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Helminth - *Mycobacterium leprae* co-infections: Facilitators of leprosy transmission and morbidity or innocent bystanders?

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

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Background: The immune derangements of helminth infections and evidence that co-infections may shift the presentation of leprosy to the lepromatous end of the spectrum suggest that they could be risk factors for both leprosy transmission and for the serious immunologic reactions. Methods: We conducted two investigations: a case-control study on helminth co-infections and leprosy reactions and a geospatial study on spatial associations of schistosomiasis and leprosy in Minas Gerais, Brazil. Adult patients with multibacillary disease were recruited from a leprosy clinic in Belo Horizonte. Cases included those with active Type 1 (T1R) or Type 2 reaction (T2R) and controls included those without reactions. Data were abstracted from charts and questionnaires, and stool and blood tested for helminth infections. Adjusted odds ratios were calculated with helminth infection as the main exposure and T1R or T2R as the outcomes. For the geospatial study, all new cases of *M. leprae* and *Schistosoma mansoni* infections from 2007-2014 were retrieved from SINAN, the Brazilian national notifiable disease network, for seven municipalities. Cases were mapped to municipality and neighborhood levels. A stratified analysis was conducted to identify spatial associations between the two infections. Results: Seventy-three patients were recruited to the case-control study. Helminth infections were found in 4 patients with reactions and 1 patient without reaction, with total prevalence of 6.9%. Helminth co-infections were not found to be associated with T1R (aOR =3.5; 95% CI 0.17, 73.15) nor T2R (aOR = 0.07; 95% CI <0.001, 80.49). The geospatial analysis found a RR of 6.80 (95% CI 1.46, 31.64) of finding new cases of leprosy in neighborhoods with schistosomiasis in one municipality. Incidence rates of leprosy per neighborhood increased with corresponding incidence rates of schistosomiasis. Conclusion: While the pilot study did not show a statistically significant association with helminth infections and reactions, the total numbers of co-infections were low. However, we found an association between leprosy and schistosomiasis on the spatial analysis, suggesting a possible role of co-infections propagating leprosy transmission. These findings call for further research with prospective studies on reactions as well as epidemiologic and immunologic studies on co-infections in areas with higher helminth endemicity.

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CHAPTER 1: INTRODUCTION

Multidrug therapy led to a significant reduction in the number of cases of leprosy (Hansen's disease) worldwide in the 1980s: 3 millions cases per year down to 300,000 cases per year. However since 2005, there has been no significant change in the annual incidence with a steady number of new cases diagnosed each year. In 2012, 232,857 cases were reported globally, with India and Brazil carrying the highest burden of disease(1). The estimated prevalence of *M. leprae* infection in Brazil in 2012 was 1.5 per 10,000 inhabitants with variable distribution and hyperendemicity in several areas of the country(2, 3). There are still large gaps in knowledge about transmission of and susceptibility to infection that continue to limit successful control of the infection(4). In addition, due to the complexity of the disease there are many clinical questions that have not been completely elucidated. One of the most pressing clinical questions is why some patients are more susceptible to leprosy "reactions" than others. Since these severe immunologic reactions are a significant cause of disability and irreversible nerve damage, studying risk factors is of utmost importance(5). Many studies have attempted to determine risk factors for reactions, but few findings have been consistent across studies and many gaps persist(6-8).

Helminth infections are co-endemic with leprosy in many areas and there is evidence to suggest that the chronic immune dysregulation of helminth infections may shift the presentation of leprosy to the more infectious form, thus providing a means of increased transmission in the community(9). Given the immune effects of helminths, it also follows that leprosy reactions could also be

influenced by co-infections. Therefore, the aims of this study were two-fold: 1. To investigate whether helminth co-infections are a risk factor for the occurrence of leprosy reactions; 2. To use geographic information systems (GIS) to study the overlap of leprosy and a helminth infection, schistosomiasis, in an endemic area of Brazil, with the goal to provide evidence on the potential influence of helminths on leprosy transmission.

CHAPTER II: LITERATURE REVIEW

Mycobacterium leprae infection and "reactions"

Leprosy is caused by the bacteria, *Mycobacterium leprae,* and infects skin, nerves and mucous membranes. It can cause irreversible nerve damage and the subsequent disability and disfigurement that are commonly associated with leprosy(10, 11). These complications are preventable if the disease is diagnosed and treated early. Overall, leprosy is not highly transmissible with estimates of only 5% of the world's population susceptible(10). In terms of presentation, leprosy involves a pathologic disease spectrum, with tuberculoid disease on one end of the spectrum and lepromatous disease on the opposite end(5, 11) (Table 1). An important and troubling part of the disease is the occurrence of immunologic "reactions" that are characterized by worsening nerve inflammation, rash and systemic symptoms(11). These can cause rapid irreversible nerve damage in some situations, and thus are an important cause of disability(5).

Reactions occur in three types: Type 1 or reversal reactions (T1R), Type 2 (T2R) or Erythema nodosum leprosum (ENL), and lucio phenomenon. The first two will be addressed in these studies. Reversal reactions are more common in borderline infections although they can occur with any type of clinical form of leprosy. They are characterized by an increase in cellular immunity and delayed hypersensitivity(12) and usually present as enlargement or increased inflammation of skin lesions, neuritis and nerve dysfunction(13). Reversal

reactions are a significant cause of nerve damage in patients with leprosy(13). Type 2 reactions (T2R) occur only in lepromatous and borderline lepromatous cases and are characterized by a systemic illness with immune complex formation and deposition microscopically(11). Symptoms include fever, arthralgias, neuritis, and classically painful erythematous skin nodules (erythema nodosum)(7). Presentations, however, can vary greatly from patient to patient, with several reports of unique clinical manifestations(14-18), including complete lack of the typical rash. T2R can often be accompanied by severe manifestations including hypotension and acute renal failure and can lead to intensive care unit admission or mortality in rare cases(18, 19). While treatment for T1R is corticosteroids, T2R often requires other immune modulating medications like thalidomide, methotrexate or other medications(5, 20). In terms of timing, both reactions can occur prior to the diagnosis of leprosy / Hansen's disease (HD), after initiation of multidrug therapy (MDT), or even after completion of MDT(5). For T1R, studies have found that the majority of cases are either present at the diagnosis of HD or occur within the first two years after initiation of MDT(12, 13). For T2R, the first occurrence typically happens within the first 3 years after MDT treatment(7, 12).

Risk factors for Type 1 and Type 2 reactions

About 30-50% of patients will experience reactions (either type) but little is known about what triggers them in certain individuals(5). Various studies have investigated risk factors – some looking at both types of reaction together, some

separately. However, most studies are small and retrospective. The pathologic / clinical type of leprosy seems to have the strongest association across studies, with borderline leprosy leading more commonly to T1R than other types, and T2R being exclusively associated with borderline borderline (BB), borderline lepromatous (BL) or lepromatous (LL) disease, with higher likelihood at the lepromatous end of the spectrum(7). Several gene polymorphisms have been studied as risk factors for both T1R and T2R reactions(12). Investigators have found certain toll-like receptors (TLR) genes more commonly in patients who develop T1R and IL-6 related genes more common in those with T2R in a different study(21) (12). Critiques of some of these studies include difficulty defining control groups in case-control studies as well as the fact that these studies have not been duplicated(12, 21). While the clinical manifestations of both reactions are distinct, there has also been substantial evidence that the cytokine profiles of both T1R and T2R are similar, with increased TNF-α, INF-γ, IL-1, IL-2, IL-6, IL-8, which could suggest common predispositions or pathways(22).

Gender and hormonal fluctuations have been proposed risk factors for reactions. In an 8-year prospective cohort study from the 1990s, Scollard et al. found a higher frequency of T1Rs in women but no difference in gender among T2Rs(8). In a 2013 epidemiologic review on risk factors for reactions, authors did not find a gender predisposition for T2R(7). One article showed a male predominance and another found a female predominance(7). However, pregnancy and lactation was associated with severe and recurrent ENL in one

study and with a higher incidence of ENL in another(23) (24). Furthermore, observational studies have shown a predilection for T1Rs to occur in the post-partum period, presumably from the recovery of full cell-mediated immunity (23). With pregnancy, lactation, and gender having possible associations with reactions, a large 2015 study set out to better understand these factors(6). Neither gender, BMI, nor menstruation was found to be associated with the occurrence of reactions in this case-control study(6).

High bacillary loads, or index, seen microscopically on skin smears have been more consistently found to be risk factors for both reactions, but especially for Type 2 reaction(12, 25). This makes intuitive sense given the highly infrequent occurrence of T1R in tuberculoid forms (low bacillary index) and the association of T2R solely in multibacillary forms (high bacillary index) (7). In fact, a 2013 systematic review of 65 papers on general epidemiologic factors associated with T2R reported odds ratios of 1.39 (95% CI 1.11-1.76) to 5.2 (95% CI 2.1-12.9) of having ENL when the bacillary index was $\geq$4 versus < 4 (26). Likewise, studies showed a higher likelihood of reactions in those with lepromatous leprosy (LL) versus those with borderline lepromatous (BL) disease(7). While T2R appears to happen most frequently during the first year after initiation of MDT, those with longer MDT regimens (24 vs. 12 months) appear to have less severe forms, possibly due to the immune modulating effects of clofazimine, a component of MDT (7). T1R has also been found to occur most frequently in the first few months of MDT(25, 27).

In terms of age, one study found a decreased incidence of T2R in those over 40 years old(26). Another also supported higher risk in younger age with higher occurrence of T2R in those who were diagnosed with leprosy in their second decade of life(8). However, may studies on T2R have not shown an age association(7). Few studies have supported a significant age association with T1R. A Thai study showed increased T1R in those over 15 years of age, but did not identify associations beyond that age (28). Scollard et al. did not find a statistically significant association between age and T1R in their 2015 study(6).

