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2011

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Abstract

Exploring poor water, sanitation, and hygiene (WASH) as factors related to leprosy transmission in Minas Gerais, Brazil

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Background: While leprosy is associated with poverty, it is not completely clear which factors, such as substandard and crowded housing conditions, unsafe drinking water, poor sanitation, or limited access to health care are driving this association. Given data suggesting that water and contaminated environments could be reservoirs for *Mycobaterium leprae* and that co-infections with water, sanitation, and hygiene (WASH)-associated helminths may increase susceptibility to leprosy, we aimed to explore the association of WASH factors, socioeconomic status (SES), and the residential environment with leprosy. **Methods:** A case-control study was conducted in the municipalities of Governador Valadares, Minas Gerais, Brazil, between June 2016-December 2018. Individuals ages three years or older were recruited as cases or community-matched controls. Cases were diagnosed by dermatologic experts with confirmatory skin slit smears for the bacillary index. Questionnaires were administered on socioeconomic status, education, occupation, and WASH factors. Descriptive and statistical analyses were conducted to identify WASH associations with leprosy and helminth infections in residential environments. **Results:** Seventy-nine cases of leprosy, 96 household contacts, and 81 controls (non-household contacts) were recruited. 51.5% were female with a mean age of 40 years. 75.2% (n=112) of the participants had piped water as their water source, and 54.5% (n=85) acknowledged they did not treat water. Multivariate logistic regression revealed an association with larger household sizes with leprosy (aOR = 1.34; 95% CI 1.07, 1.68), and an unexpected positive association with the presence of a piped sewer system (aOR=4.67; 95% CI 1.51, 14.45). In a contrast, those with leprosy were less likely to have household plumbing (versus a well or unimproved sources) (aOR=0.39; 95% CI 0.13, 1.18) or to treat their water (aOR=0.52; 95% CI 0.25, 1.06), although these did not reach statistical significance. 6.6% of individuals had positive stool exams for *Schistosoma mansoni*, 29.9% were positive for schistosoma antibodies, and 19.9% for strongyloides antibodies. The residential environment was not associated with leprosy and strongyloides; however, schistosoma was found to be associated with rural dwellers, 41.9% of those dwelling in rural community had schistosoma antibodies (p<0.001). **Conclusion:** These associations suggest that WASH factors could be related to leprosy and supports other emerging research in this field. Still, there is a need for further research on the association of WASH and leprosy disease, more specifically the potential mechanisms of bacterium exposure and viability of *M. leprae* in the environment.

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CHAPTER I: INTRODUCTION

Introduction and Rationale

Leprosy, also known as Hansen's Disease, a neglected tropical disease (NTD), still occurs in more than 120 countries, with over 200,000 new cases reported annually [1]. Neglected tropical diseases, including leprosy, tend to cluster in substandard housing areas, unsafe drinking water, poor sanitation, and limited or no access to health care [2].

Leprosy is a chronic, debilitating infectious disease caused by the bacteria, *Mycobacterium leprae*. This intracellular bacillus destroys Schwann cells, causing their death, and leading to severe neuropathies that causes deformities and physical disabilities [3]. It is an old disease that continues to be of significant public health concern in many developing countries. Some risk factors for the transmission and development of the disease include poor housing conditions lacking proper sanitation and communal spaces [3]. Socio-economic disparities in developing countries, exacerbated by poor housing conditions, low levels of education, low income, gaps in health care, and migration to urban centers, are critical obstacles to eliminating leprosy [3].

Several studies also show an association with poor water, sanitation, and hygiene (WASH) access and leprosy [3-5]. In the Brazilian state of Ceará, a study found that leprosy is related to higher levels of poverty and unregulated urbanization [4]. A survey of five municipalities in Ceará, Northeastern Brazil, identified a positive correlation between the residences of leprosy cases and water sources containing the bacterium [5]. Therefore, understanding the social determinants that affect the risk of leprosy transmission is of fundamental importance for the implementation of actions and strategies aimed at accelerating the disease elimination process in Brazil [3]. Also, WASH-related infections travel together, and one may affect the transmission of the other; therefore, it is essential to investigate these coinfections with leprosy disease.

Problem Statement

The reported global point prevalence of leprosy was 177,175 in 2019, with a corresponding prevalence rate of 22.7 per million. Two general developments that may have exerted downward pressure on leprosy incidence are improving living standards and implementation of multi-drug therapy in the 1990s [7]. Globally, the decline in the number of new cases has been steady and consistent over the last ten years. However, Brazil, India, and Indonesia reported over 10,000 new cases each [1]. India, Brazil, Indonesia, Nepal, Myanmar, Madagascar, and Mozambique account for almost ninety percent of leprosy cases worldwide. Eighty percent of all leprosy cases in the Americas occur in Brazil [4]. The WHO launched the Global Leprosy Strategy tagged "Accelerating towards a leprosy-free world," which aims to promote early diagnosis of leprosy and provide prompt care to avoid disability and minimize community-based disease transmission. This strategy seeks to reduce leprosy prevalence by improving the health service's capacity to detect cases at the early stages of the disease, provide timely curative treatment, and eradicate sources of infection [3]. Although studies have underscored the numerous ways poverty creates conditions that perpetuate leprosy risk, these findings call attention to the knowledge gaps in the associations between leprosy and socio-economic risk markers [8]. Several studies found that WASH factors, including poor water access/source, are associated with leprosy transmission; however, the extent of the association was not determined due to the small sample size [4-5, 9].

This study aims to understand better the effects of water, socio-economic status, and residential area on leprosy transmission in patients from Minas Gerais, Brazil. Through this, not only can more research focus on WASH as a factor in transmission, but policy recommendations can also target the Brazilian healthcare system to better support and assist patients with leprosy. These findings could be used to build health care services that are more inclusive and benefit patients from low-socioeconomic and marginalized communities.

Purpose Statement

This research will enhance the understanding of patients/individuals with leprosy in Brazil and recognize factors that can be targeted within the healthcare system to improve their health, treatment, and access. More precisely, this study would increase knowledge of how leprosy transmission can be influenced by WASH factors, socio-economic status, and in the setting of schistosoma co-infection, the residential environment (urban vs rural). The analysis of the source of water and the role of socioeconomics among individuals with leprosy who resides in Brazil and its relation to the stigmatized disease will be beneficial, given the large population, social ties, and significant economic diversity of the country.

Research Questions/Hypothesis

Research question one: What are the associations between access to safe water and sanitation, socio-economic status, and leprosy?

Hypothesis one: Individuals with low socio-economic status and insecure access to water are more likely to have leprosy disease.

Research question two: Are coinfections (leprosy and schistosomiasis) more common in specific residential locations, such as urban vs rural locale, and can these data better inform targeted control measures?

Hypothesis two: Rural locations are associated with coinfection and may present a unique area for targeted interventions.

Significance Statement

With leprosy still plaguing developing countries, the role of water supply and socio-economic conditions in transmission has not been a focus of much research but must be taken into consideration. The ability to consider shortfalls and challenges in leprosy-affected persons' experiences can give clinicians and public health officials great insights into where and how changes are needed.

The WHO has called for targeted diagnosis and intervention among higher-risk groups within endemic countries to decrease infection rates and prevent new leprosy-associated grade 2 disabilities (G2Ds) [8]. Delayed diagnosis can lead to severe adverse effects, such as increased risk of nerve damage. Various factors lead to delay in diagnosis, but stigma is an essential characteristic in many cultures [10]. There is evidence that increasing age, food insecurity, lower education levels, poor sanitary and socio-economic conditions are associated with a higher risk of leprosy [8].

