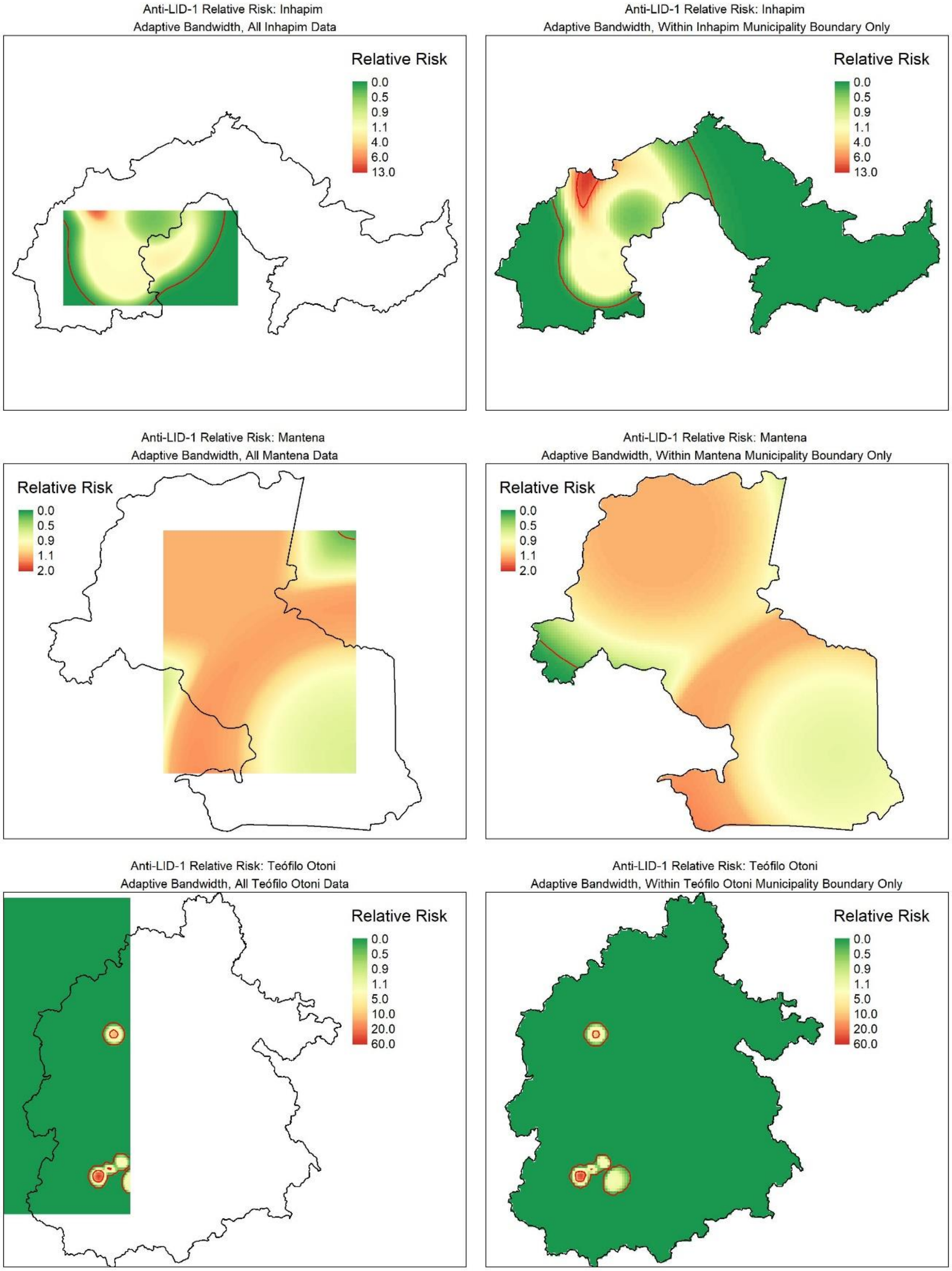


Figure 7: Relative risk surfaces comparing anti-LID-1 positivity to negativity for Governador Valadares