Specific to the region of Brazil of this study, an epidemiologic study of risk factors examined characteristics of 440 patients with leprosy and compared those with reactions to those who never had a reaction(25). Most of those with reactions (73.5%) developed them within the first three months of MDT. High bacillary index, antibody anti-PGL-1 positivity, and white race were associated with reactions, as were elevated WBC, thrombocytopenia, and elevated lactate dehydrogenase at diagnosis(25). Interestingly anemia after completion of MDT was associated with reaction episodes (25).

Co-infections and risk of reactions:

Certain co-infections have been studied as possible risk factors for leprosy reactions. A 1996 study showed no increased risks of either reaction in HIV co-infected patients(29). However, the initiation of antiretroviral therapy in co-infected patients has been shown to be associated with reversal reactions presumably consistent with an inflammatory response type syndrome(30). Other

viral infections may be associated with T1R, with a small study finding an increased prevalence of hepatitis B and hepatitis C antibodies in those with T1R as opposed to those without reaction (31). Another study found that “co-infections” were associated with reactions but this was a retrospective study that grouped many different kind of infections (mostly bacterial) into one category(32). A small cross-sectional study in India did not find a difference in malaria or filarial diagnosis in those patients with leprosy presenting with T2R and controls (although the make-up of the control group was not described)(33). While most of these studies have been small and retrospective, it does suggest that coexisting infections may play a role in the development of reactions in some individuals.

Helminth- leprosy co-infections

Helminth infections coexist with leprosy in many endemic areas in Brazil and elsewhere and are notable for chronic immune derangements in the host (34-36). However, very little data are available on the interactions between helminths and *M. leprae* infection. Conversely, in areas that have eliminated endemic transmission (such as most of Europe), helminths do not remain as major public health issues. A 1979 study showed a higher frequency of lepromatous leprosy in areas where the filarial worm, *Onchocerca volvulus*, was hyperendemic(37). Two studies in Vitoria, Brazil (2001, 2010) by Diniz et al. demonstrated an association between soil transmitted helminth infections and a shift towards the lepromatous end of the spectrum(9, 38). There have been no published studies on the interaction with *schistosoma* infection. Since

lepromatous disease is associated with a Th2 immunologic response as opposed to the Th1 (cell-mediated) response found in tuberculoid disease(11), it would make intuitive sense that the presence of helminths, which generally activate the Th2 response(39), would predispose to the Th2-mediated Type 2 reaction (36, 39). There is also evidence that helminth co-infections with tuberculosis have had effects on cell-mediated immunity and may alter the disease course of tuberculosis(36, 40). Therefore, whether one presents with paucibacillary or multibacillary disease and whether one develops T1R or T2R could be affected by helminth infections and can have significant implications on transmission and morbidity of leprosy.

Geographic information systems and leprosy

Shifting to investigating the effects of co-infections on transmission, geographic information systems (GIS) and spatial analysis can detect clusters of disease that aggregate data can miss, thus making it a very useful tool to study the transmission of infections(3, 41, 42). Furthermore, it can take into account other things such as environmental factors that may influence disease incidence(43, 44). GIS has been useful to study both leprosy and schistosomiasis in Brazil and in other endemic areas (3, 45-47). It has helped increase new case detection rates of leprosy in a hyperendemic area in northern Brazil by allowing for targeted interventions in areas of clustering (42). In fact with the increased ease of GPS (global positioning system) technology and accessible GIS programs, it has become a useful tool in describing disease

epidemiology and the WHO has encouraged its use in order to accomplish the "Final Push" in eliminating leprosy (http://www.who.int/lep/monitor/gis/en/). Given the associations of leprosy and soil-transmitted helminths found by Diniz et al, further studies investigating overlap of leprosy and helminths are warranted. GIS is a good tool to identify clustering of leprosy and schistosomiasis (the only reportable helminth infection in Brazil) that may signify a role of helminths in the transmission of leprosy. One such role is the shift towards the lepromatous end of leprosy in co-infections that then increases the infectious reservoir of infection in the community (Figure 1).

Helminth infection epidemiology in Minas Gerais

The state of Minas Gerais (MG) in Brazil, where these studies took place, is endemic for leprosy, schistosomiasis and soil-transmitted helminths, and therefore represents a unique opportunity to study the interplay of *M. leprae* infection and helminth infections(2, 41, 48). The best available data demonstrate an overall prevalence of helminth infections of 6% in MG and similar states (41). There are little published data, however, of the actual prevalence of these helminth infections and their overlap with leprosy. And while the overall prevalence of helminth infections reported in a survey of select municipalities of this state was 6%, the prevalence varies due to socioeconomic status and urban versus rural areas. Therefore, there are likely to be pockets where prevalence is much higher than 6%(41, 49).

Goals of this study

Given the many unknown risk factors for reactions and a biologically plausible mechanism of helminth induced immune dysregulation that may favor the development of either Type 1 or Type 2 reaction, the first goal of this study was to determine if helminth infections are associated with the occurrence of reactions in a population of Brazilian patients with multibacillary disease, with the hypothesis that those with either T1R or T2R are more likely to be co-infected with helminths than those without reactions. With the continued uncertainty regarding risk factors for reactions, additional factors such as age, sex, race, presence of anemia, body mass index, socioeconomic status, and rural residence were also investigated (Figure 2). The second goal was to determine if there is an association between the geographic distribution of leprosy and *Schistosoma mansoni* infection with the hypothesis that in neighborhoods with schistosomiasis, leprosy is more likely to be detected.

CHAPTER III: MANUSCRIPT

Title: Helminth - *Mycobacterium leprae* co-infections*:* facilitators of leprosy transmission and morbidity or innocent bystanders?

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Abstract:

Background: The immune derangements of helminth infections and evidence that co-infections may shift the presentation of leprosy to the lepromatous end of the spectrum suggest that they could be risk factors for both leprosy transmission and for the serious immunologic reactions. Methods: We conducted two investigations: a case-control study on helminth co-infections and leprosy reactions and a geospatial study on spatial associations of schistosomiasis and leprosy in Minas Gerais, Brazil. Adult patients with multibacillary disease were recruited from a leprosy clinic in Belo Horizonte. Cases included those with active Type 1 (T1R) or Type 2 reaction (T2R) and controls included those without reactions. Data were abstracted from charts and questionnaires, and stool and blood tested for helminth infections. Adjusted odds ratios were calculated with helminth infection as the main exposure and T1R or T2R as the outcomes. For the geospatial study, all new cases of *M. leprae* and *Schistosoma mansoni* infections from 2007-2014 were retrieved from SINAN, the Brazilian national notifiable disease network, for seven municipalities. Cases were mapped to municipality and neighborhood levels. A stratified analysis was conducted to identify spatial associations between the two infections. Results: Seventy-three patients were recruited to the case-control study. Helminth infections were found in 4 patients with reactions and 1 patient without reaction, with total prevalence of 6.9%. Helminth co-infections were not found to be associated with T1R (aOR =3.5; 95% CI 0.17, 73.15) nor T2R (aOR = 0.07; 95% CI <0.001, 80.49). The geospatial analysis found a RR of 6.80 (95% CI 1.46, 31.64) of finding new cases of leprosy in neighborhoods with schistosomiasis in one municipality. Incidence rates of leprosy per neighborhood increased with corresponding incidence rates of schistosomiasis. Conclusion: While the pilot study did not show a statistically significant association with helminth infections and reactions, the total numbers of co-infections were low. However, we found an association between leprosy and

schistosomiasis on the spatial analysis, suggesting a possible role of co-infections propagating leprosy transmission. These findings call for further research with prospective studies on reactions as well as epidemiologic and immunologic studies on co-infections in areas with higher helminth endemicity.

Introduction:

Multidrug therapy led to a significant reduction in the number of cases of leprosy (Hansen's disease) worldwide in the 1980s and 90s: 3,000,000 cases per year down to 300,000 cases per year(10). However since 2005, there has been no significant change in the annual incidence with a steady number of new cases diagnosed each year. In 2012, 232,857 cases were reported globally, with India and Brazil carrying the highest burden of disease(1). The estimated prevalence of *M. leprae* infection in Brazil in 2012 was 1.5 per 10,000 inhabitants with variable distribution and hyperendemicity in several areas of the country(2, 3). There are still large gaps in knowledge about transmission of and susceptibility to infection that continue to limit successful control of the infection(4). In addition, due to the complexity of the disease, there are many clinical questions that also have not been completely elucidated. One of the most pressing clinical questions is why some patients are more susceptible to leprosy "reactions" than others. Since these severe immunologic reactions or episodes are a significant cause of disability and irreversible damage, this question is of utmost importance.

About 30-50% of patients will experience either Type 1 reactions (T1R) or Type 2 reactions (T2R) but little is known about triggers and susceptibility(5). Most studies investigating risk factors have been small and retrospective. The

pathologic / clinical type of Hansen's disease (HD) seems to have the strongest association across studies, with borderline leprosy leading more commonly to T1R than other types, and T2R being exclusively associated with borderline borderline (BB), borderline lepromatous (BL) or lepromatous (LL) disease. Closely tied to the pathologic type of leprosy are bacillary loads in skin smears, with higher loads being associated with both T1Rs and T2Rs (12, 25). While pregnancy and the post-partum period appear to have some associations with both types of reactions(23, 24), gender and menstruation has not been consistently found to be associated with the occurrence of reactions(6, 23). Several genetic factors have also been investigated (21), with some associations found, but no findings subsequently duplicated (21).