Definition of terms

Prevalence: The proportion of a particular population affected by a specific disease at a particular time.

Incidence: The occurrence of new cases of a disease in a population over a specified period.

CHAPTER II: LITERATURE REVIEW

Overview

Neglected tropical diseases (NTDs) are a group of infectious diseases defined by the World Health Organization (WHO) that are present worldwide in 149 countries [11]. Over one billion individuals are infected with NTDs, which contain many different diseases, including but not limited to soil-transmitted helminths, schistosomiasis, and leprosy. These diseases are prevalent in low and middle-income countries (LMICs), with the most significant disease burden in disadvantaged communities [11].

In 2007, the "Global Plan to Combat Neglected Tropical Diseases 2008-2015" was released by WHO, which outlines aims to combat, reduce, eliminate, and eradicate diseases based on World Health Assembly resolutions and regional offices and is the first initiative of the WHO to battle NTDs [12]. In 2012, the WHO published Accelerating Work to Overcome the Global Impact of Neglected Tropical Diseases: An Action Roadmap that sets out NTD strategies and 2012-2020 goals for prevention, monitoring, elimination, or eradication of each of the NTDs [13]. To achieve these goals, WHO recommends five methods, including preventive chemotherapy; improved management of diseases; vector and intermediate host control; veterinary public health at the human-animal interface; and access to safe water, sanitation, and hygiene provision (WASH). For each of the NTDs, this roadmap described matched the WHO recommendations for treatment and prevention [9,13].

Leprosy

Leprosy is considered one of the earliest human endemic diseases. It is a chronic infectious disease caused by *Mycobacterium leprae* [14]. The WHO defines a leprosy case as the person showing one or many of the following symptoms: hypopigmentation or reddish skin lesions with sensation loss; peripheral nerve damage, measured by loss of sensation and hand, feet, or facial mobility; positive skin smear tests [15].

Despite all attempts by the WHO, leprosy transmission continues. In total, 60.2% of notified cases of leprosy are multibacillary (characterized by numerous infiltrated skin lesions displaying high bacillary loads, impaired peripheral nerves, and possible involvement of internal organs) [14]. The exact transmission route for *M. leprae* has yet to be clearly understood in humans, but infection with a droplet from the nasal mucosa is presumed. Prolonged contact with an infected person with a high bacterial load is a predisposing factor for the transmission of *M. leprae* [14].

Characteristics of Leprosy

The WHO describes leprosy diagnosis as one or more of the clinical signs of infection: (i) definite loss of sensation in a light (hypopigmented) or reddish skin area, (ii) thickened or swollen peripheral nerve with loss of sensation or (iii) presence of acid-fast bacilli in a slit-skin smear. Since early leprosy and paucibacillary leprosy clinical diagnosis can be a challenge, various serological and other laboratory tests have been developed to support clinical diagnostic methods [16]. Leprosy is associated with disability from nerve damage especially when diagnosis is delayed or from complications called leprosy reactions. WHO has graded leprosy-associated disability and uses it as a measure of disease burden (Table 1)

Table 1: The WHO Leprosy Disability Grading System [17]

Disability Grading	Hands and feet	Eyes
0	No anesthesia, no visible deformity or damage	No eye problem due to leprosy; no evidence of visual loss
1	Loss of sensation (anesthesia) present in the hands or feet, but no visible deformity or damage	Eye problems due to leprosy present, but vision not severely affected as a result (vision: 6/60 or better; can count fingers at 6 meters)
2	Visible deformity or damage present and complications such as claw deformities and bone resorption in the extremities	Severe visual impairment (vision worse than 6/60; inability to count fingers at 6 meters); also includes lagophthalmos, iridocyclitis, and corneal opacities

Schistosomiasis

Schistosomiasis is a parasitic NTD caused mainly by *Schistosoma mansoni*, *S. haematobium*, and *S. japonicum*, and less often, *S. mekongi* and *S. intercalatum*. Schistosomiasis is transmitted to polluted water by the release of cercariae from aquatic freshwater snails. An individual becomes infected by the cercariae through skin contact with contaminated water, usually by swimming, bathing, wading, and washing [18]. Schistosomiasis is a significant infectious disease of poverty, with more than 700 million people living in endemic areas worldwide at risk [19]. With occasional reports in the Arabian Peninsula, *S. mansoni* is found commonly in sub-Saharan Africa and some South American countries (Brazil, Venezuela, Suriname). *S. hematobium* is present in the Middle

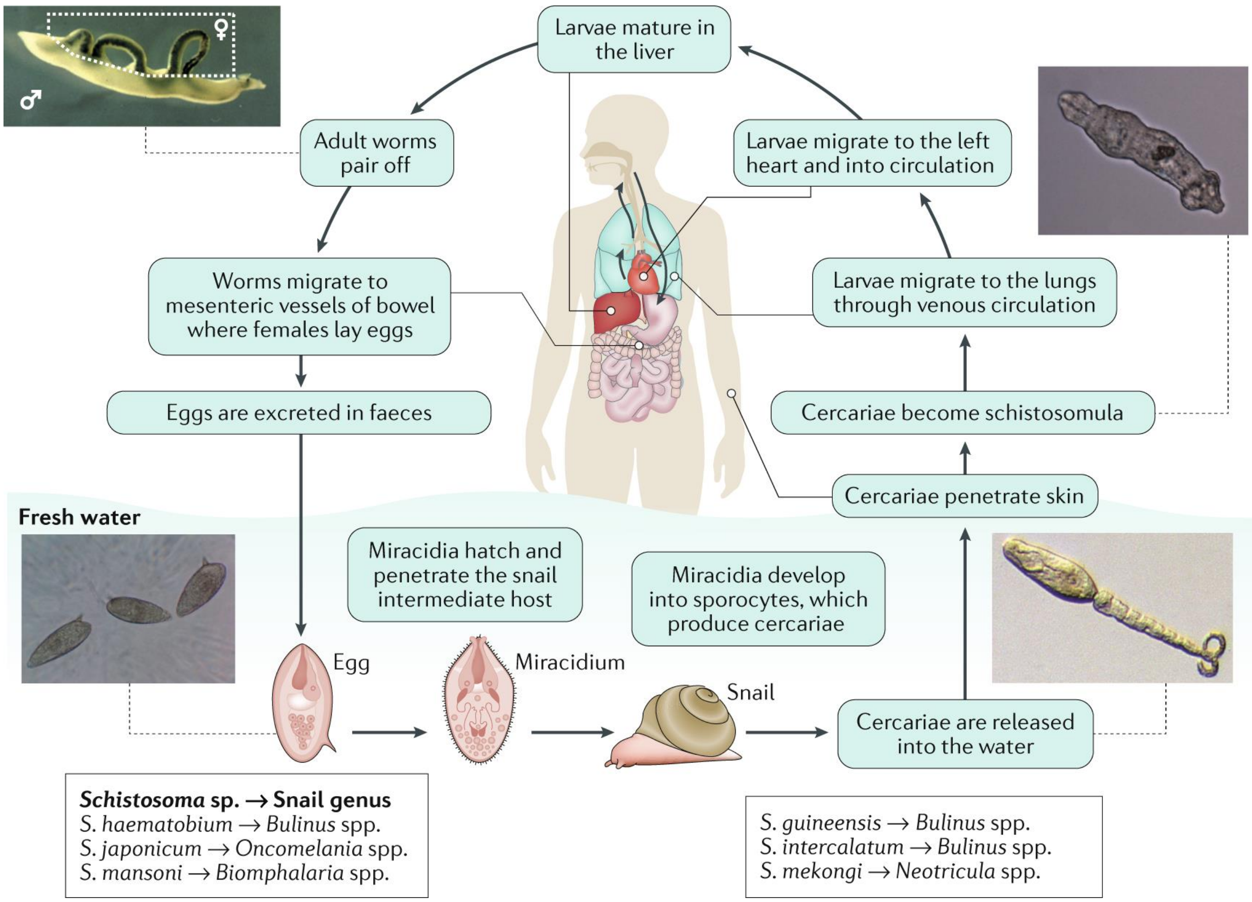
East and Africa. *S. japonicum* is found in China, Sulawesi, and the Philippines. It has long been eliminated from Japan despite its name [20-21].

