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Megan Wasson Date

Susceptibility to Leprosy: An Examination of Nutrient Deficiencies, Parasitic Coinfection, and WASH Conditions

By

Megan Wasson
MPH

Hubert Department of Global Health

_____

Jessica K. Fairley, MD, MPH
Committee Chair

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By

Megan Wasson

Bachelor of Arts
Transylvania University
2018

Thesis Committee Chair: Jessica K. Fairley, MD, MPH

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in the Hubert Department of Global Health
2021

Abstract

Susceptibility to Leprosy: An Examination of Nutrient Deficiencies, Parasitic Coinfection, and WASH Conditions

By Megan Wasson

Background

Despite extensive control measures and a declining number of human reservoirs, continued incidence of leprosy in excess of 200,000 new infections each year suggests that alternative pathways may play a role in continued endemicity. Nutritional deficiencies, parasitic coinfection, and limited water, sanitation, and hygiene (WASH) have been suggested to predispose individuals to *M. leprae* infection and were further explored in this analysis.

Methods

Leprosy cases and uninfected controls were recruited from areas around North Gondar, Ethiopia throughout 2019. Participants completed dietary and WASH surveys in addition to providing stool for Kato Katz, urine for Schisto POC-CCA^{TM} rapid diagnostic testing, and blood for micronutrient biomarker testing. Multivariate logistic regression was performed to investigate associations between the above exposures and leprosy.

Results

A total of 80 men (59%) and women (41%) participated in this study with an average age of 40 (SD 15.0 years). Most leprosy cases were multibacillary (93.3%). There was a high prevalence of undernutrition among cases and controls, with 32.1% of participants classified as underweight. Food shortage [OR 4.57, 95% CI (1.62, 12.89)] and fewer meals consumed per day in the last four weeks [OR 3.85, 95% CI (1.17, 12.67)] were both significantly associated with leprosy in the univariate analysis. Additionally, 64.1% of the study population tested positive for a helminth and WASH insecurities were widespread. On multivariate analysis, lack of soap for handwashing [aOR 2.53, 95% CI (1.17, 5.47)] and lack of toilet facilities [aOR 2.32, 95% CI (1.05, 5.12)] were significantly associated with leprosy. Positive directionality was identified for a number of other inputs, including helminth infection [aOR 3.23, 95% CI (0.85, 12.35)].

Conclusions

Taken together, these findings strengthen previous research conducted in 2018 implicating WASH as a driver of leprosy infection. Subsequent micronutrient results will be integrated to further explore nutritional risks and build upon the significant macronutrient findings from this analysis. Given that leprosy remains the leading infectious cause of disability in the world, future research should explore all of the above susceptibilities in more depth to curtail the global burden of disease.

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Acknowledgements

I am immensely grateful to my mentor and advisor, Dr. Jessica Fairley, for her guidance throughout the development of this thesis project. I would also like to thank all of the Ethiopian collaborators, especially Dr. Annisa Befekadu Tesfaye, and student volunteers, Bridgete Miller and Cassidy Whitson, who assisted with data collection, entry, and dissemination. Their time and dedication to the study is sincerely appreciated. Finally, my ability to complete this analysis and the MPH degree as a whole would not have been possible without the advice, encouragement, and support of my friends and family to whom I owe an immense debt of gratitude.

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Chapter 1: Introduction

Neglected tropical diseases (NTDs) infect over 2.7 billion people worldwide, a disproportionate number of whom reside in low- and middle-income countries (LMICs) (1). Although there have been efforts at the national level to prevent and control NTDs in recent years, countries often lack sufficient resources to reduce the disease burden and populations unable to stretch $2 a day to seek treatment. The impact of these conditions reaches far beyond the financial sector to hinder childhood survival, educational attainment, and agricultural productivity to name just a few. Perhaps even more significantly, those who recover from acute symptoms often suffer lifelong chronic disability. In recognition of this injustice, the United Nations included effective treatment and management of NTDs in Goal 3 of the Sustainable Development Goals (SDGs) with the intention of meeting this benchmark by the year 2030 (2).With less than 10 years remaining, the prevalence of NTDs continues to exert a tremendous burden on individuals across the globe, threatening the health and safety of billions.

Leprosy, also known as Hansen's disease, is one such NTD that continues to wreak havoc in certain areas of the world. Archaeological evidence suggests that this bacterial agent has been active since antiquity, with biblical and other historical references lending support to this theory (3). Thousands of years later, the global incidence of leprosy remains unacceptably high. Though effective treatments have since been developed, the long-term consequences of infection chip away at the net gains that have been made (3).

Mycobacterium leprae, a rod-shaped bacillus and the causative agent of disease, is believed to be transmitted via droplets from the nose or mouth during sustained contact with an infected individual. Though naturally infected nine-banded armadillos have been identified as an animal reservoir, leprosy is considered a disease that primarily afflicts and is exchanged between

humans (4). Once infected, incubation of the pathogen lasts an average of 1-5 years but may extend up to 20 years in some cases (5). Symptom onset generally occurs as a result of pathogenic activity in peripheral nerves, skin, eyes, mucous membranes, bones, and testes. A special affinity for keratinocytes in the skin and Schwann cells in the peripheral nervous system gives rise to some of the more characteristic symptoms of disease including skin discoloration, nodules, lesions, sensory loss, disability, and deformity. Bacterial proliferation in these areas and the potential for subsequent systemic dissemination of *M. leprae* contributes to the development of other symptoms such as nosebleeds, non-healing ulcers on the bottoms of the feet, loss of eyebrows, burning sensation of the skin, and blindness (6). Attack on the Schwann cells in the peripheral nervous system and the subsequent demyelination of neurons is a key turning point in the disease course given the more permanent consequences of this progression. Targeted multidrug therapy prior to this stage is intended to prevent permanent deformity, disability, and social stigma in addition to being curative of the initial infection (3).

The World Health Organization (WHO) classifies the different types of leprosy into two categories based on physical symptoms and the presence or absence of bacilli in slit-smear tests. Paucibacillary (PB) leprosy is characterized by five or fewer skin patches and no bacilli on a slit-smear test while multibacillary (MB) leprosy applies to cases with greater than five skin patches and visible bacilli on a slit-smear test (5). However, research suggests this differentiation is somewhat arbitrary, with other classification strategies such the Ridley-Jopling system offering a more meaningful delineation based on the cell-mediated response to disease (3). This approach proposes the division of leprosy into five categories ranked according to immune response and disease severity.

Table 1. *Ridley-Jopling classification of the disease spectrum of leprosy.*

Ridley-Jopling Classification of Leprosy		
Tuberculoid (TT)	Greatest Immune Response	Least Severe Disease
Borderline Tuberculoid (BT)	↓	↑
Borderline Borderline (BB)		
Borderline Lepromatous (BL)		
Lepromatous (LL)	Least Immune Response	Most Severe Disease

Effective cellular immunity against tuberculoid (TT) leprosy is characterized by a T helper type 1 (Th1) response which allows for complete destruction of the pathogen and, in turn, less severe disease. In contrast, lepromatous leprosy (LL) is characterized by malfunctioning cytotoxic T cells, or a Th2 immune response, which allows the pathogen to survive and multiply as demonstrated by the presence of a high bacillary load in skin lesions (7). Interestingly, individuals with LL develop a strong humoral response that is ultimately ineffective in defending against the pathogen. Together, this failure of cellular and humoral immunity results in severe disease and higher transmissibility (8).

Depending on the degree of individual immune response, some individuals with leprosy present with mild symptoms. Others with borderline versions of the disease are at a higher risk of complications, or immune reactions, which ultimately affect around 30-50% of patients. These reactions typically occur within the first few months of treatment but can develop at any point in the disease course. The resulting inflammation is capable of causing extensive peripheral nerve damage manifesting in nerve degradation, deformity, and even injury to other organ systems (3).

Immune reactions are classified into two categories: type 1 reactions, or reversal reactions (RR), and type 2 reactions, or erythema nodosum leprosum reactions (ENL). Type 1 reactions are more frequent among those diagnosed with BT, BB, or BL forms of disease while Type 2 reactions are more typical of BL and LL forms. Type 1 reactions typically emerge within the first 6 months after the initiation of treatment causing pain in existing lesions, ulcers, tenderness, and nerve damage most likely as a result of the cell-mediated T-cell response. Type 2 reactions often emerge while on the treatment regimen but can develop at any time and continue, or recur, after the initial infection is cured. Characteristics symptoms include the development of new and painful subcutaneous nodules, fever, and malaise as well as inflammation of the nerves, lymph nodes, eyes, and extremities. Nerve damage in the course of this reaction is attributed to the production and deposition of antigen-antibody complexes (3).

Management of these symptoms often requires use of anti-inflammatories in addition to the standard MDT regimen. Corticosteroids, the anti-reaction drug of choice, may be administered in excess of 20 weeks for especially severe cases leading to downstream consequences in the form of diabetes, weight gain, facial swelling, and anxiety. Killing the *M. leprae* bacteria also does not guarantee the termination of these reactions nor the reversal of nerve degeneration acquired in the course of immune dysregulation (3).

These complications on top of the standard consequences of morbidity underscore the importance of prevention efforts to limit the spread of infection. Unfortunately, these efforts have been impeded by an incomplete understanding of the transmission pathways of disease in large part due to the inability to grow *M. leprae* in the lab independent of an animal host (9). It is generally agreed that droplets expelled from the mouth or nose are the primary means of *M. leprae* colonization, but many other potential mechanisms have been proposed. In the decades

since the development of MDT for leprosy, the overall prevalence of disease has decreased but incidence in endemic areas has persisted despite aggressive control efforts. As the number of human reservoirs declines, researchers have looked to alternative transmission pathways to understand this higher-than-expected rate of leprosy infection (10). The possibility of secondary mechanisms of exposure is substantiated by the inability to link the majority of new leprosy cases to a preexisting case or household contact (11). The uncertainty surrounding leprosy transmission and the possibility of alternative environmental reservoirs opens the door for consideration of potential risk factors that may facilitate infection.

Thus, the primary objective of this thesis project was to better understand host and environmental factors that increase the risk of leprosy infection through a secondary analysis of data collected from the North Gondar region of Ethiopia. Nutritional deficiencies, parasitic coinfection, and water, sanitation, and hygiene (WASH) conditions were collectively evaluated to determine if there were associations between these susceptibility factors and disease onset. As suggested by prior research, patients with leprosy were hypothesized to lack appropriate nourishment and WASH resources compared to healthy controls. Those with leprosy were also expected to have higher rates of parasitic infection.

Chapter 2: Review of the Literature

Nutrient Deficiency

For many decades, scientists have proposed a link between undernutrition and susceptibility to leprosy. However, the inability to distinguish undernutrition as the driver of leprosy infection or rather a byproduct of the disease continues to cast a shadow over the current understanding of this relationship. For example, a case study conducted among multibacillary patients in India found that undernutrition, defined by a body mass index (BMI) less than 18.5, was more frequent in leprosy cases than controls, and this pattern was even more pronounced for women. Deformity and disability due to leprosy were also associated with higher rates of undernutrition than those of cases with no physical or functional impairments. Though the use of BMI as an indicator of undernutrition speaks to the probable longevity of insufficient dietary intake, researchers could not causally link nutritional deficiency to the onset of leprosy infection due to the long bacterial incubation period and retrospective study design (12). Findings from Diffey and colleagues further support the link between leprosy and undernutrition, though causality was again indeterminable (13). According to a study in southern India, undernutrition in individuals with leprosy is not an uncommon occurrence. An estimated 57% of leprosy patients with disability experience some degree of undernutrition with 10% of these individuals suffering from severe malnourishment (14).