In terms of co-infection, one study in Brazil study found that "co-infections" were associated with a higher likelihood of reactions, but this was retrospective study and grouped many different kind of infections (mostly bacterial) into one category (32). Viral hepatitis may also be a risk factor for reactions with a study finding hepatitis B and C antibodies at a higher frequency in those with T1R compared to those without(31). Very little has been studied, however, with regard to parasitic infections and reactions. One small cross-sectional study did not show a difference in T2R between patients with malaria or filarial infections and controls(33). In fact, little is known about the effects of helminth infections and leprosy. One group has studied soil-transmitted helminths and risk of multibacillary disease and has found a higher prevalence of co-infections in those with MB disease than in those with PB disease or in healthy controls(9).

Immunologic profiles suggest a shift to the Th2 immune response in these individuals(9). Since both T1R and T2R are associated with an immune mediated process and can have both Th1 (i.e.IL-2) and Th2 (IL-6) mediated cytokines(22), it follows that there is a biologically plausible association with chronic helminth infections and the risk of reactions.

Data from Diniz et al. also suggest that the transmission of *M. leprae* may be affected by helminth co-infections. If those who are co-infected are more likely to have lepromatous rather than tuberculoid disease, then the infectious reservoir is increased in the community and can lead to further person-to-person transmission. Studying the geographic overlap of leprosy and helminth infections can help delineate some of these potential associations. Geographic information systems (GIS) coupled with spatial analysis is a rapidly growing field and has become an important tool to study disease epidemiology. Spatial analysis can detect clusters of disease that aggregate data can miss, thus making it very useful to study the transmission of infections(44, 50). Furthermore, it can take into account environmental factors that may influence disease incidence(44). GIS has been useful to study both leprosy and schistosomiasis (separately) in Brazil and in other endemic areas (3, 44-47). It has helped increase new case detection rates of leprosy in a hyperendemic area in northern Brazil by allowing for targeted interventions in areas of clustering (42).

Therefore, given the biologically plausible hypothesis that leprosy-helminth co-infections could increase the reservoir of *M. leprae* infection, one goal of this study is to use GIS as a tool to study the relationship and spatial overlap of these

two infections, predicting that there will be a spatial association between cases of schistosomiasis and leprosy. Furthermore, given the chronic immune dysregulation of helminth infections, using a case-control study design, we predict that those with leprosy reactions will have a higher odds of helminth co-infections than those without reactions. As a secondary goal, other risk factors, such as age, gender, race, and socioeconomic status will also be investigated in the logistic regression models.

Methods:

Reactions study:

Study site and population

The case-control study was conducted in Belo Horizonte, Brazil, at the Hospital Eduardo de Menezes, the regional referral center for Hansen's disease (HD) for the state of Minas Gerais (MG). MG is an inland state in southern Brazil, north of Rio de Janeiro. Some areas have a relatively high case detection rate for HD and MG is also endemic to *Schistosoma mansoni* infection as well as other helminths(44, 46, 48). Patients come from the metropolitan area of Belo Horizonte as well as from all over the state. Since it is the main clinical site for Hansen's disease (leprosy) in the state, it often receives patients with complex symptoms that local physicians do not feel comfortable managing. Eligible participants included patients with multibacillary (MB) disease as defined in Table 1, and may include some cases of borderline tuberculoid (BT) (>5 lesions), and all cases of borderline borderline (BB), borderline lepromatous (BL) and

lepromatous (LL). Cases were defined as patients 18 years of age and over with MB disease with a current diagnosis of Type 1 or Type 2 reaction or initiating treatment for the reaction. Controls were defined as either never having had a Type 1 or Type 2 reaction or reaction-free (and no reaction treatment) for 1 year or longer. Both cases and controls could be at any point in their Hansen's disease (HD) treatment (newly diagnosed, on MDT for HD, or completed MDT). Exclusion criteria for both groups included pregnancy, age <18 years old and paucibacillary disease. For the multivariate analyses, cases and controls are defined below.

Data collection:

Patients were recruited from July through December 2015 and asked to participate at a regularly scheduled clinic visit. Since patients are seen monthly, over the course of the study, most patients cared for by this clinic were eligible to participate. Informed consent was obtained by Brazilian investigators and all questions answered. Height and weight were measured for each patient, with all investigators trained in the proper methods to measure height and weight. If the patient was wearing jeans, this was recorded and a kilogram subtracted from their weight to get a more accurate assessment. Body mass index (BMI) was then calculated for each patient. The investigator then administered a questionnaire to the patient face-to-face. Questions included basic demographic questions on race, marital status, occupation, socioeconomic status, place of residence (urban vs. rural, and district), and education. Other questions pertained

to whether the patient had ever been diagnosed with a parasitic infection, how they washed their vegetables, and their source of water. Details from the medical record such as the type of HD, the presence of nerve damage, which reaction (Type 1 or Type 2), past or current reaction, date of diagnosis of HD and reaction (where applicable) and the current treatment for reactions, where applicable, or for HD. If the patient had another infection at the time of enrollment, this was recorded. These included diverse, common infections like tooth infections or fungal skin infections.

Blood samples were taken by venipuncture to test for anemia (hemoglobin / hematocrit) and a prior or recent history *of Schistosoma mansoni* infection was determined by ELISA for *S. mansoni* IgG. The participants collected their stool samples at home on three different days to identify any soil-transmitted helminths or *S. mansoni* infection. Specific instructions were provided to the participants to ensure proper collection, storage, and delivery of stool specimens. The collection cups for stool samples can preserve stool samples for up to 30 days at room temperature. This was an ideal mode since many patients lived far away and would not be able to return with the specimens until the next monthly appointment. Anemia was defined as a hemoglobin of < 12 g /dL for women and < 13 g/dL for men per WHO guidelines(51).

Infection diagnosis:

Infection of *S. mansoni* and soil-transmitted helminths was defined as the presence of eggs in any of the three consecutive stool samples examined by the

Kato Katz and Hoffman-Pons-Janer methods of egg detection (52). The likely helminth infections for this region include *Ascaris lumbricoides*, *Trichuris trichiura, Strongyloides stercalis,* and *Schistosoma mansoni(48).* To increase the sensitivity of diagnosis for schistosomiasis and to identify any past infections that could influence the susceptibility of reactions, testing for *S. mansoni* IgG by ELISA was performed on every sample.

Statistical analysis:

There are no published data on the prevalence of helminth co-infection in patients with HD reactions; therefore a formal sample size calculation was not performed for this pilot study. Diniz et al. found co-infection in 22% of patients with lepromatous infection as opposed to 6% in those without leprosy(9). A study in Minas Gerais confirmed a helminth prevalence of 6%; however, in some parts of the state this could be much higher(44, 48). Our goal was to enroll 40-50 participants in each group during the 6 month time period, understanding that it may not be fully powered to 80%. All statistical analyses were performed in SAS version 9.4 (Cary, NC). Descriptive statistics were performed on the main study variables and p-values describing the differences between participants with and without reactions were calculated for each variable using chi-square, fisher's exact test, or t-test where appropriate. A p-value of < 0.05 was considered significant.

Multivariate analysis:

The goal of the analysis was to determine the adjusted odds ratio of helminth infection (exposure) in those with and without leprosy reactions (outcome). A graph of the hypothesized and known relationships between the exposure, outcome and potential confounders is shown in Figure 2. For the multivariate analyses, four different models were undertaken. Type 1 and Type 2 reactions were analyzed separately. Furthermore, two different exposures were used in separate analyses: helminth infection and reported history of parasitic infection. Helminth infection was defined as either a positive ova and parasite test from stool sample or a positive ELISA test for *S. mansoni* IgG. Initial confounders in the model are shown in Figure 2 and included age (continuous variable), sex, clinical HD disease (borderline vs. lepromatous), body mass index (BMI) (continuous variable), socioeconomic status based on monthly income, rural residence, race, presence of anemia, smoking, and presence of another infection. For monthly income, two groups were considered – those in the lowest category of income (< 1 x minimum wage) and those above this mark. Anemia was defined as above. Four multivariate logistic regression models were performed given the two outcomes and two exposures. For the T1R analyses, cases were those with T1R and controls were those with either no reaction or those with T2R. Mixed reactions were excluded from the T1R analyses. For T2R analyses, cases included those with T2R and controls were those with either no reaction or T1R. Since patients with borderline tuberculoid disease are not susceptible to T2R, those patients with BT disease were excluded from these

analyses. Mixed infections were kept as cases for the T2R reactions, as more than likely, the T2R was the predominant reaction. Model diagnostics included testing of collinearity and interaction, as well as an assessment of confounding using the change in estimates approach. A p-value of < 0.05 was determined to be significant. Adjusted odds ratios for the exposure variables (helminth infection or reported history of parasitic infection) as well as the other variables were calculated through logistic regression. Unadjusted odds ratio for each variable in the final models were also calculated using chi-square or fisher's exact test where appropriate. All analyses were done using SAS v9.4 and OpenEpi v3.03a.

GIS study:

Study Area:

Municipality data from seven municipalities surrounding Vespasiano, Minas Gerais, Brazil were included in this study. These included Vespasiano, Confins, Matozinhos, Pedro Leopoldo, Santana do Riacho, Lagoa Santa, and São José da Lapa. This area is endemic for leprosy, *Schistosoma mansoni* infection, and visceral leishmaniasis. For the neighborhood level analysis, the most populous municipality, Vespasiano, was used due to the availability of neighborhood population data. There were 44 neighborhoods in Vespasiano. In Vespasiano, there are no notable water sources that transmit schistosomiasis in this small geographic area, therefore, most residents have contracted the infection elsewhere, likely in nearby municipalities in Minas Gerais.