Characteristics of Schistosomiasis

Schistosomiasis signs and symptoms are not caused by the worms themselves but rather by the body's response to the larvae and eggs. Some infections are asymptomatic. After skin penetration by cercariae, a local cutaneous hypersensitivity reaction can occur with small, itchy maculopapular lesions. Acute schistosomiasis is a systemic hypersensitivity reaction that may occur weeks after the initial infection, especially with *S. mansoni* and *S. japonicum*. Systemic symptoms/signs, including fever, cough, stomach pain, diarrhea, hepatosplenomegaly, and eosinophilia, can also occur [21].

Schistosoma infections can sometimes lead to lesions in the central nervous system. Persistent infection can cause granulomatous reactions and fibrosis with associated signs/symptoms in the affected organs (e.g., liver and spleen). Pathology of *S. mansoni* and *S. japonicum* involves numerous hepatic complications in the brain or spinal cord due to inflammation and granulomatous reactions and rare embolic egg granulomas. In the brain or spinal cord, pathology of haematobium schistosomiasis includes hematuria, scarring, calcification, squamous cell carcinoma, and occasional embolic egg granulomas [21].

Figure 1: Lifecycle of Schistosome exemplified by *Schistosoma mansoni* [22]



Leprosy and Schistosomiasis in Brazil

In 2012, the prevalence rate of leprosy in Brazil was 1.5 cases per 10 000 inhabitants [6] but now declining with a total of 25,218 cases reported in 2016, representing 12% of global cases and 92% of Latin American cases [23]. The highest prevalence of the disease is in the areas of the North, Northeast, and Central West. In this context, identifying high-endemicity regions is also an essential tool for tracking and evaluating control measures' efficacy at the national level [23,24].

The Brazilian Ministry of Health (MoH) has developed various control strategies to reduce the burden of Leprosy in the country. Within the domain of the National Unified Health System (Sistema Único de Saúde [SUS]), the Brazilian guidelines for leprosy monitoring and elimination describe interventions focused on primary health care. Primary health care is characterized as being responsible for the diagnosis, treatment, prevention of disabilities, and surveillance. Simultaneously, cases with complex clinical presentations, such as leprosy reactions, children younger than 15 years of age, and relapses are referred to specialized secondary clinics and tertiary care [23]. To create a global plan, the WHO, working closely with the Regional Office for South-East Asia, released the "Global Leprosy Strategy 2016-2020, accelerating towards a leprosy-free world". This document's main objectives include zero leprosy cases in <15-year-olds with G2D, reducing new cases with G2D to <1 case per million population, and having no country with legislation allowing for discrimination on the grounds of leprosy status [9,24]. In Brazil, leprosy's geographical distribution is heterogeneous with the persistence of regions with various levels of endemicity. Higher detection rates are observed predominantly in areas with socio-economic deprivation [8].

A study in Brazil indicates that unplanned and unregulated urbanization raises social disparity by excluding people from social and material resources, rendering them vulnerable to several diseases, including leprosy [25]. In addition to poverty, it is believed that social deprivation and segregation are an obstacle to the enjoyment of citizenship and social policies – particularly those relating to education and health – making it difficult to access the services necessary to ensure good health [6].

In terms of schistosomiasis, Brazil has the largest endemic region in the South Americas, accounting for 95% of *S. mansoni* infections [26]. As a signatory of the World Health Assembly

Resolution WHA65.21 of May 2012 on the elimination of schistosomiasis [27], the MoH is committed to achieving the priorities of the Strategic Plan 2012-2020 of the WHO [26]. These aims are to reduce the prevalence of heavy-intensity infections, that is, the percentage of positive feces with 400 or more eggs per gram (EPG) to less than 5% by 2020 (morbidity control) and less than 1% by 2025 (elimination of schistosomiasis as a public health problem) [26].

The MoH guidelines for morbidity control depend primarily on early detection and early treatment of infection vectors rather than mass drug administration, given Brazil's epidemiological peculiarities and public health policies [26]. Moreover, to reduce schistosomiasis transmission, the MoH considers it necessary that preventive measures, including health education, environmental sanitation, and community mobilization, be introduced [26]. Despite the developments of the Schistosomiasis Surveillance and Control Program (PCE) of the MoH, the target set by the WHO for morbidity regulation was not achieved because data from the Schistosomiasis Control Program Information System (SISPCE) for 2013-2014 suggest that more than 5 percent of positives from 165 municipalities surveyed had heavy-intensity infections (EPG >= 400), and prevalence surpassed 5 percent in 144 (26.8 percent) of cities, disqualifying them from advancing under the PCE guidelines from the monitoring to the surveillance stage [26].

