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EXPLORING POVERTY-RELATED RISK FACTORS FOR LEPROSY TRANSMISSION IN A HIGHLY ENDEMIC AREA OF BRAZIL: FOCUS ON HELMINTH CO-INFECTIONS AND MICRONUTRIENTS

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B.A. Anthropology with a Concentration in Natural Sciences

Minor in Biology

University of Louisville

2015

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
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Abstract

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Background: Although cases of leprosy have decreased over the past 20 years, there are still many obstacles to control the infection, especially in areas of hyperendemicity such as northeast Minas Gerais (MG), Brazil. Studies have shown that leprosy, as well as helminth infections, are associated with poverty. We hypothesized that in areas with higher rates of leprosy, helminth infections and micronutrient deficiencies are risk factors for continued leprosy transmission due to effects on the immune response leading to acquisition and spread of *Mycobacterium leprae*. **Methods:** This hypothesis was examined through a case-control study in Minas Gerais, Brazil. Participants were enrolled either as newly diagnosed cases or as one of two types of controls: household contacts and negative controls. Data were collected through surveys, clinical data, and stool specimens for helminth diagnosis and blood for micronutrient analyses. Analysis was conducted through univariate analysis, Chi-square testing, adjusted odds ratios and ultimately logistic regression controlling for socioeconomic status and micronutrient status. **Results:** This study recruited 75 cases and 148 controls. Schistosomiasis infection was found in 11.8% of cases and 5.2% of controls. When looking at micronutrient deficiencies, 21.7% of cases and 10.4% of controls had vitamin D deficiency. Multibacillary infection was the prominent classification identified (73.3%) with the majority having no disabilities associated with infection (60.0%). The majority of cases in this study had at least primary education (71.4%) and lower income (92.0%). On multivariate analyses, cases were more likely to have schistosomiasis infection when compared to all controls (aOR = 3.60; 95% CI [1.06, 12.10]) and an even higher likelihood when compared to just household contacts (aOR = 6.56; 95% CI [1.16, 36.94]). When looking at micronutrient results, cases were more likely to have vitamin D deficiency compared to both control groups (aOR = 4.81; 95% CI [1.24, 18.72]). **Conclusions:** Through this case-control study we saw associations between schistosomiasis and vitamin D deficiency with leprosy. These results show the importance and relationship between co-infections and micronutrient deficiencies in the continued transmission of leprosy infection in endemic areas. Additionally, these associations could lead to potential combined control efforts in endemic areas for both schistosomiasis and leprosy infections.

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Chapter 1: Introduction

Although cases of leprosy caused by infection of *Mycobacterium leprae*, have decreased over the past 20 years, there are still many obstacles to control the infection, especially in areas of hyperendemicity such as in northeast Minas Gerais (MG), Brazil. Studies have shown that leprosy, as well as helminth infections, are associated with poverty suggesting that there are poverty-related factors like poor living conditions that may be maintaining a reservoir for continued transmission of leprosy [1-4]. Leprosy has often been described as a disease of the poor, but the specific aspects of poverty that may be leading to continued transmission are unclear. A recent study in Bangladesh found that patients with leprosy have less money to spend on food and therefore have a less diverse diet [3]. Another study in Northern Brazil found associations between low education level and experience of food shortage associated with leprosy [4]. Interestingly, this same study found an association between having bathed in open water bodies within the past 10 years which could suggest that water is a potential risk factor for continued transmission [4]. These studies and others related to micronutrient deficiencies, helminth infections and leprosy suggest that certain socioeconomic risk factors for leprosy exist and further investigation into the aspects of poverty which could be influencing these risk factors needs to be conducted.

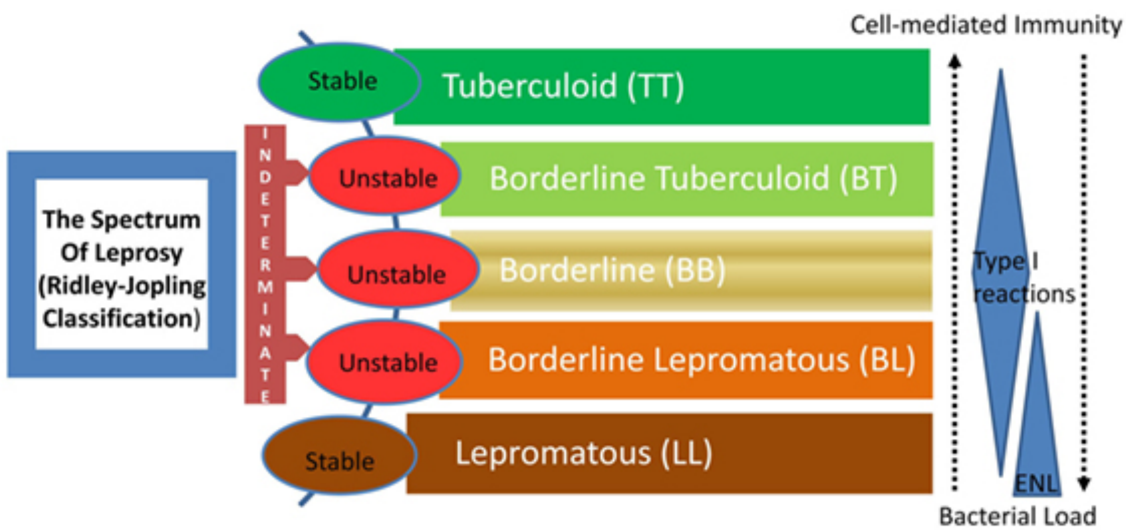
The objective of this case-control study was to examine these pockets of "hyperendemic" areas in Brazil with higher rates of leprosy, helminth infections and micronutrient deficiencies and the potential risk factors for continued leprosy transmission due to effects on the immune response leading to acquisition and spread of *M. leprae.* This study can be used to increase the body of knowledge on potential associations between parasitic worm infections and leprosy as well as micronutrient deficiencies and leprosy and to increase the understanding of the immunologic differences that are presented in co-infected versus non-co-infected individuals.