More compelling evidence for the link between undernutrition and leprosy has emerged as a result of the continued prevalence of disease in certain areas of the world. A report by Van Beers and colleagues connects the disappearance of leprosy from Northern Europe in the last century with improved socioeconomic conditions in this region (15). Countries with continued endemicity, in contrast, have been associated with lower life expectancies, education, and

standards of living culminating in a lower positioning on the human development index. Ethiopia, known for a high prevalence of leprosy, ranks 173rd out of a total 189 countries on this scale (16). The consequences of this ranking trickle down to the individual level, with many Ethiopians forced to grapple with socioeconomic vulnerabilities on a daily basis.

A study conducted in India, another country with sustained leprosy, effectively demonstrated an increased risk of infection in young, malnourished children who face the challenges of food insecurity on a regular basis (15). This association may be explained by underlying biological mechanisms that manifest as a result of prolonged undernutrition. For example, major dietary insufficiencies cause thymic atrophy and reduced production of T lymphocytes. Diminished protein and micronutrient availability impairs function in the remaining T-cells and alters production of interleukins and cytokines, some of which have been implicated as mediators of immunosuppression and immunopathogenesis (17).

Oktaria and colleagues offer additional support for the role of nutrition in cellular immunity through an examination of food shortage as a proxy for nutritional deficiency. In their study of leprosy cases in Indonesia, researchers found that 53% of patients experienced food shortage at some point in their lives. Dietary diversity and consumption of nutrient dense foods such as fruits, vegetables, eggs, nuts, and legumes were also lower among cases than controls (18). A meta-analysis of cohort, case-control, cross-sectional, and ecological studies found that food shortage was, in fact, significantly associated with a higher risk of leprosy [RR = 1.39, 95% CI (1.05, 1.85)] (19). Data from a case-control study conducted in Brazil further supports this finding with the odds of experiencing food shortage at any point in the past 1.54 times higher among individuals diagnosed with leprosy compared to controls [OR = 1.54, 95% CI (1.45, 1.63)] (11).

Others propose that food shortage may facilitate the progression from infection with *M. leprae* to clinical presentation of leprosy due to impaired immune defenses. Among newly diagnosed leprosy cases in Bangladesh, lower food expenditure, lower BMI, lower dietary diversity, and absence of household food stocks were significantly associated with increased risk of having leprosy (20). Research by Feenstra and colleagues supports the link between malnutrition and the development of active symptomatology. Among individuals included in this study, the odds of food shortage in the last year were 1.79 times higher for those with symptomatic leprosy compared to controls [OR= 1.79, 95% CI (1.06, 3.02)] (21). Food shortage in northwest Bangladesh is seasonal, lasting from the end of September through November after the conclusion of the rainy season. This period of food insecurity roughly coincided with symptom onset in the leprosy patients under study, over 70% of whom reported new symptoms within 6 months of enrollment. This pattern remained consistent when researchers reviewed monthly records of leprosy cases over 9 years prior. Per this documentation, newly diagnosed cases consistently began to rise in February, approximately four months after the start of the seasonal food shortage period, with peak rates in June six months following this window (21). Since *M. leprae* has a long incubation period and may never become active in some people, these results suggest that a lowered immune response from malnutrition could trigger the development of active leprosy, or clinical symptomology, from latent infection.

The connection between leprosy infection and depressed immunity due to malnutrition is further strengthened through the examination of a parallel mycobacterial disease, tuberculosis. An abundance of experimental evidence in this arena suggests that malnutrition causes secondary immunodeficiency which, in turn, promotes increased susceptibility to infection. A prospective ten year study of 1,717,655 Norwegian youths found that the relative risk of tuberculosis was

five times higher among individuals in the lowest body mass index category, independent of sex or age (17). Other evidence for this association comes from historical documentation of tuberculosis outbreaks during times of war and famine in addition to animal studies that confirm the presence of a physiological mechanism underlying this association. In guinea pigs, for example, cell mediated immunity was significantly diminished when feeding was limited over a prolonged period (22). Research (unpublished) conducted by our study group in Ethiopia in 2018 further substantiates the extension of this relationship to leprosy and implicates malnutrition as a possible transmission factor. In addition to an association between underweight and leprosy [aOR= 10.32 95% CI (1.79, 59.67)], skipping or reducing the size of meals [OR= 2.87, 95% CI (1.0, 8.32)] and insufficient funds for food [OR= 10.0, 95% CI (3.44, 29.06)] were identified as significant drivers of infection (23). These indicators of macronutrient malnutrition in relation to leprosy, together, lend support to the proposed pathway beginning with food insecurity and ending with increased susceptibility to infection.

As previously stated, cellular immunity is influenced by both protein malnutrition and micronutrient deficiency. Specifically, vitamin A and vitamin D have been explored in connection to leprosy susceptibility. Vitamin A plays an important role in lymphocyte proliferation and epithelial tissue function. T and B lymphocytes, macrophage activity, and the antibody response are all influenced by vitamin A. In reference to tuberculosis, vitamin A was found to inhibit replication of bacilli in cultured human macrophages. Lack of vitamin A, in contrast, increased adherence of bacteria to respiratory epithelial cells (17). A previous study of leprosy patients revealed significantly lower mean serum levels of vitamin A compared to controls, especially LL cases (24). Though the exact mechanism through which vitamin A deficiency impacts leprosy infection remains unclear, many have speculated that vitamin A

exerts an adjuvant-like effect on the immune response. Others suggest that the antioxidant status of vitamin A is the primary mechanism through which this micronutrient influences cellular immunity, with deficiencies resulting in oxidative stress and increased susceptibility to infection (25). Diminished phagocytic and natural killer (NK) cell activity coupled with an inability to suppress interleukin 12 (IL-12), tumor necrosis factor (TNF), and interferon production of Th1 lymphocytes are other explanatory mechanisms. All of these pro-inflammatory proteins are derived from white blood cells and contribute to a decreased capacity to fight off extracellular pathogens (26).

Vitamin D has also been implicated in the onset of leprosy. This micronutrient plays a similar role as vitamin A in host resistance, particularly with regards to macrophage activity. Calcitriol, the active derivative of vitamin D, enhances the ability of macrophages to control the intracellular replication of mycobacteria. Guinea pigs exposed to pulmonary tuberculosis and fed a diet devoid of vitamin D were found to have a marked depletion of calcitriol levels with an associated loss of T cell function. Adults with untreated tuberculosis have been shown to have significantly decreased blood levels of 25-hydroxy-vitamin D compared to controls, implicating vitamin D as a risk factor for disease (17). Because of the influence of vitamin D and calcitriol in macrophage activity against mycobacterium tuberculosis, many have speculated on the importance of this micronutrient in the response to leprosy infection (22).

Evidence also suggests that vitamin D plays a role in mediating immune reactions after disease onset. The presence of vitamin D receptors in monocytes, macrophages, and thymus tissue is a major indication of the importance of this particular micronutrient in immune function (26). In fact, deficiency of vitamin D is correlated with a rise in proinflammatory cytokines. One such cytokine, TNF-α, has been directly implicated in demyelination and is a probable causative

agent of many of the clinical symptoms of leprosy including nerve impairment. A significant increase in TNF-α has also been found in the skin lesions of patients with type 2 reactions. Individuals with type 2 reactions have been shown to have very low expression of the gene that controls the receptor that binds calcitriol. In addition to facilitating disease onset, low levels of vitamin D and reduced expression of its binding receptor may determine the severity of disease through modulation of immune reactivity (27). Human epidemiological studies support the ability of this micronutrient to induce immune reactions, with a direct correlation between vitamin D status and autoimmune disease (26). A prior study by our research group in Brazil further expounded upon this relationship in the context of leprosy. Among infected cases, the odds of vitamin D deficiency were 4.66 higher compared to non-household controls [aOR= 4.66, 95% CI (1.42, 15.33)]. Results from this analysis lend support to the proposed relationship between depressed host immunity and the risk of active leprosy (28). Research to determine the directionality of vitamin D as a driver of transmission as well as the role of this micronutrient in predisposing individuals to leprosy reactions is ongoing.

In sum, nutritional intake exerts a biological influence on immune function by mediating the cellular and humoral response. Deficiencies in certain macro- and micronutrients can promote the dysregulation of the immune system and increase susceptibility to infection. In turn, active infections can exacerbate deficiencies and alter metabolic utilization of available nutrient stores, making the directionality of this relationship difficult to establish via case control studies. Vitamins A and D have been researched in the context of leprosy given their integration in the immune system. Vitamin A deficiency induces inflammation and impairs the response of phagocytes and NK cells to extracellular pathogens. Vitamin D deficiency leads to a diminished localized innate immunity as well as defects in antigen recognition capabilities (26). In

combination, the consequences of deficiency in both micronutrients are suspected to increase overall susceptibility to infections like leprosy and will be explored in more detail throughout this report.

Parasitic Coinfection

In areas of the world where leprosy remains endemic, parasitic diseases simultaneously pose a major public health threat. In Ethiopia, for example, lymphatic filariasis and cutaneous leishmaniasis cases are present in higher numbers than anywhere else in Sub-Saharan Africa. Ascariasis and hookworm are also abundant and cause significant downstream health and economic consequences (1).

Prior research has demonstrated that intestinal helminths, one very common group of human parasites, invoke a strong Th2 immune response and weaken Th1 immunity, a critical defensive system against mycobacteria (7). Individuals harboring intestinal helminths have also been shown to exhibit defective immune cell signaling and greater stimulation of regulatory T cells which may further suppress Th1 activation (7, 29). Thus, preexisting parasitic infection may physiologically predispose colonization by *M. leprae*, trigger active leprosy from latent infection, and favor the more severe and infectious lepromatous end of the disease spectrum.

This hypothesis has been born out in the literature. Prost and colleagues found that among individuals living in geographic areas with a similar prevalence of leprosy, those residing in a filariasis hyperendemic region were more prone to lepromatous leprosy (30). In patients with latent pulmonary TB, researchers found that patients with concurrent filariasis infection exhibited a downregulated Th1 and Th17 response with associated increases in cytotoxic T lymphocyte antigen and programmed cell death. This immune malfunction profoundly impacted

the chance of patients shifting from latent to active tuberculosis, with other systemic helminths suspected to do the same (31).

In a study of leprosy patients in Brazil, the odds of testing positive for an intestinal helminth among cases was 1.46 times the odds of non-leprosy controls [OR= 1.46, 95% CI (1.08, 1.97)]. This association was even stronger for LL patients, with the odds escalating to 2.99 [OR= 2.99, 95% CI (1.82, 4.95)] (7). Additional work from our group in Brazil identified a strong association between helminth infection and leprosy in cases compared to household contacts [aOR= 8.69, 95% CI (1.50, 50.51)]. These findings provide further evidence of altered host immunity in individuals infected by a parasite which may, in turn, increase susceptibility to leprosy. Alternatively, suppression of the Th1 response among individuals infected with a helminth may promote the transition from latent to clinically apparent leprosy in previously asymptomatic individuals (28).