GIS mapping:

Data on new cases of leprosy, schistosomiasis, and visceral leishmaniasis, all compulsory reportable diseases in Brazil, were retrieved from the national notifiable disease surveillance system (SINAN) for the years 2007-2014 for the 7 municipalities described above. The de-identified data collected included age, sex, municipality and neighborhood of residence, class of leprosy disease (multibacillary vs. paucibacillary), and date of diagnosis. For all three diseases, the cases were first mapped to the municipality level with ArcGIS (v10.3.1) using publicly available maps of Minas Gerais (Instituto Brasileiro de Geografia e Estatística: http://www.ibge.gov.br/). The cases of leprosy and schistosomiasis were then mapped to the neighborhood level (0.5-1 km^2 on average) for Vespasiano. Visceral leishmaniasis (VL) cases were mapped to the municipality level for general incidence rates and to analyze the temporal pattern of infections. The VL cases did not have complete neighborhood level data and were not included in the Vespasiano analysis. A list of official neighborhoods with corresponding population data from 2014 was provided by the Vespasiano Secretary of Health. The boundaries of neighborhoods were determined using maps provided by municipalities where available. In areas without municipal maps, neighborhood boundaries were determined by comparing crowd-sourced maps (Wikimapia and OpenStreetMap) with at least two private local real estate companies, as well as with publicly available urban census tracts. These maps were also overlaid with 2014 purchasing power per capita data made available

through Esri's ArcGIS map service, which allowed for a comparison of one measure of poverty between neighborhoods.

Statistical analysis

Population data to the neighborhood level was available only for the most populous municipality, Vespasiano. Despite being an urban municipality, 34 of the 44 mapped neighborhoods had a population density below 3,000 persons/km^2, with the other ten neighborhoods having a population density averaging more than 6,500 persons/km^2(53). Additionally, all but six neighborhoods were in the second quintile of purchasing power per capita in Brazil (BRL 4,100 – 15,700) with the remaining six in the fifth and sixth quintiles (BRL 27,300 – 39,000 and 39,000 – 1,029,100, respectively). None of these six higher income neighborhoods were in the high population density group. Using these data and the maps generated through ArcGIS a simple stratified analysis was performed comparing neighborhoods in the same, lower, quintile of purchasing power per capita (which included 38 of 44 total neighborhoods comprising 65 cases of schistosomiasis and 46 cases of leprosy) and two different levels of population density (28 neighborhoods with lower population density and 10 with higher population density). The (unadjusted) relative risk for detecting leprosy was determined for neighborhoods with increasing numbers of cases of schistosomiasis, and average yearly incidence of leprosy was calculated for four categories of increasing incidence of schistosomiasis. For this average yearly incidence of leprosy per neighborhood comparison, two very low

population neighborhoods were identified as outliers by the modified Thompson Tau method and excluded from the chart. To compare the diseases over time, incidence rates were calculated and charted for each year for both the study area as a whole and for Vespasiano. Visceral leishmaniasis was included in these temporal charts to control for other temporal trends beyond those related to the association between leprosy and schistosomiasis. OpenEpi (v3.03a) was used for the calculations in the stratified analysis.

Ethical approval:

Ethical approval for both studies was obtained from the institutional review boards of Emory University and Faculdade da Saúde e Ecologia Humana (FASEH). In addition, ethical approval was also granted by the Fundação Hospitalar do Estado de Minas Gerais (FHEMIG), which oversees the Hospital Eduardo de Menezes.

Results:

Reactions study:

Participant characteristics:

Over the 6-month study period, 53 patients with active reaction and 20 patients without reactions were recruited. Demographic and clinical variables of the study participants are described in Table 2. Among those with reaction, 24 (45.3%) had Type 1 reaction, 21 (39.6%) had Type 2 reaction and 8 (15.3%) had a mixed reaction of both types. Four (20%) of those without active reactions had

had a reaction treated more than a year prior. The mean age among all study participants was 51.3 (SD 14.4) and 53 (72.6%) were male. Most of the patients with reaction (68.4%) were first diagnosed after MDT treatment was completed. Among the demographic and clinical study variables, only the clinical type of HD was statistically different among the cases and controls on univariate analyses. The distribution of the clinical type of HD showed a higher percentage of lepromatous disease in those with reaction and a higher percentage of borderline tuberculoid disease in those without reactions (Table 2). Other variables are outlined in Table 2 and in Appendix Table 1. Results from the stool and serum testing are outlined in Table 3. Of the 43 stool samples returned for analysis, only one was found to have a helminth infection and was identified as hookworm. This was in a patient with Type 2 reaction. For schistosoma serology, 5 out of 72 were found to be positive, four in those with reactions and one in those without reaction (Table 3). There were no differences between those with and without reactions in terms of anemia or whether they reported a history of parasitic infections (Table 3).

Results of the multivariate analysis:

Type 1 reaction: In the first model, T1R cases were compared to controls (no reaction or T2R). The exposure was helminth infection as defined above. Initial confounders included in the model are described above in the Methods section. Most of the interaction terms and BMI were associated with collinearity and were removed. Subsequently, tests of interaction showed no interaction of

the covariates with the exposure variable (helminth). After confounding assessment, the best model that retained precision was chosen. The variables retained in the model are outlined in Table 4 with unadjusted and adjusted odds ratios. While the odds ratio of those with T1R having a helminth infection was elevated at 3.5 (95% CI 0.17, 73.15), it was not statistically significant. The only variables significantly different between the cases and controls was a history of borderline disease with an adjusted odds ratio of 5.93 (95% CI 1.14, 30.76) and low monthly income with an inverse relationship with T1R (OR=0.04; 95% CI 0.02, 0.88). The T1R model results with history of parasitic infection as the exposure is outlined in Table 5. In the final model, BMI was removed due to collinearity and again no interaction was found. Using the change in estimates approach, confounding was assessed and final model variables listed in the Table 5. The adjusted odds ratio for T1R with reported history of parasitic infection as the exposure was 7.34 (95% CI 0.68, 79.61), and not significant. Again, borderline disease was found to be significantly more common in the cases as opposed to controls (OR = 13.91; 95% CI 1.41, 137.0). Consistent with the first model, socioeconomic status was also associated with T1R (Table 5). In addition, rural residence was inversely associated with T1R with an OR = 0.03 (95% CI 0.001, 0.47).

Type 2 reactions: For the model with those with T2R as cases and helminth infection as the exposure, the variables that remained after collinearity, interaction and confounding assessments are outlined in Table 6. In this case, the odds ratio of helminth infection in T2R compared to controls was low with an

adjusted OR of 0.07 (95% CI <0.001, 80.49), but this was not statistically significant. Again clinical disease was found to be significantly associated with T2R, with borderline disease much less likely in those with T2R (OR of 0.01, 95% CI <0.001, 0.35). For the second model with T2R and history of parasitic infection, unadjusted and adjusted odds ratios were calculated for the variables retained after collinearity, interaction and confounding assessments (Table 7). History of parasitic infection was not associated with T2R with an OR of 0.50 (95% CI 0.04, 6.46). However, clinical disease continued to be significantly associated with T2R with an OR of 0.01 (95% CI <0.001, 0.32), signifying a lower likelihood of borderline disease in those with T2R. No other variables were statistically significant in the models for T2R.

GIS study:

Demographic data of the three infections for all municipalities are presented in Table 8 and includes the number of cases, average age of new cases and sex. Also shown is the breakdown of multibacillary versus paucibacillary leprosy cases. Spatial comparison of cases of leprosy, schistosomiasis, and visceral leishmaniasis in the 7 municipalities studied is presented in Figure 1 of Appendix A. The same municipality (Confins) had the highest average incidence of leprosy (1.1/10k) and schistosomiasis (9.3/10k), but not of visceral leishmaniasis, which was highest in Vespasiano. Mapping at the neighborhood level for leprosy and schistosomiasis in Vespasiano is represented in Figure 3, comparing cases of leprosy and schistosomiasis in the largest

municipality juxtaposed on the population of the neighborhoods. These maps identify the similar distribution of these diseases throughout the municipality as well as areas of high burden for both infections.

Combining these case data with the population density categories listed above and at the same purchasing power per capita quintile (38 / 44 neighborhoods as described above), the adjusted relative risk for detecting leprosy in a neighborhood with reported schistosomiasis vs. those without was 6.80 (95% CI 1.46, 31.64). The unadjusted RR before stratifying was 2.90 (95% CI 1.53-5.51). Relative risk (unadjusted) was also calculated for neighborhoods with increasing case numbers of schistosomiasis, and is presented in Figure 4. A comparison of the average yearly incidence of leprosy vs. the average yearly incidence of schistosomiasis at different levels of schistosomiasis incidence is presented in Figure 5, and shows a trend of higher incidence of one infection with higher incidence of the other. Finally, changes in incidence over time for all three diseases for the study area as a whole and for Vespasiano specifically are charted in Figure 6. These charts show that incidence of *S. mansoni* and *M. leprae* infections were highest for the same time period in Vespasiano (2009-2011), and for the study area as a whole (2010-2011). In both instances, both diseases peaked in 2011 before beginning to decline again. None of these trends held true for visceral leishmaniasis, another disease endemic to this area.

Discussion:

This pilot case control study not only allowed an assessment of the association of helminth infections and reaction, but also provided a picture of the demographic and clinical aspects of 73 patients with and without reactions in a HD referral clinic in Brazil, which carries the second highest burden of HD worldwide. Our main study question on whether helminth infections are associated with reactions did not reveal a significant association using multivariate analyses. However, while only five patients were found to have helminth infections, 4/5 (80%) of them were in patients with either T1R or T2R. Given the biologic plausibility of chronic helminth infections disrupting the immune system and increasing the likelihood of these immunologic reactions, these findings warrant further investigation in a setting with higher helminth endemicity. Studying other coinfections would also be advisable given the fact that bacterial co-infections and viral hepatitides have been shown to have possible associations with reactions(32).