Chapter 2: Comprehensive Review of the Literature

Leprosy Immunology

The impacts and effects of leprosy on infected individuals has a lot to do with the host immune system. The particular pathways and exact mechanisms in the immune system of leprosy infected patients is still being researched, but studies have shown that manifestations and symptoms are highly driven by the host immune system [5, 6]. Since leprosy is impossible to culture in a laboratory, it is difficult to determine immunologic implications of infection on different individual host immune systems. Clinical representation of leprosy infection occurs on a spectrum defined as the Ridley-Jopling Classification (Figure 1).

Figure 1: The Ridley-Jopling classification spectrum of leprosy and its relationship with immune responses [7]. ENL, erythema nodosum leprosem, and Type 1 reactions in the figure below refer to immunologic complications associated with leprosy infection within the spectrum of classification.



In 1982, the WHO simplified the Ridley-Jopling spectrum of infection as multibacillary (MB) or paucibacillary (PB) to make diagnosis and treatment easier. MB infection is defined by a large number of bacilli found in the lesions. PB infections are more difficult to diagnosis with patients usually having little to no lesions on their skin and have a low bacilli count, and therefore often times have no bacilli seen on the skin smear [5]. In countries like Brazil, the Madrid classification system is still currently used where patients are defined on a scale from tuberculoid to borderline to lepromatous [5].

Undernutrition and Leprosy

Undernutrition is common in areas where helminth infections and leprosy co-exist as well as in areas of higher poverty rates and crowding. Data support a role of nutritional deficiencies and leprosy, but overall the literature is sparse. It has been demonstrated that lower vitamin A levels are present in individuals with lepromatous (LL) compared to controls [8]. Lima et al. implemented a study in Manuas, Brazil that measured serum levels in 43 leprosy patients for vitamin A and divided these patients into the Ridley-Jopling classification spectrum. The patients enrolled had not previously been submitted to drug treatment and healthy subjects (n=11) were also enrolled in the study as a control group. They found that there was a statistically significant difference in lower vitamin A levels among LL patients versus controls [8]. This is consistent with what is now known about vitamin A deficiency, which is associated with a suppression of the Th1 immune response. The authors from this study suggest that the inclusion of dietary supplements for leprosy patients could be beneficial for treatment and recovery of patients. However, this study seems to indicate that having a diet that is higher in vitamin A could prevent infection of leprosy or polarization to the more infectious lepromatous form.

Additionally, vitamin D has been shown to be influential in both the innate and adaptive immune response in humans [9]. Macrophages of the innate immune system utilize vitamin D dependent antimicrobial responses to kill intracellular microbes suggesting that vitamin D levels in patients with leprosy could determine the antimicrobial response to infection with *M. leprae* bacteria [10]. In the study conducted by Kim et al., the status of vitamin D in the activity of macrophages during *M. leprae* infection was observed. Their data show that during the cytokine IL-15 macrophage differentiation throughout an infection with *M. leprae* (over the course of 24-120 hours) there is a contribution to the vitamin D dependent antimicrobial response against *M. leprae* [10]. The vitamin D present in the immune system and throughout the process of macrophage differentiation aid in the antimicrobial response to *M. leprae* infection. Mandal et al. examined patients in Kolkata, India (30 cases and 15 healthy controls) where blood samples were collected to measure vitamin D levels and pro-inflammatory cytokines [11]. In their results they found that individuals with complex leprosy reactions at the MB end of the spectrum had lower levels of vitamin D versus healthy controls.

Poverty Related Risk Factors and Leprosy

In Bangladesh, a study of newly diagnosed patients with leprosy (n=52) and controls (n=100) were interviewed about their socioeconomic status, health and their diet. Food insecurity that was measured by the Household Food Insecurity Access Scale found that patients with leprosy suffered more food insecurity than controls ($p=0.003$) [3]. In addition, food shortage at any time in life and within the past year was recorded in the questionnaire given to patients and controls and patients were significantly more likely to have reported food shortage ($p=0.03$). In regard to socioeconomic factors, this study found that both religion and household size were significantly related to leprosy [3]. With an increase in food shortage, this study concluded that patients with leprosy have a low intake of highly nutritious foods that may then lead to the clinical manifestations of *M. leprae* infection.