Increased susceptibility to leprosy among those with concurrent parasitic infection not only influences severity and progression of the disease but may actually increase the reservoir of *M. leprae* in endemic communities. Greater bacterial exposure in susceptible individuals, then, may drive sustained leprosy transmission and continued endemicity in areas where coinfection is prominent. A study conducted across seven municipalities in Brazil put this hypothesis to the test. Geographic information systems (GIS) allowed researchers to analyze the spatial distributions of leprosy against schistosomiasis and visceral leishmaniasis, two parasitic diseases of particular prominence in the area. GIS maps revealed significant overlap and clustering between schistosomiasis and leprosy. Relative risk calculations between a community with known schistosomiasis cases compared to a community without revealed a 6.80 increased chance of contracting leprosy [RR= 6.80, 95% CI (1.46, 31.64)]. Incidence of schistosomiasis and

leprosy also peaked during the same time periods, further substantiating the relationship between these diseases (9).

Many areas with endemic leprosy infection have alternating wet and dry seasons, often resulting in times with little precipitation and stagnant pools of water. Damp environments with no flowing water can become a habitat for a variety of plant life, small animals, and parasites (11). Guinea worm, for example, is born from aquatic copepods that serve as temporary hosts while infectious larvae mature. These microscopic larvae are then transmitted to humans who drink water from stagnant sources, including ponds, pools in drying riverbeds, and unprotected shallow wells. They may also be transmitted via uncooked aquatic animals, a major risk factor for populations that rely on fish for sustenance (32).

Additional study of parasitic coinfection as a risk factor for leprosy is merited given the heightened exposure to parasites in leprosy endemic areas coupled with the reality that many individuals have no knowledge of their host status. In general, parasites are long-lived and induce asymptomatic or subclinical infections by downregulating the immune response and restricting inflammation as detailed above. When subsequently exposed to an infectious agent, individuals harboring parasites are unable to mount a full immune response (31). A better understanding of this relationship in the context of leprosy may allow for improved public health efforts to reduce the burden of parasitic disease, and in turn, leprosy infection.

Water, Sanitation, and Hygiene (WASH) Conditions

As noted above, parasitic transmission often occurs in the context of poor WASH conditions and may impact leprosy exposure, susceptibility, and progression. In fact, certain behaviors practiced in areas lacking sanitation resources and clean water are known risk factors

for parasitic transmission. A study conducted in Bihar, India, for example, found that over 95% of children practiced open defecation and 61% washed hands with soil rather than soap or ash (33). The presence of one or more soil transmitted helminths including ascariasis, hookworm, and whipworm was identified in 68% of this study population. In southern India, the odds of practicing open air defecation were found to be 5.37 times higher among children with a parasitic infection [OR= 5.37, 95% CI (1.61, 17.87)] (34).

Contact with water has also been identified as a risk factor for parasitic infection. Researchers in Brazil discovered that individuals who came in contact with bodies of water were 5.6 times more likely to be infected with schistosomiasis compared to those with no water contact [OR= 5.6, 95% CI (3.50-8.96)]. Further, the odds of positivity with *S. mansoni* infection linearly increased according to degree of water exposure (35). This association held true in Ethiopia for individuals infected with leprosy. Researchers in North Gondar found that leprosy patients living in districts bordering Lake Tana had greater odds of acquiring schistosomiasis compared to individuals with leprosy living in districts away from the lake [OR= 3.56, 95% CI (0.80, 15.85)] (36).

Also included in this previous study by our research group were WASH conditions distinct from parasitic transmission. Unimproved water source, lack of water access on the premises, lack of soap, lack of handwashing, and open defecation were associated with leprosy on unadjusted analyses (36). These risk factors are downstream consequences of low socioeconomic status and reflect the reality that leprosy infection is much more prevalent in limited resource areas. Poor housing conditions, inadequate sanitation, unsafe water, and lack of garbage collection have all been connected to leprosy suggesting that interventions targeting

poverty, and in turn WASH conditions, are necessary for the complete elimination of this disease (37).

Though prior research has certainly established the role of WASH as a facilitator of infection, others have proposed water as the primary driver of leprosy transmission. In 19th century Norway, leprosy was endemic to areas of the West Coast with most cases characterized by lesions on the lower bilateral legs and feet. During this time, it was common to walk barefoot, often through rivers and swamps, with the combination of abrasions from walking and contact with *M. leprae* in water hypothesized to sum to leprosy infection (11).

Further evidence of water as a potential disease reservoir comes in the form of epidemiological research. A case-control study in North Brazil concluded that the odds of bathing regularly in open bodies of water were 1.54 times higher among individuals with leprosy compared to controls [OR= 1.54, 95% CI (1.45, 1.63)] (11). A strong seasonal pattern of leprosy transmission was determined in India, with higher rates of *M. leprae* detectable by PCR positive nasal swabs following the monsoon rains from July to November, with salivary PCR indicative of peak levels in November (21). In Malawi, rates of leprosy similarly peaked in areas with high levels of precipitation as well as in areas near the shore of Lake Malawi (38). The relative risk of leprosy was also associated with percentage of water bodies in a study conducted in northern Brazil. Researchers in this setting discovered a clustering with regards to leprosy cases. The five primary clusters were all located in the Amazon region, an environment characterized by high temperatures and humidity (39). In the lab, scientists have shown *M. leprae* to favor warm and moist environments, indicating a possible biological explanation for these associations (40).

Potentially viable *M. leprae* has actually been detected in water sources on a number of occasions. In a highly endemic village in Indonesia, *M. leprae* DNA was detected by PCR in 21

out of 44 water sources utilized daily by villagers. Those who relied upon this water for bathing and washing were found to experience higher rates of leprosy, strongly implicating water as the source of infection (41). Over 75% of water samples obtained from reservoirs, rivers, streams, springs, and wells in northeastern Brazil tested positive for *M. leprae* mRNA. Per local health officials, sampled water sources were commonly utilized for drinking, bathing, cooking, washing clothes, and recreation (42). In endemic areas of Purulia, India, 24.2% of water samples taken from areas inhabited by leprosy patients were positive for viable bacilli. Interestingly, 25.6% of soil samples from these same areas also tested positive for *M. leprae* (43). Soil samples collected from areas utilized for washing and bathing exhibited positivity as well. In other parts of India, such as Ghatampur, even higher rates were noted, with 55% of samples collected from residential areas of leprosy patients containing bacilli. Other areas of the village located away from the residences of leprosy cases also tested positive, though in lower abundance and viability (44).

In determining the existence of environmental reservoirs of leprosy and the capacity for indirect transmission, survivability outside of a host is a primary consideration (45). Laboratory studies have demonstrated *M. leprae* survivability in soil for up to 40 days (43). This speaks to the relatively high resistance of the pathogen outside of the human body (8). Viability of the pathogen beyond this timeframe has been confirmed through bacterial replication on the foot pads of mice. Desikan & Sreevatsa experimented with the pathogen under numerous environmental conditions and discovered that viability was maintained after 46 days in wet soil, 60 days at room temperature, and 60 days upon refrigeration. The bacteria even survived for 7 days when exposed to limited levels of sunlight. Viability was maximized when the organism

was dried and kept in the shade, with survivability observed for up to 5 months under these conditions (40).

Some researchers have reported survivability above 5 months when the pathogen is phagocytized by common free-living amoeba. In environments where *M. leprae* may otherwise be unsuited, amoebic cysts have been found to provide an intracellular refuge, potentially allowing for perpetuation of disease in communities where prevalence is low. These protozoa are capable of hosting *M. leprae* for up to 8 months, protecting them from extremes in temperature, drought, and even biocides. In a laboratory study to examine viability after 35 days of encystment, mice injected with *M. leprae* from amoebas developed lesions similar to mice challenged with fresh *M. leprae* (10). Molecular analysis suggests the ancestry of mycobacteria can be traced to an environmental organism living in an aquatic habitat, potentially accounting for the ability of *M. leprae* to coexist with amoebas and generally thrive under wet conditions (46). In combination, these epidemiological findings, laboratory observations, and proposed biological mechanisms strongly implicate WASH along the pathway of leprosy infection and point to the need for further exploration of WASH insecurity as a risk factor for disease.

Target Population

In order to analyze the above susceptibilities in connection with leprosy, data was collected from North Gondar and surrounding woredas. Ethiopia continues to net a high incidence of leprosy each year with over 3,692 new cases reported in 2016 alone, or roughly an incidence of 3.52 per 100,000 individuals (47). This number is much higher when accounting for existing cases of leprosy, those who have recovered with disability, and probable underreporting due to stigma and lack of access to healthcare. Additionally, Ethiopia is endemic for parasitic

diseases, many related to poor WASH conditions, making this region uniquely suited for studying the overlap between these susceptibility factors. Schistosomiasis, for example, affects close to five million Ethiopians each year (48). Hookworm, a common soil transmitted helminth, affects over 11 million people in Ethiopia amounting to 5.6% of the hookworm burden in Sub-Saharan Africa. Ascariasis affects over 26 million Ethiopians, or 15% of the overall burden in Sub-Saharan Africa. Ethiopia ranks within the top ten countries for highest counts of both leishmaniasis and lymphatic filariasis, and over 12 million Ethiopians are at risk of onchocerciasis and subsequent blindness (1).

To make matters worse, 31% of Ethiopians live on USD $1.25 per day. Up to 44% of children are stunted and the prevalence of undernourishment reaches above 35% in children under 5 (49). Though these figures reflect significant improvement over the course of little more than a decade, individuals in Ethiopia continue to be touched by poverty, many of whom face the consequences of limited access or affordability of food on a daily basis. These conditions, together, frame Ethiopia as an ideal location for data collection.

Information gathered during the data collection period in 2019 ultimately allowed for analysis of nutritional deficiency, parasitic coinfection, and WASH factors as they relate to leprosy onset and progression. The remainder of this report outlines the specific methodology employed for data collection and analysis as well as key findings with regards to these posited susceptibility factors.

Chapter 3: Manuscript

Title: Susceptibility to Leprosy: An Examination of Nutrient Deficiencies, Parasitic Coinfection, and WASH Conditions

Megan K. Wasson[1], Cassidy Whitson[1], Bridget Miller[1], Wondwossen Abebe[3], Belay Tessema[3], Lisa E. Emerson[1], Puneet Anantharam[1], Annisa Befekadu Tesfaye[3], Jessica K. Fairley[1, 2]

Affiliations

[1]Emory University Rollins School of Public Health, Atlanta, GA, USA

[2]Emory University School of Medicine, Atlanta, GA, USA

[3]University of Gondar, Gondar, Ethiopia

For Publication in: Frontiers in Microbiology

Abstract

Background

Despite extensive control measures and a declining number of human reservoirs, continued incidence of leprosy in excess of 200,000 new infections each year suggests that alternative pathways may play a role in continued endemicity. Nutritional deficiencies, parasitic coinfection, and limited water, sanitation, and hygiene (WASH) have been suggested to predispose individuals to *M. leprae* infection and were further explored in this analysis.