Consistent with prior studies, our analyses showed borderline disease (BT, BB, and BL) to be a risk factor for T1R. On the other hand, our T2R analyses demonstrated lepromatous disease to be associated with a higher likelihood of T2R. Again, this is consistent with the literature where lepromatous disease and higher bacillary loads, which are typical of lepromatous disease, are risk factors for T2R(12). Interestingly, a bacillary index (BI) $\geq$4 found on skin slit smears was not a statistically significant risk factor for either reaction in our study, despite our findings of lepromatous disease being a risk factor for T2R.

Since many of our patients were referred from other sites, it is possible that we did not have their initial BI recorded, and thus had lower recorded initial ones due to treatment effects. Also found to be associated with T1R was socioeconomic status (SES). Those with T1R were less likely to be in the lowest category of monthly income. Likewise, rural residence was also less likely in those with T1R on one of the models. While T2R did not have any associations with SES or rural residence, it is an interesting finding and deserves more investigation. Since T1R is associated with robust cell-mediated immunity (CMI), there could be a correlation with better CMI in those with higher SES or urban residence. Since some micronutrient deficiencies, like vitamin A, are associated with depressed CMI(54), a difference in nutrition could explain these findings if those in higher categories of monthly income and those who live in urban areas have better nutrition than those with the lowest incomes and those who live in rural areas.

Other variables such as age, sex and race were not associated with reactions in our study. Prior studies provided mixed results for these variables, with age over 15 years a risk factor for T1R in one study and no associations found in other studies(6, 28). Likewise, for T2R, those in the second decade of life appeared to be at risk(8), however, again, this has not been replicated in other studies(7). The low numbers in our study precluded breaking down the age into categories, so we may not have had an accurate picture of the risk of reactions by decade, and therefore may have missed an age association by using age as a continuous variable. There have been no consistent associations with sex and either reaction, although some hormonal conditions (pregnancy and

lactation) may be related to reactions(7, 23). In our pilot case control study, the predominant sex was male, therefore, it was not possible to get an accurate assessment of menstrual affects on the risk of reactions. This may have also limited our ability to determine any differences between the sexes. Few data exist on any association with race and reactions except for a study in Minas Gerais which showed white race as a risk factor for reactions(25). Our study did not find this association, although we may have had a different reference group (mixed race vs. white or black). Also, self reported race might not accurately reflect genetic or social differences. Lastly, our patients with reactions were diagnosed more often after MDT was completed then during treatment, which is not consistent with the literature(12, 25). Since patients were referred from outside clinics, it is possible that the date of diagnosis of reactions recorded in the medical record may not have been completely accurate.

A limitation of this study is the small sample size and uneven distribution of overall cases of reactions (T1R and T2R) and controls (no reactions or none in past year). Only 20 patients without either reaction were recruited during the study period. Since the dermatology clinic at the Hospital Eduardo de Menezes is a HD referral center for the state of Minas Gerais, and since reactions are often the most difficult complication to control, it makes sense that more patients with reactions than not would be referred to this clinic. Dividing the analysis between T1R and T2Rs gave a better distribution of cases and controls and made it possible to evaluate these individually, which is important given the different mechanisms of action for these reactions. However, again, numbers were low

and limited the interpretation of the multivariate logistic regression models. The overall burden of helminth infections in this population was low, but consistent with prior studies of urban populations in this area of MG(48). It is possible that we underestimated the helminth infections in this population since only 43 / 73 (59%) patients returned their stool studies and some infections, such as strongyloides, are hard to detect on ova and parasite exam. A study in an area with higher endemicity of helminth infections would better determine if there is a link between them and both types of reactions.

On the other hand, the geospatial analysis shows a previously undescribed association between *M. leprae* and *S. mansoni* infection. In the analysis of Vespasiano, there is a clear association between leprosy and schistosomiasis, which is evident in the spatial overlap of the diseases and their similar temporal trends. In addition to the overlap and clustering visible on GIS-produced maps of *S. mansoni* and *M. leprae* infection, the relative risk of 6.80 (95% CI 1.46, 31.64) of detecting leprosy in a neighborhood with known schistosomiasis was statistically significant and high. The facts that relative risk for detecting leprosy in a neighborhood generally increased along with increasing case numbers of schistosomiasis (Figure 4), combined with the fact that average yearly incidence of leprosy increased along with incidence of schistosomiasis (Figure 5), further supports the existence of an important link between these diseases.

The observed similarities in the temporal trend of incidence for leprosy and schistosomiasis from 2007-2014, and the lack of such similarities for visceral

leishmaniasis provide another piece of evidence and a point of comparison against another endemic disease associated with many of the same traditionally understood risk factors for leprosy (poverty, lower socioeconomic status, and overcrowding)(2, 55).

Limitations of the GIS analysis included low numbers of overall cases, which limited the extent of stratification, the categorical nature of much of the available data, and limited data on other confounders such as more specific measures of poverty, crowding, sources of infection. Some of these limitations are inherent to GIS analysis, but these factors limited the ability to control for additional variables, or to control in a more precise fashion through more complex spatial regression analyses. There could also be a reporting bias if not all cases of the diseases were reported to the state health department. However, for leprosy at least, this is likely to be minimal since multidrug therapy is supplied by the government and requires reporting in order to receive the medication.

There are many important points to take away from these two pilot studies. The fact that most of the patients in the case-control study were diagnosed or at least still symptomatic for reactions after they had finished MDT demonstrates the often long period of time that patients can suffer from complications. The burden on patients and healthcare systems, especially those in low-income areas, is grossly underestimated by leprosy prevalence data, which only counts cases during the 6-12 months of MDT(10). Grade 1 or 2 nerve disability was found in the majority of these patients (Appendix Table 1), whether with reactions or not, again pointing to the long-term sequelae and the need for

better diagnostics, case finding, and management of complications. This study also shows the complex nature of leprosy reactions and the difficulties that many researchers have had in elucidating risk factors for reactions. Further studies, especially prospective ones are needed to better determine what makes some patients susceptible to these serious complications. In addition, the geospatial findings make an argument to continue looking for associations between helminth infections and *M. leprae* given the findings uncovered by this study. Identifying factors, such as helminth co-infections, that are associated with transmission of leprosy could have significant public health impact and provide innovative strategies to control this debilitating infection.

Tables and Figures:

Table 1. Ridley Jopling classification (left) and WHO classification (right) of the disease spectrum of leprosy.

Ridley-Jopling Classification			**WHO Classification**
Tuberculoid (TT)	Single or few lesions, negative or rare bacilli on histology	Very good cell-mediated immunity	Paucibacillary
Borderline Tuberculoid (BT)	Single or few lesions, rare bacilli on histology	Good cell-mediated immunity	Paucibacillary (if $\leq$ 5 lesions) Multibacillary if > 5 lesions
Borderline Borderline (BB)	Several lesions, more bacilli on histology	Fair cell-mediated immunity	Multibacillary
Borderline lepromatous (BL)	Many lesions, many bacilli on histology	Fair-poor cell-mediated immunity	Multibacillary
Lepromatous (LL)	Diffuse lesions, heavy bacillary load	Poor cell-mediated immunity	Multibacillary

Table 2. Main demographic variables of patients with either type of active reaction and those without reaction. P-values describing differences were determined from t-test, chi-square, or Fisher's exact test where appropriate, and considered significant if <0.05.

Variable	Reaction (n= 53)	No Reaction (n=20)	Total (n=73)	p-value
Age, years (mean, SD)	50.2 (14.3)	54.1 (14.2)	51.2 (14.4)	0.33
Gender, n (%)				
Male	41 (77.4)	12 (60.0)	53 (72.6)	0.14
Type of Reaction, n (%)		N/A	N/A	N/A
Type 1	24 (45.3)			
Type 2	21 (39.6)			
Mixed	8 (15.3)			
Clinical HD, n (%)				
BT	5 (9.4)	5 (25.0)	10 (13.7)	**0.02**
BB	13 (24.5)	9 (45.0)	22 (30.1)	
BL	3 (5.7)	2 (10.0)	5 (6.9)	
LL	32 (60.4)	4 (20.0)	36 (49.3)	
Stage of treatment when diagnosed w/ reaction (18 miss)				
Within first 6 mo	7 (20.0)	N/A	N/A	N/A
Second 6 mo	4 (11.4)			
After MDT completion	24 (68.6)			
Bacillary index (BI) (8 miss)				
1st recorded: Mean, SD	2.8 (1.8)	2.0 (1.4)	2.62 (1.7)	0.10
BI ≥ 4, n (%)	16 (32.7)	3 (18.8)	19 (29.3)	0.36
Race, n (%) (1 miss)				
African descent	20 (38.5)	9 (45.0)	29(40.3%)	0.57
White	15 (28.9)	3 (15.0)	18 (25.0%)	
Mixed	16 (30.8)	8 (40.0)	24 (33.3%)	
Refused	1 (1.8)	0	1 (1.4%)	
Residence, n (%)				
Urban	40 (75.5)	15 (75.0)	55 (75.3%)	0.97
Rural	13 (24.5)	5 (25.0)	18 (24.7%)	
Monthly income#, n (%)				
<1	13 (24.5)	7 (35.0)	20 (27.4%)	0.65
1 to 3	35 (66.0)	11 (55.0)	46 (63%)	
3 to 5	5 (9.4)	2 (10.0)	7 (9.6%)	
>5	0	0	0	
Smoking, n (%)				
Yes	16 (30.8)	4 (20.0)	20 (28%)	0.56

#definition: Categories of monthly income determined by Brazilian minimum wage, with <1 being below minimum wage and >5, more than five times the minimum wage.

Table 3. Hemoglobin and helminth results among those with reactions and those without reactions. P-values describing differences were determined by t-test, chi-square, or Fisher's exact test where appropriate, and considered significant if <0.05.