A larger, case-control study conducted in four municipalities in the State of Ceará, Brazil used a questionnaire to collect demographic, socioeconomic, environmental and behavioral data from cases (n=226) and controls (n=857) [4]. Kerr-Pontes et al. found that low education level, experience of food shortage at any time in life, bathing in open water bodies and low frequency of changing bed linens were significantly associated with leprosy infection. The authors conclude that, like the study in Bangladesh, that food shortage leads to nutritional deficiencies in patients of leprosy which weakens the immune system in fighting against *M. leprae* infection.

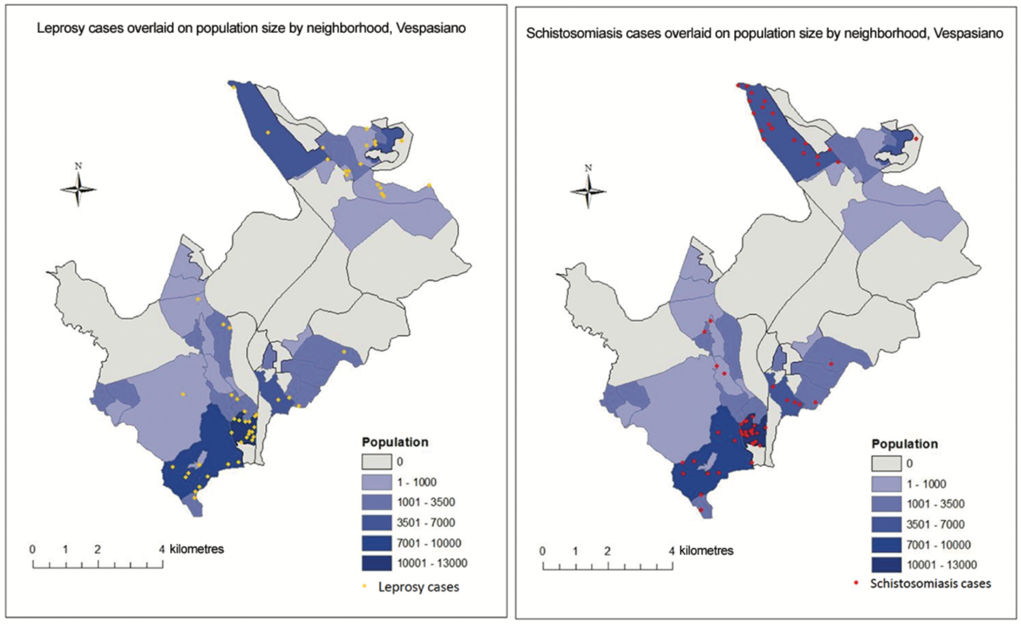
Oktaria et al. conducted a similar case-control study in Indonesia investigated the association between poverty-related diet and nutrition and leprosy. This study utilized a household-survey to examine demographic, socioeconomic status, health and diet in cases (n=100) and controls (n=200). Additionally, this study collected serum samples to obtain iron and anemia micronutrient levels. Oktaria et al. found that unstable income and higher household food insecurity were significantly associated with risk of leprosy. This study did not find a significant association between those with leprosy and iron deficiency, although, lower dietary diversity, food shortage, low serum iron and high ferritin were more common amongst those with leprosy [2]. Ultimately this study concluded that micronutrient deficiencies and their association with leprosy infections needs to be further investigated along with other factors that could be linking poverty to continued leprosy transmission.

Helminths and Leprosy

In addition to little data being found on the impact of nutritional diseases and leprosy, there are little data on co-infections of leprosy and helminths, especially schistosomiasis. A cross-sectional study in northeastern Brazil demonstrated that there was an association between soil-transmitted helminths and a shift towards the lepromatous end of the spectrum of leprosy infection ($p<0.001$) [12]. Additionally, Oktaria et al. demonstrated in their cross-sectional study in Indonesia that soil-transmitted helminths have a role in the progression towards type 2 reaction, particularly in those with MB infection [13]. Diniz et al. recruited 105 leprosy patients without treatment and 146 healthy household contacts. Between leprosy patients and household controls, leprosy patients were more likely to have at least one intestinal helminth when compared to contacts ($p=0.046$). Th1 immunity seems to be downregulated in patients co-infected with leprosy and helminth infections compared to individuals with only *M. leprae* infections [12]. In a cohort study in Nepal, Hagge et al. found that 55% of leprosy patients recruited for the study were positive for more than one soil-transmitted helminth infection [14]. These studies implicate that co-infection with helminths, like schistosomiasis, may facilitate *M. leprae* infection and the progression of its more severe forms.

Additionally, a study in 2017 utilizing Geographic Information System (GIS) found a spatial and temporal overlap with schistosomiasis and leprosy infections in Minas Gerais, Brazil. The study used municipality data surrounding Vespasiano, Minas Gerais which is an area endemic to both schistosomiasis and leprosy. A total of 139 leprosy cases and 200 schistosomiasis cases were identified in the surrounding municipalities with significant overlap between both infections [15]. This particular study also concluded that the relative risk of leprosy infection in a neighborhood with schistosomiasis was roughly 6.80 (95% CI 1.46-31.64) [15].