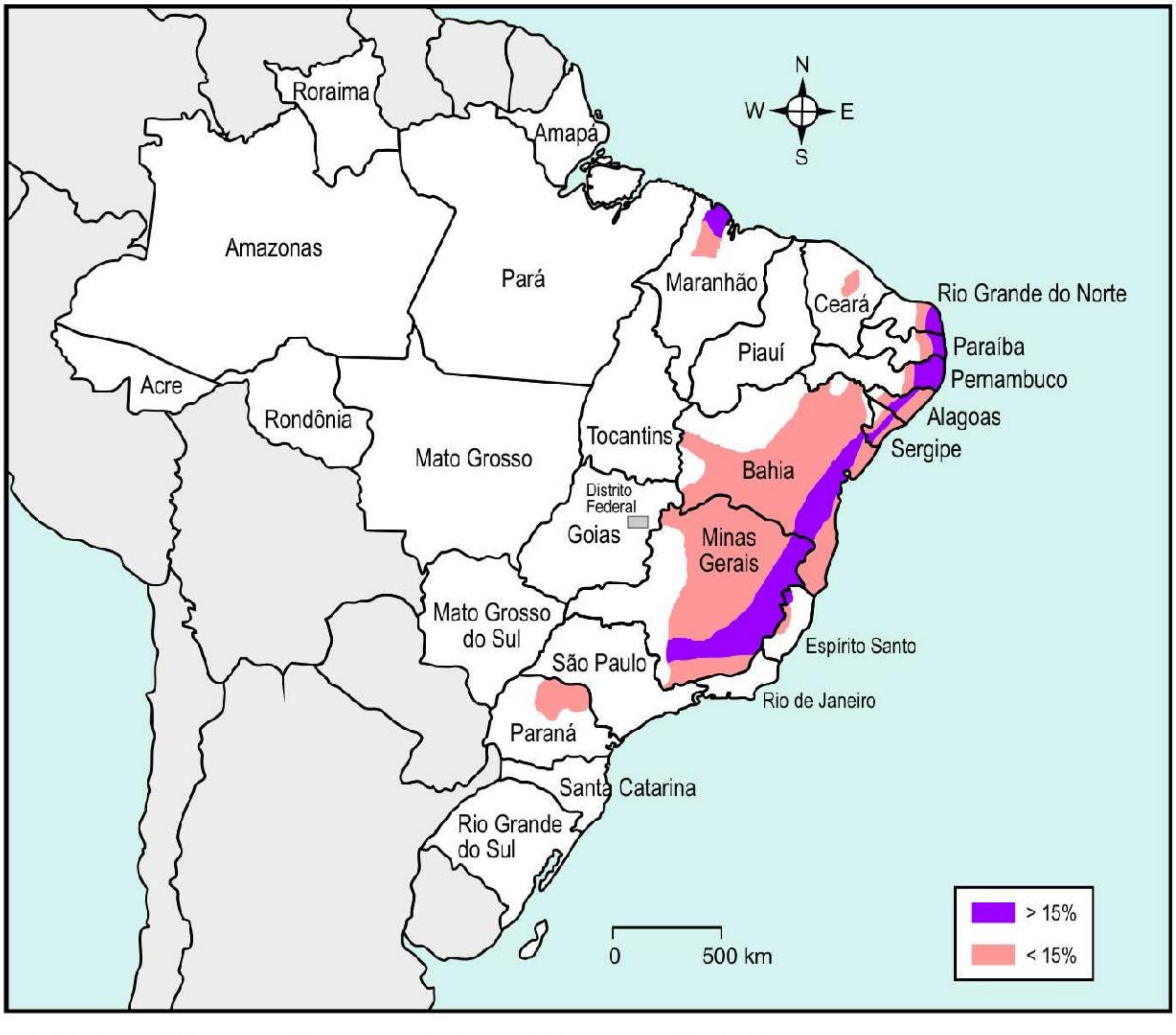
The key focus for the WHO guidelines is school-age children (6-15 years of age) since they account for the highest prevalence and severity of the infection. The management of schistosomiasis is also correlated with changes in risk behavior; thus, one way to develop awareness and thereby encourage preventive practices and behaviors in communities is health education and the implementation of educational activities into schools in endemic municipalities [26]

Current Epidemiology of *Schistosoma mansoni* in Brazil

The national control program (PCE) has focused on schistosomiasis to identify and treat infected people for more than 20 years. Over the years, the prevalence, morbidity, and mortality have decreased. However, significant cases and fatalities continue to occur [28]. The most affected areas, particularly in the Northeast and Southeast regions, are characterized by poor sanitary conditions, poverty, and low education levels. It is estimated that approximately 2.5 to 6 million people are infected (5 to 10% may develop life-threatening forms), and 25 million people living in endemic areas are at risk of infection [29].

Schistosomiasis mansoni has historically been endemic in Brazil. Its geographical distribution, however, is not homogeneous in Brazilian states, not even in endemic cities. There are 27 states in the country, and schistosomiasis is present in 19 states, where the disease is endemic in 9 states. The disease transmission is focal and linked directly to the presence of intermediate snail hosts. There is a mortality of 0.3 deaths per 100 000 population [28-30]. Increased infection rates are directly linked to the disorderly migration trends that have led many people, especially those from the north-eastern states to the south and south-eastern regions, to create new infection sources in areas that have not yet been affected. Fig 2 represents the nineteen states of Brazil with the transmission of *S. mansoni*. The North-eastern states have the highest prevalence levels alongside the States of Bahia and Minas Gerais, with Minas Gerais accounting for about 70% of the disease's endemic areas. The presence of the intermediate hosts in these regions has enabled the infection's maintenance [31,32]. The lack of infection control could also be linked to the flawed sanitation system, the lack of previous treatment, and the re-infection process [32].

Figure 2: The states of Brazil with the transmission of *Schistosoma mansoni* infection [31].

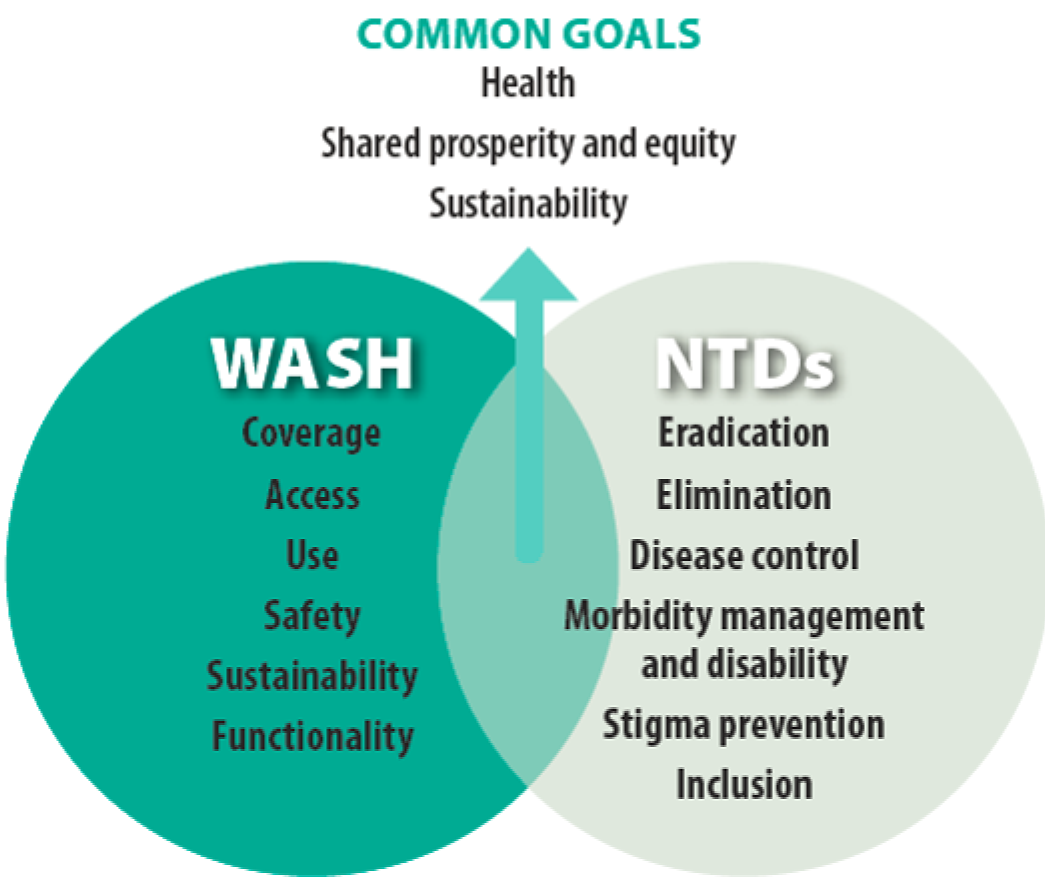


Role of WASH in Leprosy and Schistosomiasis

A primary intervention within the global NTD roadmap is safe water, sanitation, and hygiene (WASH). It is essential to prevent and provide treatment for all neglected tropical diseases, especially where transmission is closely related to poor WASH conditions like schistosomiasis [2].

Approximately one-third of the world's population (2.4 billion) lacks access to proper sanitation, with 1 billion people practicing open defecation and 663 million lacking access to improved drinking water sources. The poorest and most challenging people to reach need to be focused on to achieve equal access to safe water and adequate sanitation facilities. Sometimes, these are the same classes most affected by NTDs [2].