Variable	Reaction	No reaction	Total	p-value
Hemoglobin, mean (SD)	14.0 (2.0)	14.2 (2.0)	14.0 (2.0)	0.75
Anemic, n (%)				
Yes	17 (32.8)	4 (20.0)	14 (19.2)	0.31
Stool positive for helminth Infection, n (%) (n=43)	1 (3.0)	0	1 (2.3)	0.77
***S.mansoni* IgG, n (%) (n=72)**				
Yes	4 (7.7)	1 (5.0)	5 (6.9)	0.68
History of parasitic infection , n (%) (n=72)	13 (25.0)	2 (10.0)	15 (20.8)	0.21

Table 4. Model 1: Multivariate logistic regression model with Type 1 reaction as the outcome and helminth infection as the exposure, with crude and adjusted odds ratios. Bolded results represent significant results with a p-value <0.05.

MODEL 1	Crude OR	95% CI	Adjusted OR	95% CI
Helminth^	1.73*	0.17, 25.17	3.50	0.17, 73.15
Borderline disease	3.43	1.17, 11.89	**5.93**	**1.14, 30.76**
Female sex	0.43	0.12, 1.52	0.28	0.05, 1.71
Socioeconomic status:				
Monthly income < 1 x the minimum wage	0.43	0.12, 1.52	**0.14**	**0.02, 0.88**
Rural residence	0.49	0.14, 1.72	0.16	0.03, 1.03
Race				
African descent	1.04	0.37, 2.93	2.39	0.46, 12.49
White race	0.73*	0.20, 2.68	0.84	0.12, 5.73
Reference: mixed	1	---	1	---
Anemia	0.89	0.30, 2.67	1.76	0.23, 13.47

^ Defined as either a positive result on stool ova and parasite exam or IgG for schistosomiasis

*Fisher's exact test

Table 5. Model 2: Multivariate logistic regression model with Type 1 reaction as the outcome and reported history of parasitic infection as the exposure, with crude and adjusted odds ratios. Bolded results represent significant results with a p-value <0.05.

MODEL 2	Crude OR	95% CI	Adjusted OR	95% CI
History of parasite	1.15	0.35, 3.76	7.34	0.68, 79.61
Borderline disease	3.43	1.17,10.07	**13.91**	**1.41,137.00**
Female sex	0.43	0.09, 1.69	0.14	0.02, 1.25
Age (continuous)	--		1.00	0.94, 1.06
Socioeconomic status: Monthly income < 1 x the minimum wage	0.43	0.09, 1.69	**0.12**	**0.02, 0.91**
Rural residence	0.49	0.10, 1.92	**0.03**	**0.001, 0.47**
Race				
African descent	1.04	0.36, 2.98	4.50	0.54, 37.70
White race	0.73	0.14, 3.08	1.52	0.08, 28.11
Reference: mixed	1	--	1	--
Anemia	0.89	0.30, 2.67	0.98	0.07, 13.53
Smoking	1.42	0.49, 4.49	0.35	0.05, 2.69
Other infection	0.66	0.10, 3.42	5.06	0.33, 77.34

Table 6. Model 3: Multivariate logistic regression model with Type 2 reaction as the outcome and helminth infection as the exposure, with crude and adjusted odds ratios. Bolded results represent significant results with p-value <0.05.

Model 3	Crude OR	95 % CI	Adjusted OR	95% CI
Helminth^	0.86*	0.07, 8.08	0.07	<0.001, 80.49
Borderline dx	0.06	0.01, 0.25	**0.01**	**<0.001, 0.35**
Female sex	1.34*	0.43, 4.21	15.33	0.35, 667.57
Bacillary index ≥4	2.90	0.91, 9.29	1.37	0.14, 13.92
Socioeconomic status: Monthly income < 1 x the minimum wage	1.37	0.47, 4.10	1.03	0.05, 20.36
Rural residence	1.60	0.52, 4.89	10.71	0.20, 584.12
Race				
African descent	0.66	0.24, 1.86	0.05	0.001, 2.12
White race	2.75*	0.85, 8.92	1.41	0.06, 34.70
Reference: mixed	1	---	1	
Anemia	1.39	0.48, 4.03	11.97	0.31, 466.12
Smoking	1.69	0.55, 5.19	18.48	0.01, 7.55
Other infection	1.58	0.29, 9.22	0.26	0.01, 9.17

^Defined as either a positive result on stool ova and parasite exam or IgG for schistosomiasis

*Fisher's exact

Table 7. Model 4: Multivariate logistic regression model with Type 2 reaction as the outcome and reported history of parasitic infection as the exposure, with crude and adjusted odds ratios. Bolded results represent significant results with p-value <0.05.

Model 4	Crude OR	95% CI	Adjusted OR	95 % CI
History of parasite	1.40	0.42, 4.62	0.50	0.04, 6.46
Borderline dx	0.06	0.01, 0.25	**0.01**	**<0.001, 0.32**
Female sex	1.34	0.43, 4.21	4.83	0.22, 106.45
Age	---		1.05	0.93, 1.18
Bacillary index ≥4	2.90	0.91, 9.29	0.86	0.01, 10.78
Socioeconomic status: Monthly income < 1 x the minimum wage	1.37	0.47, 4.10	0.90	0.06, 14.73
Rural residence	1.60	0.52, 4.89	10.10	0.33, 311.13
Race				
African descent	0.66	0.24, 1.86	0.05	<0.001, 2.74
White race	2.75	0.85, 8.92	0.60	0.02, 19.08
Reference: mixed	1	---	1	---
Anemia	1.39	0.48, 4.03	6.57	0.14, 313.37
Smoking	1.69	0.55, 5.19	11.74	0.47, 291.63
Other infection	1.58	0.29, 9.22	1.31	0.07, 25.96

Table 8. Basic data of the three infections for all seven municipalities in the GIS study from 2007-2014

	Mycobacterium leprae	***Schistosoma mansoni***	**Visceral leishmaniasis**
Total Cases	139	200	315
Multibacillary, % of cases	76%	N/A	N/A
Age in years, median (range)	48 (6-97)	30 (0-84)	33 (0-89)
Sex			
Male	51%	67%	61%

Figure 1. Proposed mechanism of the effect of schistosoma-leprosy co-infections on *M. leprae* transmission

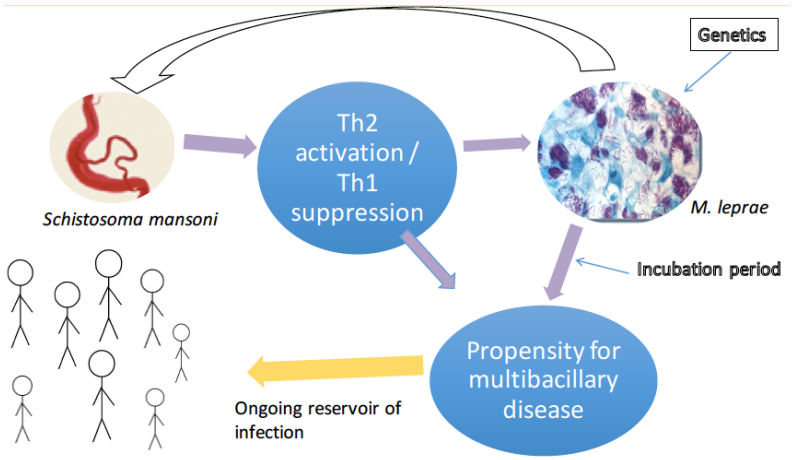


Figure 2: Directed acyclic graph of potential associations between the exposure (helminth infections) and leprosy reactions.

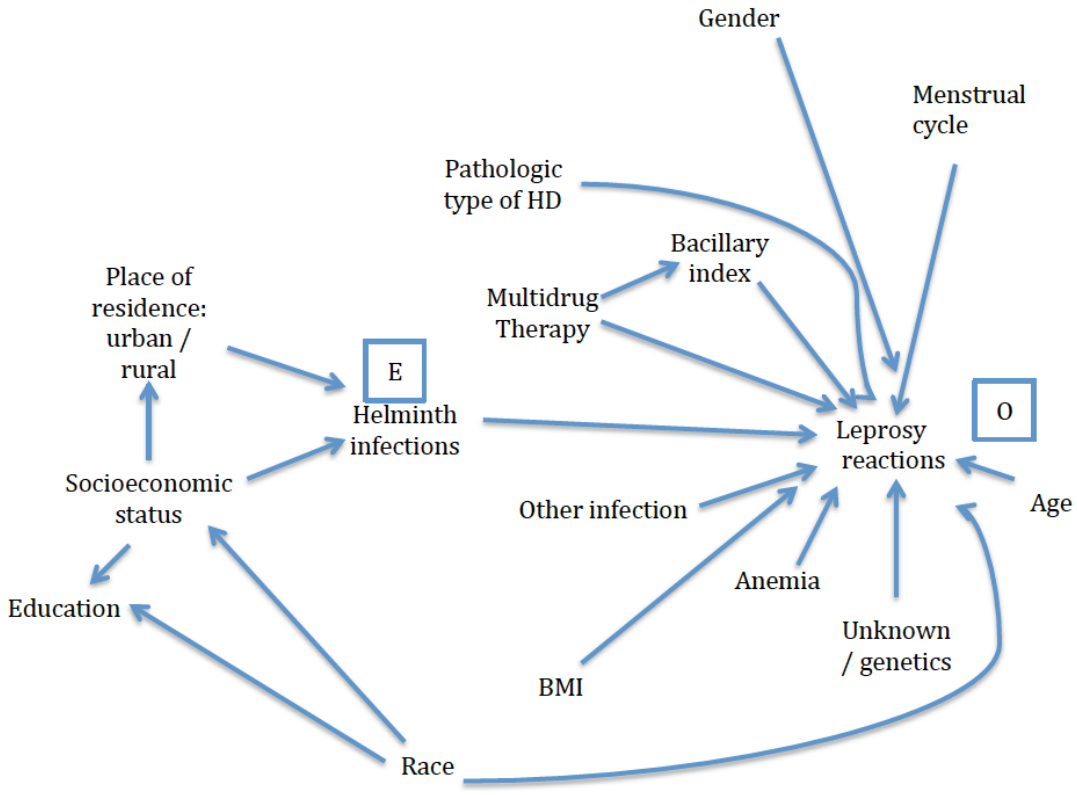


Figure 3: Comparison of cases of *M. leprae* and *S. Mansoni* infection in Vespasiano, the most populous municipality, from 2007-2014