Methods

Leprosy cases and uninfected controls were recruited from areas around North Gondar, Ethiopia throughout 2019. Participants completed dietary and WASH surveys in addition to providing stool for Kato Katz, urine for Schisto POC-CCA^{TM} rapid diagnostic testing, and blood for micronutrient biomarker testing. Multivariate logistic regression was performed to investigate associations between the above exposures and leprosy.

Results

A total of 80 men (59%) and women (41%) participated in this study with an average age of 40 (SD 15.0 years). Most leprosy cases were multibacillary (93.3%). There was a high prevalence of undernutrition among cases and controls, with 32.1% of participants classified as underweight. Food shortage [OR 4.57, 95% CI (1.62, 12.89)] and fewer meals consumed per day in the last four weeks [OR 3.85, 95% CI (1.17, 12.67)] were both significantly associated with leprosy in the univariate analysis. Additionally, 64.1% of the study population tested positive for a helminth and WASH insecurities were widespread. On multivariate analysis, lack of soap for handwashing [aOR 2.53, 95% CI (1.17, 5.47)] and lack of toilet facilities [aOR 2.32, 95% CI (1.05, 5.12)] were significantly associated with leprosy. Positive directionality was identified for a number of other inputs, including helminth infection [aOR 3.23, 95% CI (0.85, 12.35)].

Conclusions

Taken together, these findings strengthen previous research conducted in 2018 implicating WASH as a driver of leprosy infection. Subsequent micronutrient results will be integrated to further explore nutritional risks and build upon the significant macronutrient findings from this analysis. Given that leprosy remains the leading infectious cause of disability in the world, future research should explore all of the above susceptibilities in more depth to curtail the global burden of disease.

Introduction

Neglected tropical diseases (NTDs) infect over 2.7 billion people worldwide, a disproportionate number of whom reside in low- and middle-income countries (LMICs) (1). Leprosy, also known as Hansen's disease, is one such NTD that continues to wreak havoc in certain areas of the world. Ethiopia, for example, reported over 3,692 new cases of leprosy in 2016 alone (47).

Mycobacterium leprae, a rod-shaped bacillus and the causative agent of disease, is believed to be transmitted via droplets from the nose or mouth during sustained contact with an infected individual. Incubation lasts an average of 1-5 years but may extend up to 20 years in some cases (5). A special affinity for keratinocytes in the skin and Schwann cells in the peripheral nervous system gives rise to some of the more characteristic symptoms of disease including skin discoloration, nodules, lesions, sensory loss, blindness, and deformity (6).

Leprosy is generally categorized according to immune response and disease severity, with tuberculoid (TT) leprosy falling on the less severe end of the spectrum and lepromatous leprosy on the more severe side (3). Effective cellular immunity against TT leprosy is characterized by a T helper type 1 (Th1) response which allows for complete destruction of the pathogen. In contrast, the response against LL is characterized by malfunctioning cytotoxic T cells, or a Th2 immune response, which allows the pathogen to survive and multiply (7, 8).

The potential for chronic disability following leprosy infection is heightened in the event of a type 1 or type 2 reaction which causes further nerve degradation, deformity, and injury to organ systems (3). Damage to peripheral nerves in the course of a type 1 reaction is believed to occur due to the cell-mediated T-cell response while nerve damage from type 2 reactions is attributed to the production and deposition of antigen-antibody complexes (3).

These debilitating consequences underscore the importance of prevention efforts to limit the spread of infection. However, in the decades since the development of targeted multidrug therapy for leprosy and the initiation of aggressive control measures, the overall prevalence of disease has decreased but incidence in endemic areas has persisted. The majority of new leprosy cases cannot be linked to a preexisting case or household contact which further substantiates the possibility of alternative mechanisms of transmission (11). The potential for secondary routes of infection or environmental reservoirs opens the door for consideration of risk factors that may facilitate infection. A few susceptibility factors proposed in the literature include nutrient deficiencies, parasitic coinfection, and water, sanitation, and hygiene (WASH).

Decades of research have linked undernutrition to infectious disease with some exploration of macronutrient deficiency as it relates to leprosy (12-15). This association may be explained by underlying biological mechanisms that manifest as a result of insufficient macronutrient intake over a prolonged period. For example, major dietary insufficiencies are known to cause thymic atrophy and reduced production of T lymphocytes. Diminished protein and micronutrient availability further impair function in the remaining T-cells and alter production of interleukins and cytokines, some of which have been implicated as mediators of immunosuppression and immunopathogenesis (17).

In addition to facilitating leprosy transmission, others have proposed that undernutrition promotes the progression from infection to clinical presentation of disease (20, 21). Both of these hypotheses have found support in cohort and laboratory studies conducted in the context of a parallel mycobacterial disease–tuberculosis (17, 22). Research (unpublished) conducted by our study group in Ethiopia in 2018 further substantiates the extension of this relationship to leprosy and implicates malnutrition as a possible transmission factor. Along with an association between

underweight and leprosy [aOR= 10.32 95% CI (1.79, 59.67)], skipping or reducing the size of meals [OR= 2.87, 95% CI (1.0, 8.32)] and insufficient funds for food [OR= 10.0, 95% CI (3.44, 29.06)] were identified as significant drivers of infection (23). The connection between macronutrient malnutrition and leprosy lends support to the proposed pathway beginning with food insecurity and concluding with increased susceptibility to infection.

Given the role of micronutrients in mediating cellular immunity, recent research has begun to explore the impact of both micro- and macronutrient deficiencies on leprosy susceptibility. Vitamin A, for example, exerts a major influence on lymphocyte proliferation, epithelial tissue function, macrophage activity, and the antibody response (24-26). Similarly, vitamin D plays a role in host resistance, particularly with regards to macrophage activity, as well the production of proinflammatory cytokines which may cause many of the clinical symptoms of leprosy, such as nerve impairment (17, 22, 26, 27). A prior study by our research group in Brazil identified substantially higher odds of vitamin D deficiency among leprosy cases compared to non-contact controls, lending support to the proposed relationship between depressed host immunity and the risk of active leprosy [aOR= 4.66, 95% CI (1.42, 15.33)] (28).

In much the same way, parasitic coinfection has been implicated along the pathway of leprosy infection and disease. Intestinal helminths have been found to invoke a strong Th2 immune response and weaken Th1 immunity which serves as a critical defensive system against mycobacteria (7). Individuals harboring intestinal helminths are also known to exhibit defective immune cell signaling and greater stimulation of regulatory T cells which may further suppress Th1 activation (7, 29). Thus, the cumulative immune response to a parasitic infection may physiologically predispose colonization by *M. leprae*, trigger active leprosy from latent infection, and favor the more severe lepromatous end of the disease spectrum (7, 9, 28-31).

Parasitic transmission often occurs in the context of poor WASH which may further amplify leprosy susceptibility and progression (33-35). Reliance on an unimproved water source, open defecation, lack of soap, and limited handwashing have all been explored as risk factors for leprosy (36, 37). Water has also been investigated as a potential reservoir of *M. leprae,* with proximity to water bodies previously implicated in leprosy onset (11, 21, 38). Potentially viable bacilli have been detected in a number of communal water sources as well as soil samples in areas used for bathing (38, 42-44). Further, laboratory studies have demonstrated the relatively high resistance of this particular pathogen outside of the human body, with viability maintained for up to 5 months in shaded soil samples and up to 8 months when phagocytized by common free-living amoebas (10, 40).

Given the potential secondary mechanisms of transmission reviewed above, the primary objective of this case-control study was to better understand host and environmental factors as they relate to leprosy infection. As previously suggested in the literature, patients with leprosy were hypothesized to lack appropriate nourishment and WASH resources compared to healthy controls. Those with leprosy were also expected to have higher rates of parasitic infection.

Methods

Study Site and Population

This case-control study was conducted in metropolitan areas of Gondar, Ethiopia with a focus on North Gondar and surrounding woredas. North Gondar has a population of approximately 3,225,022 individuals, most of whom reside in rural or agricultural areas (84.21%) (50). An average of 5000 new cases of *M. leprae* are diagnosed at health facilities in Ethiopia each year resulting in one of the highest burdens in Sub-Saharan Africa (1). Accordingly, the number of annual cases identified in North Gondar is disproportionately high. Data utilized in the course of this analysis was collected from June to December of 2019. Cases were defined as adults over the age of 18 with a clinical diagnosis of multibacillary or paucibacillary leprosy made by a practicing physician in a dermatology or family health center surrounding Gondar.

Potential subjects were identified from a leprosy registry and recruited from local dermatology and health clinics. Patients included in the study primarily resided in the North Gondar region, had an active leprosy infection, and were on MDT for less than a year. Unconfirmed cases, pregnant women with leprosy, and patients who had completed MDT were excluded from evaluation. Controls were sampled from adult members of surrounding communities with no current or previous leprosy diagnosis and no known leprosy exposures. Controls were excluded from the study if there was a history of an unconfirmed neurological or dermatological disease or known proximal contact with a leprosy case.

A previous case-control study of leprosy was conducted by our study team in North Gondar from May to October of 2018 (36). The case definition, exclusion criteria, recruitment protocols, and methods of obtaining WASH and schistosoma data were consistent between the

2018 and 2019 collection periods. However, stool for Kato Katz ova and parasite detection was not obtained in 2018. Thus, in select analyses, both years of schistosoma infection and WASH data were combined to allow for a more comprehensive exploration of leprosy susceptibilities.

Data Collected

Participants were asked to complete a combination of surveys and clinical assessment measures after consenting to participate in the study. Demographic and other key health information, such as leprosy type, date of diagnosis, and treatment protocol, supplemented data obtained directly from patients. Disability status was also assessed in individuals with leprosy by assigning participants a score on the basis of leprosy-induced disability of the eyes, hands, and feet per the World Health Organization (WHO) grading guidelines. Individuals without anaesthesia, or pain insensitivity, visible damage to the hands or feet, eye problems, or vision loss were assigned a score of 0. Those with anaesthesia but no deformity to the hands or feet as well as those with vision problems but insignificant vision loss were assigned a disability grade of 1. Cases characterized by visible deformities or damage to the hands or feet and severe visual impairment were assigned a score of 2 (51). On site medical personnel assisted with the administration of surveys in Amharic as well as the collection of biological specimens.

A. Evaluation of Nutritional Status

Anthropometric measures including height, weight, and middle upper arm circumference (MUAC) were obtained as proxy indicators of macronutrient status. Body mass index (BMI) was determined according to the calculation weight (kg) / height^2 (m) with the following classifications: underweight (BMI less than 18.5), normal (BMI greater than or equal to 18.5 and

less than 25), overweight (BMI greater than or equal to 25 and less than 30), and obese (BMI greater than 30). MUAC was calculated by locating the midpoint between the participant's elbow and shoulder, wrapping a measuring tape around his or her arm at the midpoint, and recording the measurement in centimeters. Severe risk of malnutrition was assigned to adults with a MUAC below 18 cm, moderate risk to those with an MUAC between 18 cm and 21 cm, and normal risk to adults with an MUAC greater than 21 cm (52).