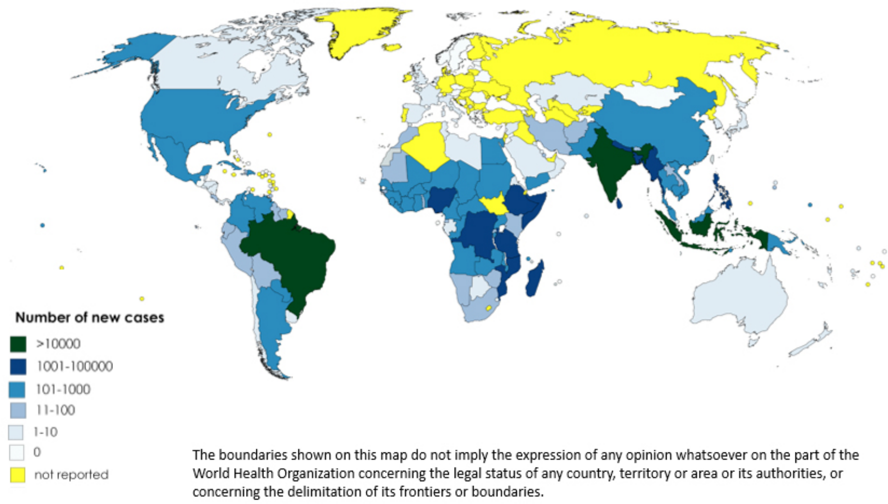
Figure 2: Cases of leprosy and schistosomiasis (2007-2014) per neighborhood in Vespasiano, Minas Gerais [15]



Study Relevance

Leprosy remains a public health issue in many parts of the world. Multidrug therapy is available to all countries through the World Health Organization, but control strategies have not continued to move forward. In 2017, 210,671 new cases were reported globally with India, Indonesia and Brazil having the highest burden of disease [16]. Brazil is known to have "hyperendemic" pockets throughout the country with > 40 new cases/100,000 persons per year [17]. Many factors like weak surveillance programs, stigma and lack of funding have caused efforts for control to be halted.

<u>Figure 3:</u> Geographical distribution of new leprosy cases reported in 2017 [16].



After observing studies and literature surrounding potential factors for continued transmission of leprosy, there appears to be a complicated web of factors that could be influencing transmission. Poverty-related factors in different endemic areas of leprosy have been conducted and show associations between leprosy and food shortage which is supported by literature on undernutrition and its impacts on the immune system. Micronutrient deficiencies could be driving continued transmission, however, co-infection with helminths related to poverty could also be driving transmission by propagating a sustained reservoir of infection in the community, especially if helminth co-infections are related to MB disease. These studies show that further investigation and research into the potential modes of transmission in endemic areas of leprosy need to be addressed and considered in conjunction with one another. Limitations present in all studies appear to be small numbers, since leprosy is often underdiagnosed and is overall still a rare infection in most places. There are still many unknown answers to the transmission of leprosy including factors associated with host susceptibility like socioeconomics and micronutrient deficiencies which need to be considered in further research.

Chapter 3: Manuscript

Abstract

Background: Although cases of leprosy have decreased over the past 20 years, there are still many obstacles to control the infection, especially in areas of hyperendemicity such as in northeast Minas Gerais (MG), Brazil. Studies have shown that leprosy, as well as helminth infections, are associated with poverty. We hypothesized that in areas with higher rates of leprosy, helminth infections and micronutrient deficiencies are risk factors for continued leprosy transmission due to effects on the immune response leading to acquisition and spread of *Mycobacterium leprae*. **Methods:** This hypothesis was examined through a case-control study in Minas Gerais, Brazil. Participants were enrolled either as newly diagnosed cases or as one of two types of controls: household contacts and negative controls. Data were collected through surveys, clinical data, and stool specimens for helminth diagnosis and blood for micronutrient analyses. Analysis was conducted through univariate analysis, Chi-square testing, adjusted odds ratios and ultimately logistic regression controlling for socioeconomic status and micronutrient status. **Results:** This study recruited 75 cases and 148 controls. Schistosomiasis infection was found in 11.8% of cases and 5.2% of controls. When looking at micronutrient deficiencies, 21.7% of cases and 10.4% of controls had vitamin D deficiency. Multibacillary infection was the prominent classification identified (73.3%) with the majority having no disabilities associated with infection (60.0%). The majority of cases in this study had at least primary education (71.4%) and lower income (92.0%). On multivariate analyses, cases were more likely to have schistosomiasis infection when compared to all controls (aOR = 3.60; 95% CI [1.06, 12.10]) and an even higher likelihood when compared to just household contacts (aOR = 6.56; 95% CI [1.16, 36.94]). When looking at micronutrient results, cases were more likely to have vitamin D deficiency compared to both control groups (aOR = 4.81; 95% CI [1.24, 18.72]). **Conclusions:** Through this case-control study we saw associations between schistosomiasis and leprosy and vitamin D deficiency and leprosy. These results show the importance and relationship between co-infections and micronutrient deficiencies in the continued transmission of leprosy infection in endemic areas. Additionally, these associations could lead to potential combined control efforts in endemic areas for both schistosomiasis and leprosy infections.