Figure 3: Common goals for WASH and NTDs collaboration [2]



While the cause of leprosy is known to be a slow-growing bacillus (*Mycobacterium leprae*), the mode of transmission has not been identified; thus, there is no established WASH-related primary prevention strategy. Improved WASH conditions can make communities and individuals less vulnerable to leprosy, as WASH leads to more hygienic conditions, improved well-being, and a better immune status [2]. Leprosy can cause permanent damage to the skin, nerves, limbs, and eyes. The resulting disabilities can make tasks difficult, such as carrying water over a stretch. Wound management by self-care using clean water is required to accelerate wound healing and minimize disability. People with leprosy may be subject to stigma and exclusion from society and may be excluded from water and sanitation services. Restricted access to water and sanitation can lead to poor cleanliness and treatment, contribute to isolation and exclusion; therefore, the role of WASH is to ensure water availability for facility-based treatment and self-care and put in measures to avoid exclusion from water and sanitation facilities based on stigma [2,33].

Access to safe water supplies was found to be associated with significantly less infection with Schistosoma infection, and adequate sanitation was found to be associated with significantly reduced odds of infection with both *S. mansoni* and *S. hematobium*, therefore, improving access to clean water and proper sanitation practices are necessary measures to control schistosomiasis [2,34]. People are infected during farming, domestic, occupational, and recreational activities that expose them to infested water. The lack of hygiene and certain playing habits of school-aged children, such as swimming or fishing in infested water, make them particularly susceptible to infection [20]. Several studies have suggested water and soil as reservoirs of infection for *M. leprae* [5,9,35-36]. A 2020 study by Emerson et al., done in Ethiopia found an association between WASH factors, including water source, open defecation, and soap access, with leprosy infection. It also suggests that schistosomiasis in highly endemic area may be associated with leprosy in regions nearer to the lake [9]. The role of WASH in schistosomiasis prevention is improved sanitation across the communities to avoid contaminated feces and urine from reaching surface water can minimize or eliminate transmission by preventing worm eggs in feces and urine from entering the water. Protecting the freshwater from animals/animal waste is essential because some schistosome species can be transmitted through animal feces or urine [2,33]. Water contact, and thus schistosome transmission, occurs typically outside the household (public exposure) and not inside the home (domestic exposure). The relation between water and sanitation can be assumed to be more strongly correlated with schistosome infection in the community rather than in the household; hence sanitation facilities across the communities should be encouraged [34].

WASH as a risk factor for Coinfections

In rural, impoverished, and disadvantaged communities, coinfections are more prevalent. The same populations with the least access to sustainable, sufficient, and affordable water and sanitation facilities are thus highly exposed to disease [2]. A systematic review and meta-analysis of water, sanitation, hygiene, and soil-borne helminth infection (STH) found WASH access and behaviors were correlated with 33–70% lower chances of STH infection; for example, after defecation, those who had washed their hands were less than half as likely to be infected as those who did not [2,37]. Another study suggests that the transmission of *M. leprae* may be affected by helminth coinfections, those with leprosy having higher rates of STH infection than household contacts [38]. And more recently from our own group, a 2021 study by Dennison et al., found significant association between active helminth infection and leprosy compared with household contacts, which were consistent with the findings from Diniz et al., [38-39]. With STH and schistosomiasis both being WASH pathogens, it would surmise that WASH factors could increase the risk of coinfection, which may increase the risk of leprosy through immune-mediated mechanisms, in addition to WASH being a possible direct reservoir of *M. leprae*. To prevent worm eggs from moving through the feces of infected persons from reaching the soil, food, or hands, prevention of open defecation, proper sanitation facilities, and feces management are essential. Acceptable hygiene practices such as handwashing with soap minimize transmission, and such initiatives are required beyond the household level [2].

Other risk factors for Leprosy and Schistosomiasis

Armadillos are natural reservoirs for *M. leprae* and contact with these animals is considered a risk factor for leprosy. The same genotype of *M. leprae* was found in armadillos and human leprosy cases in studies carried out in the south-eastern United States [39,40]. In the state of Ceará, armadillos are commonly found in nature; hunting these animals is a recreational activity, and their meat is widely eaten in the countryside [39]. Studies in Ceará, with animals caught at various locations, showed that 21% of the animals had *M. leprae* DNA, and another study in the state suggested that interaction with armadillos were a risk factor for transmission [39,40].

Another study in Ceara found that leprosy is associated with uncontrolled urbanization and frequent contact with water bodies [4]. Higher incidence rates were found in municipalities that have larger populations (over 50 000 inhabitants) and with lower socio-economic indicators (high rates of illiteracy, the high average number of dwellers per room, a high percentage of households with inadequate sanitation) and with greater social inequality [6].

There is also an influence of environmental and social conditions on the occurrence of schistosomiasis. Level of education, family income, contact with water, and the presence of the intermediate host snail are major risk factors associated with this infection, according to a systematic review and meta-analysis study in Brazil [41]. A significant correlation was identified between agricultural and fishing activities and *S. mansoni* prevalence in most rural communities [42]. Major risk factors in the prevalence of schistosomiasis among agricultural populations were rural living, poor living conditions, and low educational levels [42]. In Brazil, the economic burden of *S. mansoni* is high and results in the loss of productivity. It is a challenge to public health and requires inter-sectoral interventions in indoor water supply, basic sanitation, and education [28]. Thus, the geographic overlap of these two infections, combined with prevalence of poverty and likely insecure WASH, make studying these factors important for better understanding leprosy epidemiology and transmission.

CHAPTER III: MANUSCRIPT

Abstract

Background: While leprosy is associated with poverty, it is not completely clear which factors, such as substandard and crowded housing conditions, unsafe drinking water, poor sanitation, or limited access to health care are driving this association. Given data suggesting that water and contaminated environments could be reservoirs for *Mycobaterium leprae* and that co-infections with water, sanitation, and hygiene (WASH)-associated helminths may increase susceptibility to leprosy, we aimed to explore the association of WASH factors, socioeconomic status (SES), and the residential environment with leprosy. **Methods:** A case-control study was conducted in the municipalities of Governador Valadares, Minas Gerais, Brazil, between June 2016-December 2018. Individuals ages three years or older were recruited as cases or community-matched controls. Cases were diagnosed by dermatologic experts with confirmatory skin slit smears for the bacillary index. Questionnaires were administered on socioeconomic status, education, occupation, and WASH factors. Descriptive and statistical analyses were conducted to identify WASH associations with leprosy and helminth infections in residential environments. **Results:** Seventy-nine cases of leprosy, 96 household contacts, and 81 controls (non-household contacts) were recruited. 51.5% were female with a mean age of 40 years. 75.2% (n=112) of the participants had piped water as their water source, and 54.5% (n=85) acknowledged they did not treat water. Multivariate logistic regression revealed an association with larger household sizes with leprosy (aOR = 1.34; 95% CI 1.07, 1.68), and an unexpected positive association with the presence of a piped sewer system (aOR=4.67; 95% CI 1.51, 14.45). In a contrast, those with leprosy were less likely to have household plumbing (versus a well or unimproved sources) (aOR=0.39; 95% CI 0.13, 1.18) or to treat their water (aOR=0.52; 95% CI 0.25, 1.06), although these did not reach statistical significance. 6.6% of individuals had positive stool exams for *Schistosoma mansoni*, 29.9% were positive for Schistosoma antibodies, and 19.9% for strongyloides antibodies. The residential environment was not associated with leprosy and strongyloides; however, schistosoma was found to be associated with rural dwellers, 41.9% of those dwelling in rural community had schistosoma antibodies ($p<0.001$). **Conclusion:** These associations suggest that WASH factors could be related to leprosy and supports other emerging research in this field. Still, there is a need for further research on the association of WASH and leprosy disease, more specifically the potential mechanisms of bacterium exposure and viability of *M. leprae* in the environment.