Direct indicators of nutritional status were gathered via micronutrient biomarker test kits. These kits specifically measured vitamin A and D through the assessment of retinol binding protein (RBP) and 1,25 hydroxy vitamin D, respectively. The use of an ELISA assay to assess retinol binding protein further served as a representation of total retinol and allowed for the differentiation of total retinol concentration between cases and controls. Food security questions from the United States Department of Agriculture (USDA) provided supplementary dietary information. These questions were primarily designed to evaluate daily food intake, sources of food, nutritional knowledge, and socioeconomic status (53).

B. Evaluation of Parasitic Coinfection

The Schisto POC-CCA^{TM} rapid diagnostic test was used to detect *Schistosoma mansoni* infection. Schisto POC-CCA^{TM} rapid diagnostic tests identify active infections in urine specimens with the ability to ascertain positive cases in burdens as low as 50 worms. The sensitivity reaches 100% at higher burdens of 400 eggs per gram of feces or more (54). The presence of other helminths (including eggs for *S. mansoni*) and protozoa was discerned from one stool sample per individual via the Kato Katz technique. The sensitivity of this approach ranges from 74% to 95% with greater accuracy in high transmission settings such as Ethiopia

(55). Sensitivity also improves upon replication. Thus, any invalid or inconclusive tests were repeated. All participants who tested positive for a parasitic infection were immediately notified and referred for treatment.

C. Evaluation of WASH Conditions

A second survey adapted from the WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation (JMP) core questions on water, sanitation and hygiene for household surveys was also collected from all participants to better understand water usage, water contact, water treatment, access to soap, handwashing, and other general sanitation practices (56).

Information collected regarding drinking water source, cooking water source, and toilet type was classified according to improved and unimproved categories for analysis. According to the WHO, improved water sources are located on premises, available when needed, and free of fecal and priority chemical contamination (57). Improved water sources adequately protect water from outside contamination through avenues such as a household connection, public standpipe, borehole, protected dug well, protected spring, and rainwater collection. Unimproved sources include unprotected dug wells, unprotected springs, surface water, vendor-provided water, and tanker truck water. Because the majority of participants did not have access to a water source on premises, the time it took participants to collect water was further subcategorized from improved and unimproved into basic and limited-service groups. According to WHO criteria, water collection involving a round trip of 30 minutes or less merits a classification of basic service while a round trip to collect water exceeding this cutoff is considered limited water service (58).

In terms of sanitation, improved facilities are defined by the hygienic separation of human excreta from human contact through mechanisms such as a sewer connection, septic system connection, pour-flush latrines, ventilated improved pit latrines, and pit latrines with a slab or covered pit (57). Unimproved toilet facilities include pit latrines without slabs or platforms, hanging latrines, bucket latrines, and open defecation. Handwashing data, a key indicator of hygiene, was dichotomized into limited service and no service groupings based on the presence or absence of functional hand hygiene stations according to JMP guidance (59). Finally, previous studies have linked proximity to bodies of water as a potential susceptibility factor to leprosy (35, 36, 38). Thus, village residency reported by each participant in the study was recategorized on the basis of proximity to the local water source, Lake Tana, with individuals assigned to “on the lake” or “not on the lake” groupings as was done in previous studies (36).

In sum, BMI and MUAC measurements coupled with dietary questionnaire results and micronutrient biomarker data were utilized to evaluate nutritional susceptibilities. The schistosoma rapid diagnostic test and stool samples allowed for the assessment of parasitic coinfection while the WASH questionnaire provided insight into environmental conditions suspected to facilitate leprosy transmission.

Statistical Analysis

Data to inform sample size calculations for the association between nutritional deficiencies and leprosy is lacking. More literature on the relationship between helminths and leprosy is available and accordingly served as a guide for the sample size calculations completed for this study. Drawing on previous publications, an alpha of 0.05 and power of 0.8, coupled

with an estimated helminth prevalence in the North Gondar region between 20-25% and a predicted odds ratio of 3-4 for the association of helminth infection with leprosy, yielded a total goal sample size of 80 split evenly between cases and controls (50).

Following data cleaning, all analyses were completed using SAS 9.4. Descriptive statistics, univariate comparisons, and logistic regression were the primary mathematical outputs. T-tests and chi-square tests were utilized during univariate procedures to describe differences between cases and controls. Odds ratios were calculated to indicate associations between the exposures of interest (nutritional factors, parasitic coinfection, and WASH status) and the outcome (leprosy). A p-value of 0.05 or below was employed as the threshold for significance in all of the above computations.

Variables significantly associated with the outcome were included in a multivariate logistic regression model controlling for potential confounders such as age, sex, and socioeconomic status. Socioeconomic status was evaluated on the basis of monthly income, education, and household size. Monthly income was dichotomized into two groups after reviewing the frequency and distribution of the data. A large right skew influenced the selection of the first quartile as the cutoff value with participants separated into “above Q1” and “below Q1” groups. Education was categorized on the basis of junior secondary school completion. In Ethiopia, junior secondary school is completed by grade 8 (60). Thus, participants were divided into “below grade 8” and “above grade 8” categories. Household size was dichotomized into “crowded” and “normal” living conditions in accordance with a systematic review and meta-analysis of socioeconomic risk markers of leprosy which linked crowded living conditions, characterized by 5 or more individuals residing in a single household, to leprosy infection (19).

Variables with sustained significance featuring a p-value of 0.05 or below are reported in the following tables. A Hosmer-Lemeshow goodness-of-fit test was conducted at the conclusion of the analysis to investigate the suitability of the final logistic regression models. A p-value of 0.05 was again employed as the cutoff with any value below this point prompting rejection of the model. Collinearity was also examined and there was no evidence to suggest correlations between variables included in the final models.

Ethical Approval

This study was approved by the Institutional Review Boards of Emory University and the University of Gondar in Ethiopia. Involvement in the study was voluntary, and informed written consent was obtained from all participants. Data collection presented very little risk to patients given the minimal invasiveness of testing procedures. All data containing private or identifying information was stored in a locked room at the University of Gondar or on a password protected computer. Data utilized for the purpose of this secondary analysis was obtained with permission from the principal investigator and deidentified before being accessed in the US.

Results

Descriptive Statistics

A total of 80 participants, 31 cases and 49 controls, were enrolled in the study. The average age of enrollees was 40 with a standard deviation of 15 years. Approximately 59% of participants were male and 41% were female. The majority of cases were diagnosed with MB leprosy (93.3%) and had a positive bacillary index (70.4%). Participants ultimately varied in terms of their disability grade with 39% reporting a score of 0, 23% with a disability grade of 1, and 39% with a grade 2 disability. Thus, 61% of cases experienced some degree of chronic impairment as a result of leprosy infection.

In terms of education, over 87% of participants did not complete junior secondary school or attain an education above the eighth grade. Additionally, 30% of the population reported a monthly income below 300 Birr. The mean monthly income was 500 Birr (Figure 1). For reference, the national absolute poverty line in Ethiopia is set at 3,781 Birr per adult equivalent per year, or 315 Birr per adult equivalent per month (61). Coupled with the fact that 63.8% of participants reported living in households of five or more individuals, poverty is likely even more pervasive than reflected in these statistics. Additional demographic, socioeconomic, and clinical markers are presented in Table 2.

Figure 1. *Monthly Income (Birr) reported by leprosy cases and controls.*

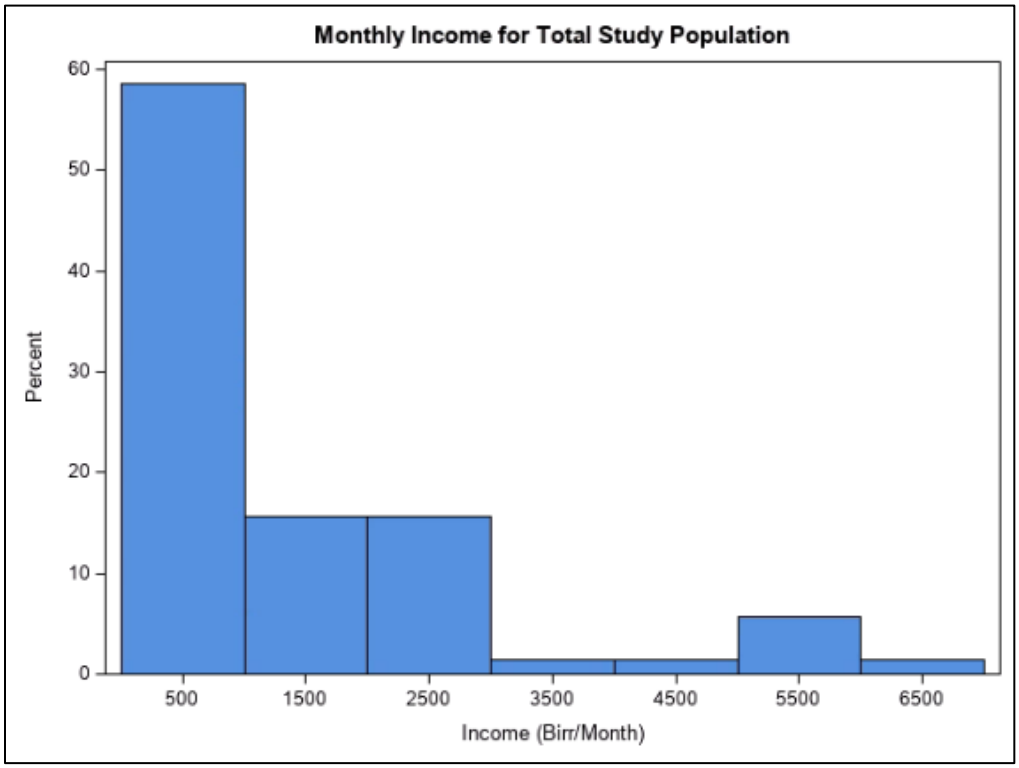

Table 2. *Main demographic and clinical data collected in 2019 for cases and controls. P-values were considered significant if <0.05. Significant values are indicated by an asterisk (*).*

Variable	Cases (n=31)	Controls (n=49)	Total (n=80)	Odds Ratio (95% CI)	P-Value (α = 0.05)
Age (years), mean (SD)	41.9 (16.9)	38.7 (13.7)	40.0 (15.0)	1.01 (0.98, 1.05)	0.3611
Sex, n (%) Male Female	 16 (51.6) 15 (48.4)	 31 (63.3) 18 (36.7)	 47 (58.8) 33 (41.3)	 0.62 (0.25, 1.54) (ref)	0.3037
Education, n (%) Less than Grade 8 Grade 8 and Above Missing n=9	 27 (93.1) 2 (6.9)	 35 (83.3) 7 (16.7)	 62 (87.3) 9 (12.7)	 2.70 (0.52, 14.06) (ref)	0.2380
Household Size, n (%) Crowded (≥ 5) Normal (< 5)	 18 (58.1) 13 (41.9)	 33 (67.4) 16 (32.7)	 51 (63.8) 29 (36.3)	 0.67 (0.27, 1.70) (ref)	0.4011
Monthly Income, n (%) Below Q1 Above Q1 Missing n=10	 6 (20.7) 23 (79.3)	 15 (36.6) 26 (63.4)	 21 (30.0) 49 (70.00)	 0.45 (0.15, 1.36) (ref)	0.1575
WHO Classification, n (%) Paucibacillary (PB) Multibacillary (MB) Missing n=1	 2 (6.7) 28 (93.3)	N/A	N/A	N/A	N/A
Bacillary Index, n (%) Positive Negative Missing n=4	 19 (70.4) 8 (29.6)	N/A	N/A	N/A	N/A
Grade of Disability, n (%) Grade 0 Grade 1 Grade 2	 12 (38.7) 7 (22.6) 12 (38.7)	N/A	N/A	N/A	N/A

Univariate Analysis

A. Nutritional Deficiencies

As reflected in table 3, there was a high prevalence of undernutrition with 25 (32.1%) participants identified as underweight (BMI < 18.5). Similarly, 15 (19.0%) participants were found to have an elevated risk of malnutrition (MUAC < 21 cm). Though univariate analysis did not yield significant differences for BMI or MUAC between cases and controls, these findings generally reflect the frequency and severity of undernutrition in the study population.