Anti-LID-1 Relative Risk: Inhapim
Adaptive Bandwidth, All Inhapim Data

Anti-LID-1 Relative Risk: Inhapim
Adaptive Bandwidth, Within Inhapim Municipality Boundary Only

Anti-LID-1 Relative Risk: Mantena
Adaptive Bandwidth, All Mantena Data

Anti-LID-1 Relative Risk: Mantena
Adaptive Bandwidth, Within Mantena Municipality Boundary Only

Anti-LID-1 Relative Risk: Teófilo Otoni
Adaptive Bandwidth, All Teófilo Otoni Data

Anti-LID-1 Relative Risk: Teófilo Otoni
Adaptive Bandwidth, Within Teófilo Otoni Municipality Boundary Only

Figure 8: Relative risk surfaces comparing anti-LID-1 positivity to negativity for Inhapim, Mantena, and Teófilo Otoni

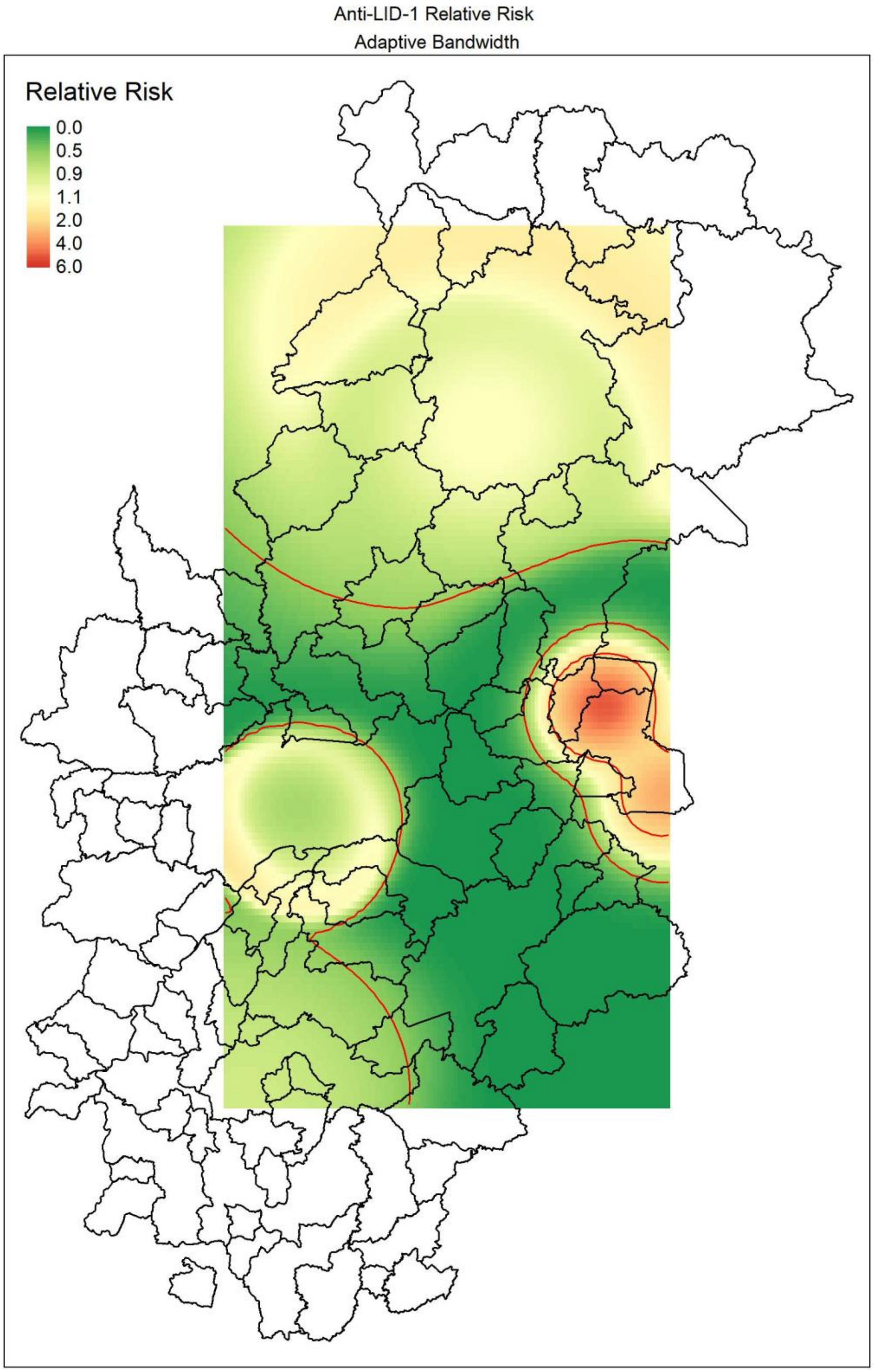


Figure 9: Relative risk surfaces comparing anti-LID-1 positivity to negativity for full study region

CHAPTER III: CONCLUSIONS

Summary

This study investigated spatial distribution of anti-LID-1 positivity and associated WASH factors in four municipalities of Minas Gerais, Brazil. We found spatial heterogeneity of anti-LID-1 positivity at the municipality level, with the highest rate in Mantena, which agrees with observed patterns of reported HD cases. Mapping anti-LID-1 positivity provides additional insight into the true underlying risk of *M. leprae* infection, which will be useful to researchers when developing future studies. We did not find a significant association between the selected household WASH factors and anti-LID-1 positivity, and further research is needed to clarify what role, if any, WASH factors have on *M. leprae* transmission. Rural residence and living in Mantena were found to be significantly associated with anti-LID-1 positivity during univariate analysis. When controlling for municipality, rural residence did not retain a significant association, indicating that there may be relevant municipality-level environmental or structural factors that are not inherent to a rural environment.

Public Health Implications

Water, Sanitation, and Hygiene

Hansen's Disease continues to be a significant public health issue in Brazil, making up 11.6% of worldwide HD cases[42]. Dedicated HD control programs in endemic countries and the availability of effective multidrug therapy have notably reduced HD cases, but progress towards elimination has stagnated in recent years[1]. Animal and environmental factors in *M. leprae* transmission, which are not yet fully established, may be responsible for this lack of advancement