Introduction

The control of leprosy, a neglected tropical disease (NTD) caused by Mycobacterium leprae, continues to present many obstacles. With India and Brazil recording most cases, more than 200,000 new cases are reported annually [43]. With > 40 new cases/100,000 persons per year, Brazil has persistent, high levels of leprosy occurrence, including eastern Minas Gerais in the southeast of the country [44]. Leprosy is associated with poverty, conditions of poverty like poor access to safe water, sanitation, and hygiene, can drive transmission of leprosy [4,45]. Considering their effects on the immune system, helminth coinfections can also be a variable, and this has been supported by the literature and our prior work [38,46].

Various studies have shown a significant association between the incidence of leprosy and socio-economic factors: poor living conditions and aggregation of people in households, sharing of homes with leprosy cases and experienced food shortages in the past, as well as poverty, indicating that improved socio-economic conditions could lead to reducing the incidence of the disease [4,6,8].

In controlling leprosy, socio-economic trends, including adequate measures to improve housing, sanitation, and education, often need to be addressed in addition to proper treatment when dealing with cases [6]. These studies suggest a potential mode of transmission through the environment, and thus improvement in WASH may lead to reduced leprosy transmission.

Eastern Minas Gerais is strongly endemic to schistosomiasis and leprosy and has significant rural and urban poverty [47]. A 2017 study found a spatial and temporal correlation between co-endemic schistosomiasis and leprosy in Minas Gerais, Brazil [48]. Another study in Northern Brazil found a correlation between bathing in open water bodies over the past ten years, suggesting that water is a possible risk factor for continued transmission [4]. In Brazil's co-endemic region, an association of active overlapping schistosomiasis and leprosy was reported [9]. Environmental factors and susceptibility to poor WASH conditions are associated with several NTDs, including schistosomiasis [2].

This study investigated the association of water source and socio-economic status with leprosy transmission through a case-control study. Further, it explored schistosomiasis leprosy coinfections in specific residential locations (urban vs. rural) in Minas Gerais, Brazil. We hypothesized that individuals with low socio-economic status and lack of access to water are more likely to have leprosy disease, and certain residential locations (urban vs. rural locale) are associated with coinfection.

Methods

Study population and Design

Brazil has one of the highest burdens of leprosy in the world, with multiple regions of transmission. Between June 2016-December 2018, there was a case-control study in the municipalities of Governador Valadares and Mantenha in eastern Minas Gerais, Brazil. This study site was chosen because it is an area that is endemic to both schistosomiasis and leprosy. The population recruited in this study were adults and children aged three years or older who were living in Minas Gerais. The population was divided into three categories:

1. Newly diagnosed, untreated leprosy cases enrolled either at a leprosy specialty clinic in Governador Valadares or through contact tracing of previously known leprosy cases in rural communities outside of the city.
2. Close contacts of cases were enrolled as one of the two control groups and defined as family members who had lived with the index case or who lived nearby and had regular weekly contact with the case for at least the past year.
3. The second control group included those with no prior contact with someone with leprosy and were matched by sex, age (within five years older or younger), and community of residence.

Socioeconomic and demographic exposures included educational level, monthly income, and household conditions (household size, water supply, and sewer system). Dermatologists with expertise in leprosy confirmed cases; skin slit smears for bacillary index were done on all cases.

Cases were classified as indeterminate, tuberculoid, borderline, or lepromatous based on the Madrid Classification [49]. All controls were clinically evaluated to ensure they had no signs or symptoms of leprosy infection. Pregnant women were excluded from this study.

Data Collection

Demographic and clinical data were extracted from medical records and a clinical exam. A detailed questionnaire was administered on socio-economic status, education, occupation, and WASH factors. Questions on WASH were adapted from standardized JMP questions from the joint WHO-UNICEF project [50]. Antibody reactivity to *S. mansoni* and *Strongyloides species* was used as a marker of co-infection with these two common helminth infections to assess active, recent, and prior infections in order to capture the overall exposure history of the patient to these helminths. Serum samples were drawn from participants and shipped to the Centers for Disease Control and Prevention, Atlanta, GA, where they were run on the Luminex multiplexed beaded platform for total IgG reactivity to antigens to the following antigens: *S. mansoni* egg antigen (SEA) and *Strongyloides stercoralis* (NIE). Results were reported as the median fluorescence intensity (MFI) minus background (MFI-BG), with positive values as those that were 3x higher than those of non-endemic controls. Active helminth infection measured by Kato Katz stool exams was also assessed and results reported previously [39].

Statistical Analysis

Because household contacts would have the same or very similar WASH settings, two sets of analyses were done: cases vs. non-household contacts (for WASH) and cases vs. all controls (for urban/rural exposure vs coinfection). Descriptive and univariate statistics were conducted through frequency and chi-square testing. A series of chi-square tests of associations between various potential risk factors and the subject's leprosy status were conducted to test bivariate associations. After that, factors for which any significant association with leprosy status was confirmed, as well as factors the inclusion of which to a multivariate model was justified theoretically, were included to a series of binary logistic regression models, which allowed to test the effects or risk factors simultaneously, while controlling for some of potential confounding factors (ceteris paribus). Multinominal logistic regression was used to compare the number of people in the family with leprosy to the cases and household contacts (using the controls as the reference). The presence of schistosoma infection in patients with leprosy was compared to the presence in controls using multivariate logistic regression, schistosoma antibodies were also compared to geographical location. The resulting odds ratios obtained for risk factors from the logistic regressions were thus adjusted for other explanatory variables.

All statistical analyses were performed in IBM SPSS software version 26 using an alpha of 0.05 to determine statistical significance.

Ethical considerations

The 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 six and up gave written assent.

Results

The total study sample included 79 cases, 96 household contacts, and 81 non-contact controls with 132 females (51.5%), 122 males (47.7%), and 2 missing (0.8%). Of the leprosy cases, 26.5% were females, and 35.3% males. The mean age for cases, household contacts, and non-contact controls was 40, ranging from 5 to 85 years old. 71.3% (112) had fully piped sewer compared to 28.7% (45) that had less developed sewer systems. Of the leprosy cases, 75.3% (58) had piped sewer than 24.7% (19) without a piped sewer system. The water source was dichotomized as running, municipal-water and non-running water; 75.2% (112) had running water as their water source, while 24.8% (37) had other water source types. Seventy-one (45.5%) acknowledged treating their water compared to 54.5% (85) who did not; however, only 37.2% (29) of leprosy cases treated water while 62.8% (49) of the cases did not (p=0.037). Comparing socioeconomic status like income and education, income was dichotomized into less than 1x minimum wage and >=1x minimum wage. With Brazilian minimum wage <1 being below minimum wage, 39% (30) of the cases survived on less than 1x minimum wage. Education was compared between grade 1-8 and completing grade 9 and above, most of the cases had more than primary education (completed grade 9 or above) (n=67, 85.9%). While only 16 (6.6%) individuals had positive stool exams for *Schistosoma mansoni*, 29.9% (n=66) were positive for schistosoma antibodies and 19.9% (44) for *Strongyloides antibodies*. Demographic information is described in Table 1.