Additionally, recent changes in diet were not significantly different between cases or controls nor significant upon univariate analysis. This aligns with previous research suggesting that more prolonged periods of depressed macronutrient intake are associated with increased odds of disease (12-14). However, household food shortage during the past two months [OR=4.57, 95% CI (1.62, 12.89)] and consumption of fewer meals per day [OR= 3.85, 95% CI (1.17, 12.67)] were both significantly associated with leprosy.

In terms of micronutrient malnutrition, preliminary analysis of the ELISA assay results for retinol binding protein revealed no apparent observed difference in the vitamin A concentrations between cases and controls. However, future verification of these findings is warranted due to an issue with the ELISA equipment and delays as a result of the COVID-19 pandemic. As such, the prevalence of vitamin A deficiency could not be definitively concluded, and total concentrations were unable to be incorporated into the logistic regression models. Unfortunately, challenges due to the COVID-19 pandemic also delayed laboratory analysis of the vitamin D data. As of April 2021, Ethiopia has reported 250,955 confirmed cases of COVID-19 and 3,531 deaths (62). Accordingly, laboratory efforts have fully shifted to support public health needs during this crisis. When resources once again become available, the micronutrient

results will be integrated with the significant macronutrient findings from this analysis to further explore nutritional risks in the context of leprosy. Additional outcomes relating nutritional status to leprosy in the present study are documented in Table 3.

Table 3. *Univariate analysis of nutrition data collected over 2019 among leprosy cases and controls. P-values were considered significant if <0.05. Significant values are indicated by an asterisk (*).*

Variable	Cases (n=31)	Controls (n=49)	Total (n=80)	Odds Ratio (95% CI)	P-Value (α = 0.05)
Body Mass Index (BMI), n (%)					
Underweight (<18.5)	11 (36.7)	14 (29.2)	25 (32.1)	1.41 (0.53, 3.71)	0.4906
Normal & above (≥ 18.5)	19 (63.3)	34 (70.8)	53 (68.0)	(ref)	
Missing n=2					
Middle Upper Arm Circumference (MUAC), n (%)					
Elevated Risk (< 21 cm)	4 (12.9)	11 (22.9)	15 (19.0)	0.50 (0.14, 1.74)	0.2737
Normal Risk (≥ 21 cm)	27 (87.1)	37 (77.1)	64 (81.0)	(ref)	
Missing n=1					
Food Shortage in Household, n (%)					
Yes	15 (48.4)	8 (17.0)	23 (29.5)	4.57 (1.62, 12.89)*	0.0041
No	16 (51.6)	39 (83.0)	55 (70.5)	(ref)	
Missing n=2					
Fewer Meals per Day, n (%)					
Yes	11 (35.5)	5 (12.5)	16 (22.5)	3.85 (1.17, 12.67)*	0.0266
No	20 (64.5)	35 (87.5)	55 (77.5)	(ref)	
Missing n=9					
Diet Change, n (%)					
Yes	5 (16.7)	7 (15.6)	12 (16.0)	1.09 (0.31, 3.80)	0.8977
No	25 (83.3)	38 (84.4)	63 (84.0)	(ref)	
Missing n=5					

B. Parasitic Coinfection

With regards to parasitic disease, a majority of participants (64.1%) were host to one or more helminths (Table 4). A total of 41 participants (52.6%) were infected with *S. mansoni*, making this parasite the primary driver of the total helminth prevalence in the study population. Approximately 16 (22.5%) of participants tested positive for a protozoan. The most frequently identified protozoan was *E. histolytica*, infecting 11.3% of participants. Though univariate analysis did not yield significant differences for helminth or protozoa infection between cases and controls, these findings underscore the frequency of parasitic infection within the general population. Of the 31 cases, 22 (71.0%) tested positive for a helminth and 7 (22.6%) were infected with a protozoan. Similarly, 28 controls (59.6%) were identified as having a helminth infection and 9 (22.5%) tested positive for a protozoan out of the entire group of 49 people. Thus, nearly all cases and the majority of controls tested positive for a helminth, protozoa, or both. Additional outcomes relating parasitic infection to leprosy are presented in Table 4.

Table 4. *Univariate analysis of parasitic coinfection data collected over 2019 among leprosy cases and controls. P-values were considered significant if <0.05. Significant values are indicated by an asterisk (*).*

Variable	Cases (n=31)	Controls (n=49)	Total (n=80)	Odds Ratio (95% CI)	P-Value (α = 0.05)
Helminths, n (%) Yes No Missing n=2	22 (71.0) 9 (29.0)	28 (59.6) 19 (40.4)	50 (64.1) 28 (35.9)	1.66 (0.63, 4.38) (ref)	0.3065
S. mansoni , n (%) Yes No Missing n=2	16 (51.6) 15 (48.4)	25 (53.2) 22 (46.8)	41 (52.6) 37 (47.4)	0.94 (0.38, 2.33) (ref)	0.8913
Ascaris (roundworms), n (%) Yes No Missing n=9	4 (12.9) 27 (87.1)	8 (20.0) 32 (80.0)	12 (16.9) 59 (83.1)	0.59 (0.16, 2.19) (ref)	0.4320
E. vermicularis (pinworms), n (%) Yes No Missing n=9	1 (3.2) 30 (96.8)	1 (2.5) 39 (97.5)	2 (2.8) 69 (97.2)	1.30 (0.08, 21.64) (ref)	0.8549
Hookworm, n (%) Yes No Missing n=9	3 (9.7) 28 (90.3)	1 (2.5) 39 (97.5)	4 (5.6) 67 (94.4)	4.18 (0.41, 42.29) (ref)	0.2260
Protozoa, n (%) Yes No Missing n=9	7 (22.6) 24 (77.4)	9 (22.5) 31 (77.5)	16 (22.5) 55 (77.5)	1.01 (0.33, 3.09) (ref)	0.9936
Amoeba, n (%) Yes No Missing n=9	3 (9.7) 28 (90.3)	1 (2.5) 39 (97.5)	4 (5.6) 67 (94.4)	4.18 (0.41, 42.29) (ref)	0.2260
E. histolytica , n (%) Yes No Missing n=9	1 (3.2) 30 (96.8)	7 (17.5) 33 (82.5)	8 (11.3) 63 (88.7)	0.16 (0.02, 1.35) (ref)	0.0920
Giardia , n (%) Yes No Missing n=9	3 (9.7) 28 (90.3)	1 (2.5) 39 (97.5)	4 (5.6) 67 (94.4)	4.18 (0.41, 42.29) (ref)	0.2260

As detailed in the methods section of this report, a subset of parasitic coinfection data (*S. mansoni* POC-CCA data but not stool samples) were available for both the 2018 and 2019 study periods. Thus, additional univariate analysis was conducted with this combined dataset. Results are recorded in Table 5 below.

Table 5. *Univariate analysis of parasitic coinfection data collected over 2018 and 2019 among leprosy cases and controls. P-values were considered significant if <0.05. Significant values are indicated by an asterisk (*).*

Variable	Cases (n=71)	Controls (n=90)	Total (n=161)	Odds Ratio (95% CI)	P-Value (α = 0.05)
S. mansoni , n (%)					
Yes	24 (33.8)	38 (43.2)	62 (39.0)	0.67 (0.35, 1.28)	0.2290
No	47 (66.2)	50 (56.8)	97 (61.0)	(ref)	
Missing n=2					

C. WASH Conditions

As illustrated in Table 6, many participants did not have access to improved WASH services. A total of 14 participants (17.7%) collected water from an unimproved drinking water source while 15 (18.8%) obtained cooking water from an unimproved source. Close to 95% of participants did not have access to water on premises. The majority of individuals with access to water off premises reported a roundtrip time to collect water under 30 minutes, with only 6.9% exceeding this demarcation. In terms of water treatment, 34 (41.3%) of participants consistently treated their water while 43 (55.8%) did not. Access to handwashing facilities, a key metric of hygiene, was only noted for 9 (13.0%) participants with the remaining 87% of the population having no functional handwashing stations. Only 38 (48.7%) participants reported consistent access to soap. Though univariate analysis did not yield significant differences between cases

and controls for the above WASH conditions, these findings underscore the prevalence of WASH insecurity within the general study population.

Similarly, proximity to a body of water was positively associated with leprosy [OR= 2.74, 95% CI (0.93, 8.01)]. These findings build on previous reports of increased leprosy among those living near Lake Tana compared to those geographically distanced from this exposure (36). The type of toilet facilities available to participants was also found to differ between cases and controls. Approximately 37 (49.3%) of participants exclusively relied upon unimproved toilet facilities that did not appropriately segregate waste. A total of 31 (41.3%) people practiced open defecation, a disproportionate number of whom were also diagnosed with leprosy. In fact, the odds of open defecation were 2.6 times higher in leprosy cases compared to controls [OR= 2.6, 95% CI (1.01, 6.73)]. Additional outcomes relating WASH to leprosy are presented in Table 6.