Introduction

Although cases of leprosy caused by infection of *Mycobacterium leprae*, have decreased over the past 20 years, there are still many obstacles to control the infection, especially in areas of hyperendemicity such as in northeast Minas Gerais (MG), Brazil. Studies have shown that leprosy, as well as helminth infections, are associated with poverty suggesting that there are poverty-related factors like poor living conditions that may be maintaining a reservoir for continued transmission of leprosy [1-4]. Leprosy has often been described as a disease of the poor, but the specific aspects of poverty that may be leading to continued transmission are unclear. A recent study in Bangladesh found that patients with leprosy have less money to spend on food and therefore have a less diverse diet [3]. Another study in Northern Brazil found associations between low education level and experience of food shortage associated with leprosy [4]. Interestingly, this same study found an association between having bathed in open water bodies within the past 10 years which could suggest that water is a potential risk factor for continued transmission [4]. These studies and others related to micronutrient deficiencies, helminth infections and leprosy suggest that certain socioeconomic risk factors for leprosy exist and further investigation into the aspects of poverty which could be influencing these risk factors needs to be conducted.

Methods

Study Region

Brazil has one of the largest burdens of leprosy in the world with several different areas of transmission. This particular study site in Governador Valadares in the state of Minas Gerais was chosen because it is an area that is endemic to both schistosomiasis and leprosy. This case control study was evaluated in Governador Valadares in people ages 3 and older served by the family health centers in different municipalities within the area (Santa Rita do Itueto, Itabirhinha de Mantena, Mendes Pimentel, and Cuparaque).

Recruitment

Cases were enrolled in rural clinics and recruited at a specialty clinic in the study area, CREDENPS. Patients considered to be cases were identified by a physician and referred to study staff for potential inclusion in the study. Contacts were enrolled based on diagnosis by physicians. In the rural community, cases and contacts were identified using contact tracing by inviting contacts of previous leprosy cases to the clinic to be examined by a trained dermatologist and enrolled. Cases were patients ages 3 and older with a new diagnosis of leprosy (within past 30 days) and no history of prior treatment for leprosy. Exclusion criteria for cases and controls included pregnant women and children less than 3 years old. Close contacts of patients with leprosy were enrolled as one of two control groups for this study. Inclusion criteria were age 3 years or older, no current or prior diagnosis of leprosy and being a close contact or a family member of a patient with leprosy enrolled in the study. When controls were examined by a physician they were examined to ensure they had no signs or symptoms of leprosy infection. The second control group included those with no prior contact with someone with leprosy. Those enrolled in this group were also 3 years of age or older and had no current diagnosis of leprosy. These controls were matched to cases by age (within 5 years older or younger) sex and community of resident.

Data collection

After enrollment into the study, the cases had additional data extracted from their medical records onto a case report form for each participant that provided their age, gender, place of residence, type of leprosy, clinical signs and symptoms and any treatment provided. A questionnaire for all participants was administered by a member of the study team to gather information on the participants' socioeconomic status, education, occupation, place of residence (including places where water is taken from and where patients are more likely to be in contact with water), history of helminth infections and treatment of prior infections as well as a full food diary recording the types of food consumed within the last 24 hours. After answering the questionnaire, the participants were then measured for their weight and height and body mass index calculated (kg / m^2). Venipuncture for blood collection was done for each participant to evaluate micronutrient status (vitamin A, vitamin D, and iron), complete blood count (for anemia), and immunologic profiles. The amount of blood collected for both adults and children was collected based on the National Institute of Health (NIH) guidelines for "minimum risk" to participants. Following blood collection, the participants were given containers for stool collection. They were instructed to collect stool on 3 consecutive days after their enrollment. These samples were sent to a microbiology laboratory at the Universidade Federal de Juiz de Fora for evaluation. Helminth infection was diagnosed by the presence of eggs being present in any of the stool samples collected over the course of three-day collection.

Thresholds for micronutrient variables were determined before data analysis. Ferritin at a level of 15 mg / dL or below was considered iron deficient. Anemia measured by haemoglobin levels was determined as 11 mg / dL or below for children under the age of 5, 11.5 and under for ages 5-11, 12 for ages 12-14, 12 and below for women over the age of 15 and 13 and below for men ages 15 or older [18]. Vitamin A deficiency was determined by measure of retinol units where a patient was deficient at <10 mg / dL. For every newly diagnosed case of leprosy that was enrolled into the study, a skin smear was performed to determine the bacillary load of infection. This was used to correlate to the correct clinicopathologic type of leprosy.