Table 1. Description and analyses of demographic information and select variables in cases, household contacts, and non-contact controls

		Total		Classification					
				Leprosy case		Household contact control		Non-household contact control	
Variable		Count	Column N %	Count	Column N %	Count	Column N %	Count	Column N %
S. mansoni based on stool exam	Negative	225	93.4%	67	89.3%	86	97.7%	72	92.3%
	Positive	16	6.6%	8	10.7%	2	2.3%	6	7.7%
Residence	Urban	108	43.5%	36	49.3%	37	38.5%	35	44.3%
	Rural	140	56.5%	37	50.7%	59	61.5%	44	55.7%
Household size	1.00	10	4.0%	2	2.6%	1	1.1%	7	8.8%
	2.00	60	23.8%	20	26.0%	13	13.7%	27	33.8%
	3.00	52	20.6%	15	19.5%	23	24.2%	14	17.5%
	4.00	63	25.0%	17	22.1%	29	30.5%	17	21.3%
	5.00	39	15.5%	13	16.9%	15	15.8%	11	13.8%
	6.00	15	6.0%	5	6.5%	6	6.3%	4	5.0%
	7.00	11	4.4%	4	5.2%	7	7.4%	0	0.0%
	8.00	2	0.8%	1	1.3%	1	1.1%	0	0.0%
Ever had a family member with leprosy	1.00	82	32.8%	23	30.3%	54	57.4%	5	6.3%
	2.00	11	4.4%	6	7.9%	5	5.3%	0	0.0%
	3.00	1	0.4%	0	0.0%	1	1.1%	0	0.0%
	>=4.00	3	1.2%	0	0.0%	3	3.2%	0	0.0%
	Does not apply	153	61.2%	47	61.8%	31	33.0%	75	93.8%
Treat water	Yes	113	45.2%	29	37.2%	42	44.7%	42	53.8%
Fresh water contact	Yes	64	25.6%	23	30.7%	22	23.2%	19	23.8%
Strongyloides Antibodies	Yes	44	19.9%	16	21.9%	18	21.7%	10	15.4%
Schistosoma Antibodies	Yes	66	29.9%	23	31.5%	25	30.1%	18	27.7%
Education	Incomplete/Complete fundamental (1-8)	67	26.6%	11	14.1%	31	32.6%	25	31.6%
	Completed >=9	185	73.4%	67	85.9%	64	67.4%	54	68.4%
Income>=1 minimum wage	Less than 1 minimum wage	80	31.9%	30	39.0%	25	26.3%	25	31.6%
	>=1 minimum wage	171	68.1%	47	61.0%	70	73.7%	54	68.4%
Piped sewer	Non-piped sewer	67	26.5%	19	24.7%	22	22.9%	26	32.5%
	Piped sewer	186	73.5%	58	75.3%	74	77.1%	54	67.5%
Water source (running water)	Non-running water	66	27.0%	20	27.0%	29	30.5%	17	22.7%
	Running water	178	73.0%	54	73.0%	66	69.5%	58	77.3%

In univariate analysis, fewer cases reported treating their water (OR= 0.51, p=0.037) which implies that those who treat water are less likely to have leprosy (Table 2). Other WASH factors on univariate analysis are outlined in Table 2.

Table 2. Univariate analyses between cases and non-household contacts of variables of SES, WASH factors, and Coinfections

Variable	Cases	Controls	Total n	OR	95% CI	P-value
Education	67 (55.4%)	54 (44.6%)	157	2.78	(1.32, 6.25)	0.009
Monthly income>=1 minimum wage	47 (46.5%)	54 (53.5%)	156	0.72	(0.37, 1.41)	0.339
Water source (running water)	54(48.2%)	58 (51.8%)	149	0.89	(0.45, 1.79)	0.743
Treat water	29 (40.8%)	42 (59.2%)	156	**0.51**	**(0.27, 0.96)**	0.037
Contact with water (water activity)	23 (54.8%)	19 (45.2%)	155	1.43	(0.70, 2.86)	0.333
Piped sewer	58 (51.8%)	54 (48.2%)	157	1.47	(0.73, 2.94)	0.278
Stool exam	8 (57.1%)	6 (42.9%)	153	1.43	(0.47, 4.35)	0.524
Schistosoma Antibodies	23 (56.1%)	18 (43.9%)	138	1.20	(0.58, 2.50)	0.625
Strongyloides Antibodies	16 (61.5%)	10 (38.5%)	138	1.54	(0.65, 3.70)	0.327

There was no statistically significant association between antibody reactivity to *S. mansoni* or strongyloides and leprosy (Table 2), but interestingly, when looking at helminth co-infections (schistosoma and strongyloides), independent of leprosy, there was some evidence (at the 10% significance level) of an association between strongyloides antibodies and schistosoma antibodies (p=0.058).

In multivariate analyses, household size as a numeric variable was used to explain the probability of belonging to each group based on leprosy (categorical variable classification). Multinominal logistic regression was used to test this effect, and controls (non-household contacts) were used as the reference category. Increase in household size was associated with increase in odds of leprosy (aOR = 1.34; 95% CI 1.07, 1.68), and increase in odds of having contacted someone with leprosy in the family (OR = 1.52; 95% CI 1.22, 1.88).

Logistic regression was done, and multiple factors were adjusted simultaneously for education, income, water source, water treatment, sewer, and household size. Though not statistically significant, having piped, municipal water (aOR=0.39) and water treatment (aOR=0.52) decrease the odds of being in the leprosy case group (p<0.1), therefore they appeared protective of leprosy disease. The sanitation measure had an opposite point estimate, showing that presence of a household sewer systems was associated with cases versus controls (Table 3).

The model was also run for schistosoma - strongyloides coinfection using leprosy case vs control group. There is no evidence of interaction effects of schistosoma or strongyloides antibodies on group membership and adding household size and education as control variables did not change the significance of antibodies indicators (p=0.914).

Table 3. Logistic regression of WASH and SES factors using cases vs non-contact controls

Variable	**aOR**	**Lower limit CI**	**Upper limit CI**	**P-value**
Education	2.89	1.21	6.92	0.017
Income>=1 minimum wage	0.82	0.38	1.76	0.612
Piped sewer	4.67	1.51	14.45	0.008
Water source (running water)	**0.39**	**0.13**	**1.18**	**0.096**
Treat water	**0.52**	**0.25**	**1.06**	**0.073**
Household size	**1.33**	**1.05**	**1.69**	**0.019**

Urban/rural as exposure was tested with leprosy cases, schistosoma based on stool exam, and positive schistosoma and strongyloides antibodies. Schistosoma based on stool exam and positive schistosoma antibodies were found to be significantly associated (at the 1% significant level) with the geographic zone (had p<0.001): 12.1% in rural areas compared to 1.5% in urban areas have schistosoma based on stool exams (OR=0.11, 95% CI 0.02, 0.50) while schistosoma antibodies was found in 41.9% among rural dwellers compared to 19.3% in urban dwellers (OR=0.33, 95% CI 0.18, 0.61), therefore the odds of having schistosomiasis is higher among rural dwellers.