towards HD elimination. Within the broad spectrum of environmental exposures, this study sought to identify household-level WASH factors that may contribute to *M. leprae* transmission. Although this analysis did not produce conclusive results about WASH associations with anti-LID-1 positivity, it did provide insight that could be used to inform future research into the connection between local *M. leprae* transmission and WASH. Identifying these associations and relevant interventions is critical to effective control of HD, given the observed inability for multidrug therapy alone to reach the goal of HD elimination.

Spatial Patterns

A key contribution of this study is the spatial mapping of anti-LID-1 positivity in the municipalities of Governador Valadares, Inhapim, Mantena, and Teófilo Otoni, which provides insight into the underlying geographic distribution of *M. leprae* infection that cannot be gained by reported HD cases alone. Gaining a better understanding of *M. leprae* distribution can inform future research into environmental factors contributing to HD, as accurately describing locations with high rates of *M. leprae* may indicate likely environmental influences. This mapping also has direct public health applications. Knowing where there are underlying high rates of *M. leprae* infection will allow healthcare workers to target active surveillance and get treatment to infected individuals before the disease progresses.

Future Directions

This analysis could be enhanced through collection of anti-LID-1 positivity data from a larger area of Minas Gerais, which would allow for more informative spatial analyses. We would also like to attempt to map the outstanding data points that were not able to be geocoded and include these in the existing disease mapping. For WASH factors, future analyses could involve incorporating a broader range of both household and individual WASH exposures. We could also examine exposures that are not explicitly WASH but are nevertheless associated, such as previous parasitic infection and nutrition variables. We would like to incorporate a spatial component more explicitly into our analysis of WASH factors by including household WASH exposures of nearby homes. Given the observed positive association between municipality and anti-LID-1 positivity, additional research should examine likely factors driving this association, including both structural and environmental factors.