Data Analysis

The statistical analyses for this study was a combination of univariate analysis and multivariate analysis. The prevalence of helminth infections in newly diagnosed patients with leprosy along with healthy controls was shown through Chi-square testing, adjusted odds ratios and ultimately logistic regression controlling for socioeconomic status and micronutrient status. In addition to this analysis, a similar one was calculated to compare the prevalence of helminth infections and micronutrient deficiencies in multibacillary and paucibacillary cases to understand associations between pathologic type of leprosy and the presence of helminth infections or micronutrient deficiencies. Analyses were also calculated on data collected from the questionnaire to determine associations between potential risk factors of infection and transmission. To compare logistic regression models, the Akaike information criterion (AIC) was used since it is an estimator of the relative quality of statistical models for a given set of data. All statistical analyses were performed in SAS version 9.4 and an alpha of 0.05 was used to determine statistical significance for all calculations.

Ethical Considerations

This case control study was approved by the institutional review boards of Emory University and Universidade Federal de Juiz de Fora. All participants or parents of participants gave written informed consent and children ages 7 and up gave written assent.

Results

The total study sample included 75 cases and 148 total controls. Most of the cases that were enrolled in the study presented with multibacillary infection versus paucibacillary (n= 55, 73.3%). When comparing socioeconomic factors like income and education, the majority of cases had a primary education (grades 1-9) (n = 55, 71.4%) and lower income status (n= 39, 52.0%). Around 50 percent of cases and controls were of mixed race and 16 percent of newly diagnosed cases were under the age of 15 (Table 1).

Table 1: Demographic information and select laboratory results from cases and all controls.

Variable	Cases (n=75)	Controls (n=148)	P-Value (Chi-Square)
Age	Mean = 43.5 years	Mean = 38.4 years	0.20
Under age 15, n (%)	12 (16.0)	31 (21.0)	0.38
Sex, n (%) Male	41 (54.7)	68 (45.9)	0.08
Race/Ethnicity, n (%)			
White	13 (17.3)	33 (21.4)	0.03
Black	12 (16.0)	24 (15.6)	
Asian Descent	0 (0.0)	3 (1.9)	
Mixed	37 (49.3)	82 (53.2)	
Indigenous	1 (1.3)	0 (0.0)	
Education, n(%)			
Higher Education	1 (1.3)	9 (6.1)	0.50
Secondary	10 (13.0)	29 (19.6)	
Primary	55 (71.4)	94 (63.5)	
None	9 (11.7)	14 (9.5)	
Family Income⁺, n (%)			
<1	30 (40.0)	50 (33.8)	0.40
1 to 3	39 (52.0)	90 (60.8)	
3 to 5	4 (5.3)	6 (4.1)	
>5	1 (1.3)	0 (0.0)	
Leprosy Classification, n (%)			
Multibacillary*	55 (73.3)	N/A	N/A
Paucibacillary**	20 (26.7)		
Disability, n (%)			
No disability	45 (60.0)	N/A	N/A
Grade 1 +	29 (38.7)		
Grade 2 ++	1 (1.3)		

[+]Categories of monthly income determined by Brazilian minimum wage with <1 being below minimum wage and >5 more than 5 times minimum wage

*Multibacillary classification includes borderline (≥5 lesions) and lepromatous cases

**Paucibacillary classification includes intermediate, tuberculoid and borderline (<5 lesions)

+Disability grade 1 defined as eye problems with vision not severely affected (can count fingers up to 6 meters)

++Disability grade 2 defined as severe visual impairment (cannot count fingers up to 6 meters)

Table 2: Univariate analysis of schistosomiasis and micronutrient deficiency results between cases and all controls.

Variable	Cases (n=75)	Controls (n=148)	OR	95 % CI	P-Value (Chi-Square)
Schistosomiasis, n (%)	8 (11.8)	7 (5.2)	2.44	(0.84, 7.04)	0.09
Vitamin A, n (%)	0 (0.0)	0 (0.0)	-	-	-
Vitamin D, n (%)	15 (21.7)	15 (10.4)	2.39	(1.09, 5.23)	0.03
Anemia, n (%)	15 (10.1)	12 (16.9)	1.82	(0.80, 4.12)	0.15
Iron, n (%)	3 (4.4)	4 (2.9)	1.56	(0.34, 7.16)	0.57

The nutritional deficiency that showed the highest burden among cases was vitamin D deficiency (21.7%). Among controls, 10.4% had vitamin D deficiency. Interestingly, the controls in this study had a higher burden of anemia than leprosy cases (16.9% vs. 10.1%, respectively). Other soil transmitted helminths, like *Ascaris lumbricoides*, *Trichuris trichiura and Strongyloides stercolis*, were included in the collection of stool samples, but only one case with *A. lumbricoides* infection was found within a negative control participant in the study. The predominate soil-transmitted helminth infection from this study was identified to be schistosomiasis, making it the soil-transmitted helminth examined in regard to analysis. Schistosomiasis infection identified from stool sample analysis was found in 11.8% of cases and 5.2% of controls. There were no cases of vitamin A deficiency among cases or any controls.