Discussion

This study hypothesized that individuals with low socioeconomic status and insecure access to water are more likely to have leprosy disease. Leprosy has usually been associated with lower socioeconomic status. In most studies, high levels of education were associated with lower leprosy rates. An ecological study by Kerr et al found an association between low socioeconomic status in terms of low income and lower level of education and a high prevalence of leprosy. Ponnighaus et al also described an increased risk of leprosy is associated with lower level of education and low income in a study in Malawi [25,51]. Another study done in Egypt found the degree of education to be unlikely to increase the risk of leprosy [52]. Low income was found to be associated with increased leprosy risk in a Brazilian cohort study [53]. Our findings suggested a clear growth of leprosy incidence among cases who have completed at least grade 9 education, showing a positive but not statistically significant association for high education level (OR=2.89, 95% CI 1.21, 6.92) and no significant association with monthly income as well (p=0.612) Table 3. With low level of education usually from the lowest income stratum of the population, the lack of association between these socioeconomic markers could be related to late diagnosis because of unavailability and inaccessibility of healthcare facilities. Also, cases and controls were not perfectly matched by age, thus there could have been different education levels represented due to the presence of children in the study.

Water has been identified as a possible source and reservoir for *M. leprae* infection. The sources of water, water contact, and treatment of water before use have all been linked to being associated with the risk of leprosy infection by several studies. Matsuoka et al. had previously added evidence to this theory, showing that in Indonesia, leprosy prevalence among people who bathed, washed clothes, or did dishes in water contaminated with *M. leprae* was substantially higher than among people who used water free of *M. leprae*, thereby suggesting water treatment could reduce the risk of leprosy infection [4,54]. From our analysis, not treating water was found to be statistically correlated with leprosy cases upon unadjusted analysis. On adjusted analysis, water source and water treatment were associated with leprosy cases. With several studies finding an association between the water source and leprosy risk, only a few studies have reported a correlation between water treatment and an increase in leprosy risks. Emerson et al. found water source to be statistically correlated with leprosy cases upon unadjusted analysis; however, it did not find an association between water treatment and leprosy infection [9]. Although Pescarini et al. found no association between lack of access to clean water and leprosy, a study by Andrade and colleagues found an association between running water and a lower incidence of leprosy [8, 55]. A report on correlation analysis between household hygiene and sanitation by Prakoeswa et al. found a significant association between clean water facilities and female leprosy. Previous research conducted by Nurcahyati et al. also had a similar result reporting the relationship between water sources and leprosy incidence [56].

Our analysis showed a more improved sewer/household sanitation and nothing to explain this, with most studies suggesting otherwise. This finding should be further explored but given that most of the study sample had a traditional piped sewer, we may not have been able to detect a difference. Furthermore, perhaps just the presence of these systems did not mean that they had an improved or fully functioning system.

The risk of developing leprosy is most significant among household contacts of leprosy patients. Our analysis showed that an increase in household size and ever having a known family member with leprosy is strongly associated with an increase in the odds of developing the disease. Pescarini et al, and several other studies looked at the association between a leprosy patient's household contact to any other type of contact, a higher risk of leprosy with household contacts was established. Household exposure to leprosy cases is associated with increased leprosy risk [8,57-59]. While many studies only found an association with household contacts but not household size, a few studies saw a correlation between household size and an increase in leprosy transmission. Lockwood suggests that population growth may cause overcrowding, increasing the risk of leprosy transmission. Another study done in Malawi showed that living in a crowded household increases leprosy risk [51,60]. There was evidence of over-crowding significantly being associated with increased leprosy risk with another cohort study done in Indonesia [61]. Going through several studies and with the result from our analysis, household contacts and household size are important associations in the transmission of leprosy.

Schistosoma antibodies were found to be significantly associated with rural dwellers. Schistosomiasis is known to be more prevalent in rural communities due to poverty, lack of infrastructure/access to clean water, sanitation, and hygiene, and more water contact among rural dwellers. Several studies found *S.mansoni* prevalence was correlated with fishing activities and water contact in most rural communities [42-43]. A recent study in Ethiopia by Hussen and colleagues, prevalence was higher in rural areas than urban areas which is similar to previous findings from Kinshasha, Congo [62-63]. In contrast, a study by Kloos et al. surmised that despite generally improved conditions in urban than in rural areas, the prevalence of schistosomiasis in many Brazilian cities is moderately high as infected people migrate from endemic rural areas, transmission sites persist, and new snail habitats are established in an urban environment [25, 42].

While exploring the relationship between leprosy and helminth infections (schistosoma and strongyloides), it was observed that there was an association between the two helminth infections but not with leprosy when compared to non-household contacts. This was not much of a surprise because if they are exposed to one, they can be exposed to another. It is not uncommon for people to be coinfected with both in these regions. The helminth infections schistosomiasis and strongyloidiasis are endemic to parts of Africa, Asia, and Latin America. Previous epidemiological studies on helminth coinfections in humans indicate positive associations between schistosomes and soil-transmitted helminths. A mouse model showed that a primary *S. mansoni* infection led to a decrease in the recovery of strongyloides venezuelensis [64-65]. This present study is built on previous study from Dennison et. al using the same cohort, where an association was found between schistosoma infection on stool exam and leprosy when compared to *household contacts* who had similar socioeconomic status and living conditions [39]. The high prevalence of these antibodies in this group, the fact that they are WASH associated pathogens, and our WASH associations when compared to non-household contacts all demonstrate a complex dynamic related to SES and WASH that likely affect the risk of leprosy. This needs to be further studied given the other published papers on the topic.

Although these results suggest an association between WASH factors and leprosy cases, other transmission routes and factors should be considered. Our study faced key limitations, including overall small sample size and the observational nature of the study. It was not powered to identify differences in specific age groups, so generalizing these results to all ages may be difficult. Furthermore, the two control groups were distinct, with one having close contact with cases and the other not. Thus, they may have other unmeasured confounders that could explain their differences compared to the variables. For instance, the household contacts may have had similar exposure to *M. leprae* infection in the household, thus more susceptible to leprosy than the non-contact controls.

Conclusion

In conclusion, our study found that socioeconomic factors, WASH factors, including water source, water treatment, increase in household size, and household contacts, are related to leprosy infection. Schistosomiasis antibodies, however, were found to be significantly associated with urban dwellers. While exploring the relationship between leprosy and history of schistosoma and strongyloides between cases and non-household contacts, an association was found between the two helminth infections but not leprosy. Overall, this study supports the hypothesis that WASH factors are associated with leprosy transmission, and there is a need for increased research and further investigation. Further investigations with larger sample sizes will be required to determine the extent of the association between leprosy and WASH factors.

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CHAPTER IV: PUBLIC HEALTH IMPLICATIONS AND RECOMMENDATIONS

Despite its long history, there are many unknowns about the transmission of leprosy. Leprosy continues to infect people of all ages, necessitating increased awareness and research. Several studies found association between socioeconomic status, WASH, and leprosy disease. This case control study explored the association between socioeconomic status, WASH factors, and leprosy disease, and co-infections with residential environment. Our study found that WASH factors including water source and water treatment are related to leprosy disease, while household size and the number of household members with leprosy are associated with leprosy disease. Leprosy and strongyloides were not associated with residential location, however, schistosomiasis was found to be associated with rural dwellers.

This study adds to the existing body of literature that investigates risk factors for leprosy transmission and may help to explain WASH associations. It also contributes to the growing body of information about WASH and leprosy, as well as residential location and schistosomiasis. Case control studies and cohorts with large sample sizes in hyperendemic areas should be considered in future studies and analysis on leprosy transmission to further explore associations between risk factors including socioeconomic status, WASH factors such as water source, water treatment, piped sewage, and leprosy disease.

In the future, it is hoped that, with WASH factors as an instrument for reducing transmission, this project will help inform strategies to eliminate leprosy disease. We hope that this study will not only inform future projects but will also serve as a reminder of the importance of adequate WASH access in preventing disease transmission to those working to control and eliminate leprosy.

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