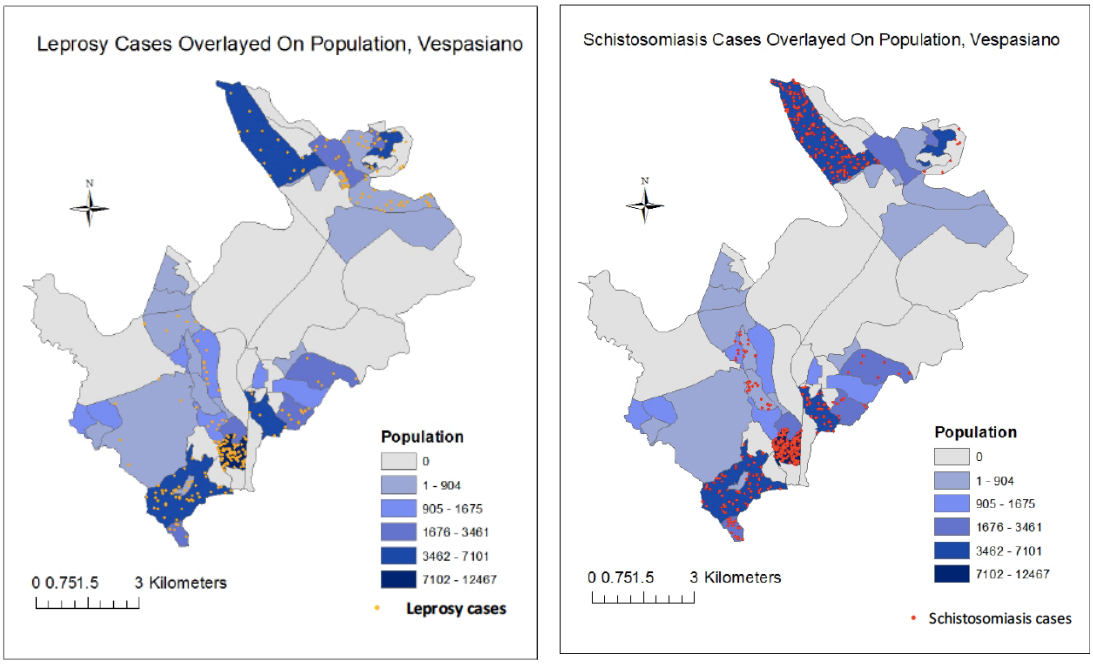


Figure 4. Relative risk (unadjusted) of detecting leprosy in a Vespasiano neighborhood with increasing case numbers of schistosomiasis (error bars represent 95% confidence intervals).

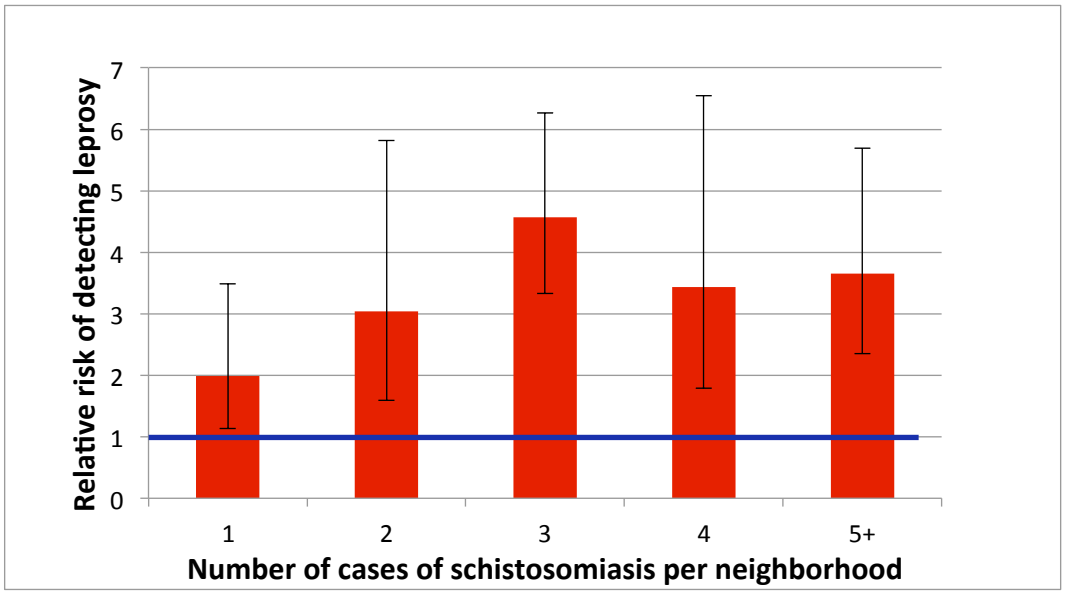


Figure 5. Average yearly incidence of leprosy in neighborhoods categorized by increasing average yearly incidence of schistosomiasis

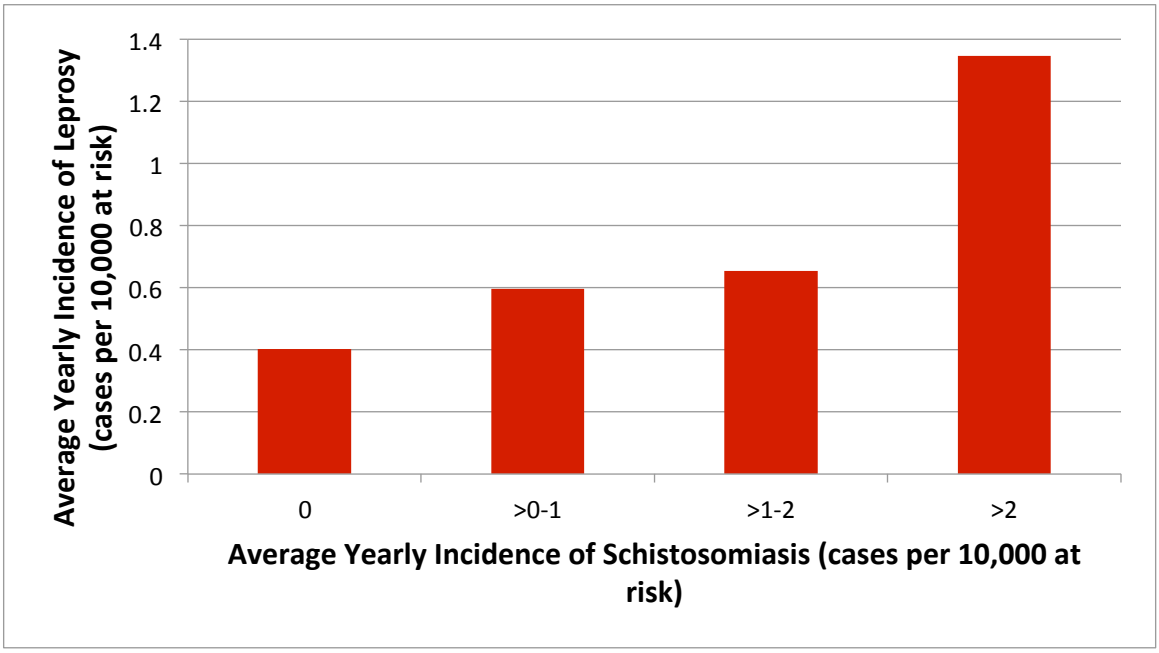
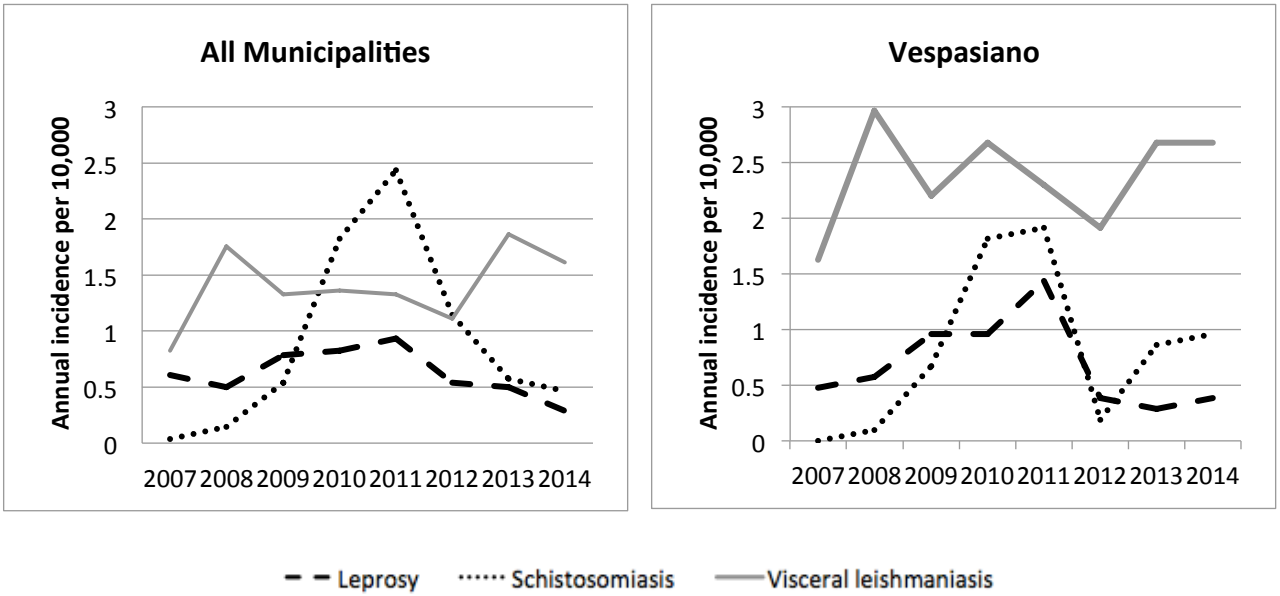