When performing multivariate analysis between cases and both control groups as well as between cases and contacts, schistosomiasis and age were associated with leprosy infection (Tables 3-5). Cases were more likely to have schistosomiasis infection than contacts (aOR= 6.56; 95% CI [1.16, 36.94]). Micronutrient deficiencies were not shown to have an association with leprosy infection in the presence of other variables except for vitamin D deficiency between cases and negative controls (aOR= 4.81; 95% CI [1.24, 18.72]). The overall AIC measure was 225.13 when all variables were included in the model between cases and all contacts. When comparing the model between cases and contacts, the overall model fit with all variables included had a model fit measure of 166.05. The model between cases and negative controls with all variables included had a model fit measure of 153.00. As variables were removed from the models, the overall model fit increased significantly showing that the best model fit for all regression models were when all variables were included.

Table 3: Multivariate analysis between cases and all controls with all variables included.

Variable	aOR	95% CI
Schistosomiasis	**3.60**	**1.06, 12.10**
Age (in years)	**1.02**	**1.00, 1.04**
Income*	2.48	0.46, 13.42
Iron Deficiency	1.95	0.38, 9.88
Anemia	2.22	0.84, 5.88
Vitamin D Deficiency	2.03	0.83, 5.00
Primary Education (1-9)	0.48	0.19, 1.26

*Low Income Level (<1 times the minimum wage)

Table 4: Multivariate analysis between cases and contacts with all variables included in model.

Variable	aOR	95% CI
Schistosomiasis	**6.56**	**1.16, 36.94**
Age (in years)	**1.03**	**1.01, 1.05**
Income*	1.28	0.22, 7.37
Iron Deficiency	0.94	0.18, 5.03
Anemia	1.99	0.63, 6.28
Vitamin D Deficiency	1.23	0.45, 3.36
Primary Education (1-9)	0.46	0.16, 1.35

*Low Income level (<1 times the minimum wage)

Table 5: Multivariate analysis between cases and negative controls with all variables included in model (Iron Deficiency and Income removed due to undefined values).

Variable	aOR	95% CI
Schistosomiasis	2.43	0.59, 10.04
Age (in years)	1.00	0.98, 1.03
Anemia	2.70	0.76, 9.62
Vitamin D Deficiency	**4.81**	**1.24, 18.72**
Primary Education (1-9)	0.48	0.14, 1.60

Discussion

When looking at potential transmission factors of leprosy in an endemic area of Brazil, there appears to be a distinct association with schistosomiasis co-infections between cases and all control groups (aOR = 3.60; 95% CI [1.06, 12.01]). Interestingly, there was an even stronger association of schistosomiasis co-infection when comparing cases to contacts (aOR = 6.56; 95% CI [1.16, 36.94]). These statistical associations between leprosy infection and schistosomiasis suggests that co-infection between the two diseases is apparent in this endemic area, and could be a continued risk factor for infection of leprosy in individuals. This provides support to previous spatial and temporal analysis by Phillips et al. that indicated that these two infections were occurring in the same areas at similar times [15]. The association between the two infections suggests that patients infected with leprosy may have an altered immune response which may alter the susceptibility to clinical presentation of the other infection, which has been seen in the case of soil-transmitted helminths [12]. Therefore, in areas where schistosomiasis is endemic, it is a potential risk factor for continued transmission of leprosy infection where leprosy is also endemic. In the study by Diniz et al., there was a statistically significant association between soil transmitted helminth infections in leprosy patients when compared to healthy household controls (OR = 3.95; 95% CI [1.77, 8.83]) supporting that there is a potential relationship between leprosy and helminth infections in co-endemic areas [12]. Additionally, Diniz et al. measured the cytokine profiles of leprosy patients with and without intestinal helminths. Patients that had tuberculoid or lepromatous classification of leprosy that were co-infected with intestinal helminths produced significantly lower levels of INF-γ and IL-4 when compared to leprosy patients that were not co-infected [12]. The Th1 immune response is associated with the production of pro-inflammatory cytokines, like INF-γ, to fight off infections like intracellular parasites. Schistosomiasis infections have been shown extremely down-regulate cell-mediated immunity in mice models through a study conducted by Pearce et al. [19]. Co-infections with helminths and leprosy show a decrease in Th1 immune response (INF-γ) meaning that the immune response that would normally help to fight off both of these infections is not working efficiently [12, 20]. In a cohort study by Hagge et al., 55% of patients with leprosy infection were found to be positive for more than one type of soil transmitted helminth infection in an area of Nepal [14]. From this study and others mentioned, these two infections together in a patient could perpetuate the continued transmission of these infectious diseases within the community.

In this study, cases and controls predominately had a primary education as well as an income that is either under or slightly over the minimum wage level. In Indonesia, Oktaria et al. looked at associations between poverty-related diet and nutrition and leprosy infection. In this case-control study unstable income, anemia, and higher household food insecurity were all significantly associated with leprosy infection [2]. The highest association in this study was seen in those with unstable income where those with leprosy were almost five times more likely to have unstable income versus controls (aOR = 5.67; 95% CI [2.54-12.64]). Factors such as low income and low education could suggest that these areas are also lacking in micronutrient availability due to lack of knowledge or lack of access to micronutrients [10, 11]. Even though our particular study in Brazil did not find any cases or controls in the area with vitamin A deficiencies, the lack of these micronutrients in conjunction with certain socioeconomic factors could play a role in the continued transmission of leprosy in endemic areas.