Table 6. *Univariate analysis of WASH data collected over 2019 among leprosy cases and controls. P-values were considered significant if <0.05. Significant values are indicated by an asterisk (*).*

Variable	Cases (n=31)	Controls (n=49)	Total (n=80)	Odds Ratio (95% CI)	P-Value (α = 0.05)
Village Location, n (%)					
On Lake	16 (55.2)	9 (31.0)	25 (43.1)	2.74 (0.93, 8.01)	0.0665
Not on Lake	13 (44.8)	20 (69.0)	33 (56.9)	(ref)	
Missing n=22					
Drinking Water Source, n (%)					
Unimproved	5 (16.7)	9 (18.4)	14 (17.7)	0.89 (0.27, 2.96)	0.8477
Improved	25 (83.3)	40 (81.6)	65 (82.3)	(ref)	
Missing n=1					
Cooking Water Source, n (%)					
Unimproved	5 (16.1)	10 (20.4)	15 (18.8)	0.75 (0.23, 2.45)	0.6335
Improved	26 (83.9)	39 (79.6)	65 (81.3)	(ref)	
Time to Collect Water, n (%)					
Unimproved	27 (87.1)	46 (100.0)	73 (94.8)	0.15 (0.02, 1.38)	0.9711
Improved	4 (12.9)	0 (0.0)	4 (5.2)	(ref)	
Missing n=3					
Time to Collect Water (Unimproved), n (%)					
Limited	4 (14.8)	1 (2.2)	5 (6.9)	7.83 (0.83, 74.12)	0.0729
Basic	23 (85.2)	45 (97.8)	68 (93.2)	(ref)	
Water Treatment, n (%)					
No	19 (65.5)	24 (50.0)	43 (55.8)	1.90 (0.73, 4.92)	0.1864
Yes	10 (34.5)	24 (50.0)	34 (44.2)	(ref)	
Missing n=3					
Toilet, n (%)					
Unimproved	17 (54.8)	20 (45.5)	37 (49.3)	1.46 (0.58, 3.67)	0.4242
Improved	14 (45.2)	24 (54.6)	38 (50.7)	(ref)	
Missing n=5					
Open Defecation, n (%)					
No Toilet Facilities	17 (54.8)	14 (31.8)	31 (41.3)	2.60 (1.01, 6.73)*	0.0486
Toilet Facilities	14 (45.2)	30 (68.2)	44 (58.7)	(ref)	
Missing n=5					
Handwashing Facilities, n (%)					
None	26 (89.7)	34 (85.0)	60 (87.0)	1.53 (0.35, 6.70)	0.5729
Limited	3 (10.3)	6 (15.0)	9 (13.0)	(ref)	
Missing n=11					
Soap, n (%)					
No	19 (61.3)	21 (44.7)	40 (51.3)	1.96 (0.78, 4.94)	0.1532
Yes	12 (38.7)	26 (55.3)	38 (48.7)	(ref)	
Missing n=2					

As previously detailed, WASH data was available for both the 2018 and 2019 study periods. Thus, additional univariate analysis was conducted with this combined dataset. Results, again, illustrate widespread WASH insecurity within the study population. Close to 18% of participants relied on an unimproved source for drinking water, 85.9% traveled off premises for all water needs, and 77.4% lacked access to handwashing facilities. As illustrated in Table 7, a total of 57% of the study population did not treat their water. Consequently, individuals with leprosy had greater odds of not treating their water compared to controls [OR= 2.24, 95% CI (1.10, 4.55)]. Lack of soap for handwashing was also significantly associated with leprosy [OR= 2.19, 95% CI (1.1.6, 4.15)]. Consistent with findings from the 2019 data, 32.9% of the population did not have access to toilet facilities, a disproportionate number of whom were also diagnosed with leprosy. Among leprosy cases, the odds of open defecation were 2.81 times greater than the odds of this exposure in controls [OR=2.81, 95% CI (1.40, 5.61)]. Additional outcomes relating WASH to leprosy are presented in Table 7.

Table 7. *Univariate analysis of WASH data collected over 2018 and 2019 among leprosy cases and controls. P-values were considered significant if <0.05. Significant values are indicated by an asterisk (*).*

Variable	Cases (n=71)	Controls (n=90)	Total (n=161)	Odds Ratio (95% CI)	P-Value (α = 0.05)
Village Location, n (%)					
On Lake	25 (37.3)	24 (34.3)	49 (35.8)	1.14 (0.57, 2.30)	0.7118
Not on Lake	42 (62.7)	46 (65.7)	88 (64.2)	(ref)	
Missing n=24					
Drinking Water Source, n (%)					
Unimproved	15 (21.4)	13 (14.4)	28 (17.5)	1.62 (0.71, 3.67)	0.2513
Improved	55 (78.6)	77 (85.6)	132 (82.5)	(ref)	
Missing n=1					
Cooking Water Source, n (%)					
Unimproved	15 (21.1)	14 (15.6)	29 (18.0)	1.45 (0.65, 3.26)	0.3626
Improved	56 (78.9)	76 (84.4)	132 (82.0)	(ref)	
Time to Collect Water, n (%)					
Unimproved	56 (86.2)	72 (85.7)	128 (85.9)	1.04 (0.41, 2.63)	0.9392
Improved	9 (13.9)	12 (14.3)	21 (14.1)	(ref)	
Missing n=12					
Time to Collect Water (Unimproved), n (%)					
Limited	6 (10.7)	4 (5.6)	10 (7.8)	2.04 (0.55, 7.61)	0.2886
Basic	50 (89.3)	68 (94.4)	118 (92.3)	(ref)	
Water Treatment, n (%)					
No	43 (67.2)	32 (47.8)	75 (57.3)	2.24 (1.10, 4.55)*	0.0257
Yes	21 (32.8)	35 (52.2)	56 (42.8)	(ref)	
Missing n=30					
Toilet, n (%)					
Unimproved	43 (60.6)	46 (54.8)	89 (57.4)	1.27 (0.67, 2.41)	0.4670
Improved	28 (39.4)	38 (45.2)	66 (42.6)	(ref)	
Missing n=6					
Open Defecation, n (%)					
No Toilet Facilities	32 (45.1)	19 (22.6)	51 (32.9)	2.81 (1.40, 5.61)*	0.0035
Toilet Facilities	39 (54.9)	65 (77.4)	104 (67.1)	(ref)	
Missing n=6					
Handwashing Facilities, n (%)					
None	47 (74.6)	42 (80.8)	89 (77.4)	0.70 (0.29, 1.71)	0.4326
Limited	16 (25.4)	10 (19.2)	26 (22.6)	(ref)	
Missing n=46					
Soap, n (%)					
No	42 (59.2)	35 (39.8)	77 (48.4)	2.19 (1.16, 4.15)*	0.0157
Yes	29 (40.9)	53 (60.2)	82 (51.6)	(ref)	
Missing n=2					

Multivariate Analysis

On multivariate analysis of the primary study exposures collected in 2019 (Table 8), several susceptibilities remained connected to leprosy. Controlling for monthly income (indicator of SES), leprosy was significantly associated with lack of soap [aOR= 6.09, 95% CI (1.53, 24.24)] and open defecation [aOR= 1.22, 95% CI (0.38, 3.89)]. An association was also sustained between leprosy and lack of water treatment [aOR= 1.43, 95% CI (0.45, 4.53)]. Thus, markers of water, sanitation, and hygiene all exhibited a positive relationship with the outcome. Other WASH indicators, such as village location, were dropped from the model due to missing data. Though not statistically significant, positive directionality was maintained for the association between helminth infection and leprosy [aOR= 3.23, 95% CI (0.85, 12.35)].

As detailed above, several nutritional markers were strongly associated with the outcome upon univariate analysis. However, variables such as food shortage, fewer meals per day, and BMI were found to exhibit very little influence on other inputs when initially incorporated in the models, suggesting that nutrition either independently drives leprosy or exerts less of an impact compared to WASH factors. In any case, the small study size and need to include a marker of SES constrained the total number of variables able to be included in the model resulting in the exclusion of a nutritional variable. Age and sex were also removed from the final 2019 model after confirming these variables did not act as significant confounders. Given the exploratory nature of the study and the limited size of the dataset, interaction terms were not investigated but may be considered as this line of research progresses going forward.

Table 8. *Model 1: Multivariate logistic regression model of data collected during 2019 featuring leprosy as the outcome and parasitic coinfection, lack of toilet facilities, lack of water treatment, and lack of soap as the exposure variables. Results were controlled for monthly income. P-values were considered significant if <0.05. Significant values are indicated by an asterisk (*).*

Variable	Adjusted Odds Ratio (95% CI)
Monthly Income (ref=Above Q1)	0.16 (0.03, 0.74)
Helminths	3.23 (0.85, 12.35)
Open Defecation (ref=Facilities)	1.22 (0.38, 3.89)
Lack of Water Treatment	1.43 (0.45, 4.53)
Lack of Soap	6.09 (1.53, 24.24)*

On multivariate analysis of *S. mansoni* infection and WASH data collected over 2018 and 2019 (Table 9), open defecation [aOR= 2.32, 95% CI (1.05, 5.12)] and lack of soap [aOR= 2.53, 95% CI (1.17, 5.47)] maintained a positive relationship with leprosy when controlling for age, sex, and education (indicator of SES). Drinking water was included as a metric of water quality but did not prove significant in this particular model. Other WASH data, such as water treatment, was incomplete and therefore unable to be included in the combined model. In terms of parasitic coinfection, schistosomiasis was not significantly associated with leprosy in the present study. However, education below 8th grade was strongly related to the outcome, suggesting that low socioeconomic status may increase the risk of disease [aOR= 3.02, 95% CI (1.02, 8.98)]. Older age (60 years and above) also exhibited positive directionality which may be explained on the basis of declining immunity with advancing age [aOR= 1.28, 95% CI (0.41, 3.98)].

Table 9. *Model 2: Multivariate logistic regression model of data collected over 2018 and 2019 featuring leprosy as the outcome and schistosomiasis infection, lack of toilet facilities, unimproved water, and lack of soap as the exposure variables. Results were controlled for age, sex, and education. P-values were considered significant if <0.05. Significant values are indicated by an asterisk (*).*

Variable	Adjusted Odds Ratio (95% CI)
Old Age (ref=Young Adults)	1.28 (0.41, 3.98)
Middle Age (ref=Young Adults)	0.85 (0.36, 2.03)
Gender (ref=Female)	0.88 (0.40, 1.91)
Education (ref=Grade 8 and Above)	3.02 (1.02, 8.98)*
S. mansoni	0.77 (0.35, 1.68)
Open Defecation (ref=Facilities)	2.32 (1.05, 5.12)*
Unimproved Drinking Water (ref=Improved)	0.93 (0.33, 2.62)
Lack of Soap	2.53 (1.17, 5.47)*

Discussion

Elimination of leprosy as a public health threat, defined as a prevalence of 1 case per 10,000 individuals, was achieved in 2000, yet there were still over 208,619 incident cases of leprosy reported worldwide in 2018 alone (5). In recognition of this alarming trajectory, the WHO launched the *Global Leprosy Strategy 2016–2020* to advance leprosy control globally. The primary objective of this campaign was to facilitate treatment in children and prevent or reduce disability as a result of disease (5). However, this approach failed to incorporate measures of disease prevention targeting susceptibility factors such as undernutrition, parasitic coinfection, and WASH (63).

Support for the presence of alternative pathways of transmission, and in turn risk factors for disease, has come in many forms ranging from animal models to epidemiological case studies. Further, in the decades since the introduction of MDT, the prevalence of leprosy has declined but new incident cases have remained high. This continued transmission despite a diminishing human reservoir implicates other factors such as "ineffective detection of early infection, case reporting deficiencies, or a lack of a thorough examination of potential environmental sources of the bacillus" (10).

Interestingly, an estimated 95% of the world's population is not genetically susceptible to leprosy (3). Most diseased persons are also not infectious given that the mycobacteria remain intracellular in the majority of cases. Only the small percentage of untreated individuals with LL actively excrete *M. leprae* from their nasal mucosa and skin. Together, these factors further depress the explanatory power of human-to-human transmission as the sole driver of continued leprosy incidence. Unfortunately, the inability to culture the bacteria in vitro, the lack of an

effective animal model, and the long incubation period of the pathogen has limited the ability to concretely understand leprosy transmission (64).