Figure 6. Incidence of leprosy, schistosomiasis, and visceral leishmaniasis in all 7 municipalities per year from 2007-2014



CHAPTER IV: SUMMARY AND FUTURE DIRECTIONS

The overall goal of the reactions study was to identify ways to reduce the morbidity and potential permanent disability of those with leprosy. In low and middle-income countries, where leprosy is most commonly found, effective treatment and monitoring of complications are limited by poor access to healthcare and sometimes inefficient healthcare systems. Chronic corticosteroids, thalidomide, and other therapies for reactions can have significant adverse events and often require close clinical and laboratory monitoring, which may be very difficult in low-resource settings. Therefore, it is of paramount importance not only to investigate ways to improve the control of *M. leprae* infection but to identify ways to reduce serious complications such as T1R and T2R. While we did not find any significant associations beyond the clinical type of HD and socioeconomic status, it did show that co-infections with helminths are present in patients with leprosy and further studies in areas with higher endemicity are needed to study these interactions. Prior evidence shows a shift towards the lepromatous end of the spectrum in co-infected patients with cytokine profiles revealing a predominant Th2 response(9). Therefore, it follows that reactions, which are predominantly immune mediated, would also be affected by the presence of a chronic helminth infection. Further supporting the need for larger studies investigating the morbidity of leprosy – helminth co-infections lies in the results of the high and statistically significant odds ratio of finding leprosy in neighborhoods with reported schistosomiasis in the GIS study.

Aside from the data on helminths there are other findings worthy of further investigation. The association with socioeconomic status, residence and T1R may be a marker for better cell-mediated immunity in those with less poverty and who live in urban areas, possibly explained by undernutrition. Therefore, those with fewer resources may have nutritional deficiencies that affect their ability to mount a T1R. Evaluation of patients' sera for vitamin A, vitamin D and iron deficiencies are planned and will be correlated to the socioeconomic results.

While both investigations were pilot studies and the geospatial analysis cannot prove a causal relationship between *S. mansoni* or helminth coinfection and *M. leprae* coinfection, they will serve as a starting point for further research efforts on the effects of co-infections on the transmission of leprosy and occurrence of reactions. The next phase of investigation into the question of helminths and leprosy coinfection will involve a larger GIS study from a more highly endemic area of Brazil, that also has known water sources for *S. mansoni* transmission. Georeferencing cases and higher order spatial analyses will allow for more specific comparison and greater use of the analytic tools of GIS. Direct investigations of leprosy, schistosomiasis, and other helminths in the form of co-infections will also be necessary to better delineate these associations. A larger, powered case-control study in Governador Valadares, Minas Gerais, Brazil, will attempt to address these questions. This area has a prevalence of *S. mansoni* infection of over 15% in many areas and is also endemic for other helminth infections(44, 48). Combining these clinical evaluations of co-infections and nutrition, including immunologic testing, with GIS epidemiologic findings will

improve the body of knowledge of leprosy transmission and have the potential to truly impact disease control by increasing the tools at our disposal to reduce the reservoir of infection. These studies and results could also expand to other chronic infections of poverty that are often found in co-infections, and which have posed significant public health challenges to elimination efforts.

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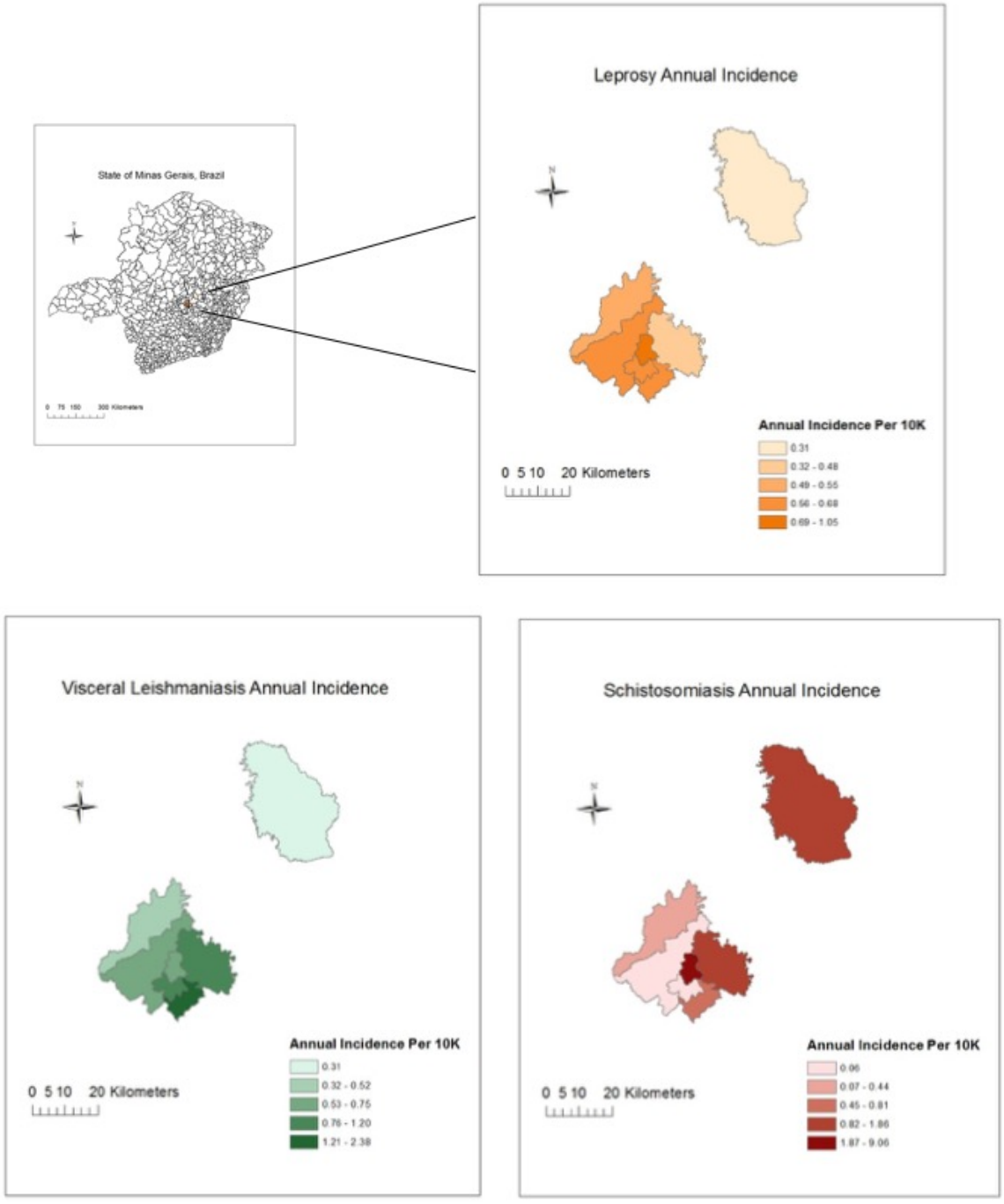
APPENDICES

Appendix A: Additional Figures

Appendix Figure 1. Average annual incidence for all seven municipalities of *M. leprae* infection, schistosomiasis, and visceral leishmaniasis, from 2007-2014.



Appendix B. Additional Tables

Appendix Table 1. Additional demographic and clinical variables among those with reaction and those without in the case-control study.

Variable	Reaction (n= 53)	No Reaction (n=20)	Total (n=73)	p-value
Body mass index, kg/m^2, mean (SD)	26.2 (4.0)	24.9 (4.4)	25.8 (4.1)	0.29
Stage of treatment (18 miss)		N/A	N/A	N/A
Within first 6 mo.	7 (20.0)			
Second 6 mos.	4 (11.4)			
After MDT completion	24 (68.6)			
Education, n (%) (1 miss)				
None	3 (5.8)	2 (11.8)	5 (6.9)	0.76
Primary	40 (76.9)	12 (70.6)	55 (76.4)	
Secondary	7 (13.5)	3 (17.7)	10 (13.9)	
Beyond	2 (3.9)	0	2 (2.8)	
Material status^, n (%)				
1	18 (34.6)	5 (25.0)	23 (31.4%)	0.52
2	26 (50.0)	13 (65.0)	39 (54.2%)	
3	8 (15.4)	3 (10.0)	10 (13.9%)	
Other infection, n (%) (15 miss)				
Yes	8 (18.9)	2 (13.3)	10 (17.2)	0.64
Disability grade, n (%)				
0	17 (32.1)	8 (40.0)	25 (34.3%)	0.80
1	14 (26.4)	5 (25.0)	19 (26.0%)	
2	22 (41.5)	7 (35.0)	29 (39.7%)	

^definition: 1 = Can satisfy one's needs adequately; 2 = Can satisfy one's needs partially; 3 = Difficulty satisfying one's needs