When comparing models including micronutrient variables and select socioeconomic variables, the best fitting model was one with all variables included between cases and negative controls. In this model vitamin D deficiency was shown to be significantly associated with leprosy infection (aOR = 4.81; 95% CI [1.24, 18.72]). Bone deformities can occur in patients with leprosy and this low calcium absorption suggests that vitamin D deficiency could have a role in leprosy infection and manifestation [21]. When looking at the immunologic response to *M. leprae* infection, Kim at al. found that the presence of sufficient levels of vitamin D prior to infection reduced the viability of the pathogen in macrophages [10]. Additionally, Mandal et al. found that individuals with complex leprosy reactions have low levels of active vitamin D [11]. Vitamin D deficiency appears to have an effect on the way that leprosy manifests within patients infected by the bacterium. A main source of vitamin D comes from sunlight and in Brazil there is a high amount of sunlight suggesting that the lack of a sufficient amount of vitamin D in patients could be attributed to dietary and malnutrition factors [2]. As found in Kim et al., the immune response to leprosy infection when sufficient levels of vitamin D are occurring, there is a reduction in the viability of that pathogen to impact macrophages meaning that a diet rich in vitamin D could mediate the response to infection. From these previous studies and this new case-control study from Brazil, it can be seen that vitamin D has some sort of role in leprosy patients. These studies make it hard to determine whether or not vitamin D deficiency is the cause of increased transmission, making people more likely to get an infection, or if leprosy infection causes the immune system to absorb lower levels of vitamin D.

In this study region, there was a significant amount of childhood cases of leprosy (16.0%) suggesting that transmission is occurring at an even larger scale in this area than previously thought. *M. leprae* is slow growing and usually does not present symptoms until 5-10 years after transmission which is why rates of leprosy are more typically found in adults. A high rate of leprosy infection among children shows an active circulation of the bacterium [22]. Infections in children should especially be monitored and utilized within endemic areas such as Brazil to better control efforts and to further understand the potential risk factors for continued transmission of this bacterium in communities. In one particular community during the study period, 41 newly diagnosed cases of leprosy were recruited for the study over a two-year period. In addition to the burden of childhood cases, the recruitment of the cases in this study through contact tracing also shows the high burden of undiagnosed cases of leprosy in this already hyperendemic area.

The findings from this study suggest that schistosomiasis infections play a role in continued leprosy transmission and that certain micronutrient deficiencies, like vitamin D deficiency, could also play a role in continued transmission of the disease, or be a consequence of infection. Although there was no statistical significance in any of the models with socioeconomic factors such as income and education status, these factors could tie into the vitamin D deficiency that is being seen among cases versus negative controls. A lack of education and income could cause patients of leprosy to suffer from vitamin D deficiency compared to negative controls. Further investigation into the associations between micronutrient deficiencies and socioeconomic factors in the area studied should be investigated in future studies.

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<u>Chapter 4: Implications and Recommendations</u>

Even with its deep history, there are still many unknowns on the continued transmission of leprosy around the world. This slow growing bacterium continues to infect individuals of all ages and there is a need for increased awareness and research into the continued transmission. As previously mentioned, there is not a lot of research regarding continued transmission factors for leprosy infection in the current endemic areas of the world. Select studies have shown that there is an association between poverty and leprosy infection, but the exact associations remain unclear. This particular case control study adds to the current body of literature that investigates the potential risk factors for leprosy transmission and may explain in part poverty-related associations. This study helps to increase the body of knowledge on potential associations between parasitic worm infections and leprosy as well as micronutrient deficiencies and leprosy. Future studies and continued research in leprosy transmission factors should include case control studies as well as cohorts with large enough sample sizes to further investigate associations between certain risk factors like poverty and micronutrient deficiencies and leprosy infection.

The effects of leprosy on the human immune system are intricate and still being researched today. With it being impossible to grow the bacterium in a laboratory setting, research is difficult to conduct on an immunological scale. Even though culturing the bacterium is impossible to do in a laboratory setting, continuing to study and further understand the effects of leprosy infection on the immune system would help to determine whether or not leprosy infection suppresses the immune system and makes it more susceptible to schistosomiasis co-infection, or vice versa.

In reviewing the literature on leprosy infections in endemic areas of the world as well as the results from this particular case control study, it is apparent that leprosy transmission is still occurring and that there is a need for continued research. With the associations seen between schistosomiasis infection and leprosy, control efforts in both of these infections combined could decrease the burden of both diseases and would be a cost-effective way to address these infections. Research into the transmission factors of leprosy with larger studies would help to determine ways in which to better diagnose patients through early detection mechanisms and ways in which contacts of cases could be less at risk for contracting infection of the bacterium. Recently, in 2016, the WHO released the Global Leprosy Strategy for 2016-2020 in an effort to control leprosy transmission and to lower rates of disabilities. One of the key interventions listed for this strategy mentions the detection of leprosy infection among higher risk groups through campaigns or control strategies [23]. Larger case-control studies in hyperendemic areas where both schistosomiasis and leprosy infection are occurring could help to address how integrated control efforts in both of these diseases could decrease the rates of transmission and burden of disease.

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