In order to chip away at this uncertainty, the present study set out to explore the contributions of other susceptibility factors to the perpetuation of leprosy infection. Findings suggest that host and environmental considerations play a significant role. In terms of nutrition, both food shortage and having fewer meals to eat per day were strongly associated with leprosy upon univariate analysis which aligns with previous research linking prolonged dietary insufficiencies to leprosy onset (11, 15, 19). In a study conducted in Indonesia in 2018, for example, Oktaria and colleagues discovered that household food insecurity was significantly associated with leprosy [OR= 1.13, 95% CI (1.06, 1.21)] (18). Another study of leprosy in Bangladesh found that the odds of food shortage in last year were 1.79 times higher for those with symptomatic leprosy compared to controls [OR= 1.79, 95% CI (1.06, 3.02)] (21). The fact that associations between food shortage, fewer meals per day, and leprosy were even stronger in the present analysis indicates that food insecurity may be more widespread or more severe within this population. Further, these findings build on our study team's data from 2018 (unpublished) in which reducing or skipping meals [OR= 2.87, 95% CI (1.00, 8.32)] and insufficient funds for meals [OR= 10.0, 95% CI (3.44, 29.06)] were significantly associated with leprosy. Other measures of micronutrient intake, such as BMI [OR= 7.20, 95% CI (2.34, 22.11)] and MUAC [OR= 6.82, 95% CI (1.78, 26.13)], were also strongly related to the outcome (23). Though these indicators were not significant in the present analysis, the results substantiate the prevalence of undernutrition in the study population.

As detailed above, limitations as a result of equipment failure and the COVID-19 pandemic restricted the availability of micronutrient data. However, previous evidence details

the importance of both vitamin A and vitamin D in the immune response to leprosy. Multiple studies have identified significantly lower serum levels of vitamin A in leprosy cases relative to controls (24, 25). Similarly, vitamin D deficiency has been implicated in the antimicrobial response to infection with *M. leprae* (65). Coupled with recent research connecting vitamin D deficiency to leprosy in Brazil, concurrent micro- and macronutrient examination in the Ethiopian context remains a top priority (28). Thus, every effort will be made to obtain these micronutrient results when time and resources are once again able to be directed towards areas of study outside the scope of COVID-19.

In terms of coinfection, the abundance of parasitic disease was very high among both cases and controls. Helminth infection was particularly prevalent with 64.1% of the total population testing positive for an intestinal parasite. *S. mansoni* was identified as the primary driver of infection which aligns with current schistosomiasis prevalence estimates in Ethiopia in the amount of five million cases per year (48). As observed in the course of univariate analysis, multivariate logistic regression modeling revealed a positive relationship between helminths and leprosy when controlling for potential confounders [aOR 3.23, 95% CI (0.85, 12.35). Though this finding was not sustained after combining the 2018 and 2019 datasets, prior literature affirms the connection between parasitic coinfection and leprosy as well as other mycobacterial diseases such as tuberculosis (7, 30, 31). A previous study conducted in Brazil, for example, identified a 6.80 increased chance of contracting leprosy in a community with known schistosomiasis cases compared to a community without [RR= 6.80, 95% CI (1.46, 31.64)] (9).

Another analysis by our study team determined that the odds of helminth infection (predominantly *S. mansoni*) were 8.69 times higher in leprosy cases compared with household contacts [aOR= 8.69, 95% CI (1.50, 50.51)]. However, this relationship was not sustained when

the comparison group was made up of non-contact controls [aOR= 1.27, 95% CI (0.38, 4.26)] (28). Given that households contacts more stringently control for SES and other common exposures, this finding suggests that the present study may have benefited from a more robust recruitment and matching scheme involving household contacts of leprosy. Results from the 2-year model may have been further attenuated by the availability of only *S. mansoni* infection data rather than information regarding all helminth exposures in 2018. Unlike nutritional deficiency and WASH, helminth infection is a product of both the host response and the environment. The true nature of the relationship between parasitic coinfection and leprosy, then, may have been blurred by the inability to include all possible, relevant confounders. Going forward, a deeper examination of the epidemiological triangle and the dynamic relationships between host, pathogen, and environment may be needed to fully elucidate the impact of helminth infection on leprosy.

Parasitic coinfection is also known to occur in tandem with poor WASH conditions. For example, a study conducted in southern India found that the odds of practicing open air defecation were 5.37 times higher among children with a parasitic infection [OR= 5.37, 95% CI (1.61, 17.87)] (34). Parasitic coinfection and WASH insecurity have also been linked to leprosy transmission. In the present study, open defecation was positively associated with leprosy in the 2-year model [aOR 2.32, 95% CI (1.05, 5.12). This finding strengthens previous research conducted by our study team in 2018 which identified a connection between open defecation and leprosy infection [aOR= 19.9, 95% CI (2.2, 176.3)] (36).

Additionally, lack of water treatment [aOR= 2.24, 95% CI 1.10, 4.55)] and lack of soap [aOR 2.53, 95% CI (1.17, 5.47)] were significantly associated with the outcome. These findings build on work from our study group in 2018 linking lack of soap to leprosy [aOR= 7.3, 95% CI

(1.1, 49.9)] (36). In combination with previous literature connecting unimproved water, lack of water access on premises, and lack of handwashing with infectious disease risk, it is likely that these conditions meaningfully contribute to leprosy susceptibility (36, 37).

It is also important to note that these risk factors are a product of low socioeconomic status. In order to truly achieve leprosy control, improvements in poverty must occur in tangent with other risk reduction measures. The fact that 30% of the population in this study were found to live on less than 300 Birr per day substantiates the prevalence of poverty in Ethiopia and underscores the need for multifactorial public health interventions that address both physical and socioeconomic contributions to leprosy infection. Future research involving larger sample sizes may generate more confidence in these findings and advance understanding of the interplay between poverty, WASH, and leprosy transmission.

That said, the persistence of significant relationships between WASH conditions after controlling for socioeconomic status lends credence to proposed existence of environmental reservoirs of *M. leprae* and the role of poor water, sanitation, and hygiene in driving continued transmission. Research has shown the presence of *M. leprae* in communal water sources as well as soil samples in highly frequented areas (41, 42). Survivability and viability of the pathogen outside of a human host has also been demonstrated in excess of 8 months under certain circumstances (10). The prevalence of open defecation in this study population coupled with the lack of water treatment and soap for handwashing is strongly suggestive of an environmental pathway connecting exposure to *M. leprae* as a result of limited WASH to the transmission of leprosy. As Ethiopia continues to net a high incidence of leprosy each year despite extensive control measures, additional research to substantiate this pathway is needed.

In addition to sustained leprosy endemicity, Ethiopia also continues to suffer from high rates of food insecurity, parasitic infection, and poor WASH. This study is unique in that it combines both host and environmental risk factors for leprosy in this setting. A major strength of the above investigation lies in the utilization of a variety of different data streams including self-report questionnaires, information from medical records, anthropometric measures, and biological specimens. Further, this study builds on previous research in the realm of parasitic coinfection by investigating a number of helminths and protozoa in addition to *S. mansoni*. Efforts were also made to collect both macronutrient and micronutrient data to better understand the role of nutritional status in disease susceptibility. To date, most of the literature linking nutrition to leprosy has been limited to analysis of information from dietary questionnaires or clinical metrics such as BMI and MUAC. The micronutrient evaluation initiated in this study adds another dimension to the relationship between nutrition and leprosy infection in an effort to affirm and advance several previously proposed causal mechanisms.

However, as with all case control studies, establishing temporality between exposure and disease can prove challenging. This study was no exception, leaving many lingering uncertainties regarding the prospective relationship between undernutrition, parasitic coinfection, and high WASH insecurity. The long incubation period of *M. leprae* further complicates things by extending the window between transmission and symptom onset, thus opening the door to extraneous influence.

Fortunately, existing literature speaks to the presence of underlying biological mechanisms that lend credence to the proposed forward directionality of the above exposures and leprosy onset. For example, major dietary deficiencies are known to cause thymic atrophy, T-cell impairment, and altered production of interleukins and cytokines which play a major role

in immune reactivity to *M. leprae* (17). Similarly, parasitic infection results in the downregulation of cellular and humoral responsiveness to all pathogens, paving the way for the onset of active leprosy after exposure to the disease-causing bacterium (7). Perhaps even more compellingly, laboratory studies have confirmed the survivability of *M. leprae* outside of a human host for many months (8, 40). The existence of potentially viable *M. leprae* in highly frequented areas, such as community water sources, has been detected through DNA sampling and may serve as a common environmental exposure driving sustained leprosy transmission in endemic areas (41). Finally, the collection of physiological data in real time, such as the stool testing and micronutrient assays, provides greater confidence in the prospective nature of these relationships.

Despite the above assurances, the small sample size of the study poses another possible limitation, especially as the number of cases did not quite reach the projected sample size. These concerns are attenuated in light of the supplemental *S. mansoni* and WASH data obtained from 2018 which allowed for a larger database through which to explore exposure and outcome relationships as well as the opportunity to build on previous findings. Nevertheless, future investigations should emphasize the recruitment of additional cases in order to maximize statistical power. Focus should also be directed towards the collection of complete data given that certain variables in the present analysis, such as village location, were unable to be examined in depth due to the number of missing values.

Finally, while this study measured a number of different nutrition, parasitic coinfection, and WASH variables, other host and environmental conditions are known to exert influence over the immune response and may alter the risk profile for leprosy infection. In the same vein, it is possible that these susceptibilities not only facilitate transmission, but also accelerate the

progression of disease from latent to clinically symptomatic in previously infected individuals. Alternatively, one or more of these conditions may predispose individuals to the multibacillary form of leprosy which is associated with less cell-mediated immunity and a more severe disease course. The fact that 93.3% of cases in the present study were diagnosed with multibacillary leprosy lends credence to this theory. Future research should explore these potentialities in more depth in order to gain a better understanding of leprosy transmission and inform prevention efforts.

Chapter 4: Public Health Impact

Leprosy remains the leading infectious cause of disability in the world and, as such, efforts to better understand and prevent transmission must be accelerated (64). A cohort study in Ethiopia showed that 47% of new cases already had established nerve function impairment at diagnosis, with an additional 8% reporting recent nerve damage. Another 12% developed nerve damage after the start of MDT, leaving only 33% of cases without clinical evidence of nerve involvement at any time (64). Women continue to be disproportionately impacted, with rates of delayed diagnosis and disability even higher in this demographic.

Time of diagnosis relative to the progression of disease and treatment adherence are key determinants of disability status. Lack of prompt identification of leprosy varies by setting but generally comes about due to a combination of slow clinical decision-making on the part of healthcare providers and delayed presentation to clinic on the part of leprosy patients (64). Though grossly inhumane, the concentration of positive cases in leprosy colonies up to the mid-20th century resulted in a number of doctors and researchers who specialized in the disease, often using individuals in these communities as involuntary test subjects to advance scientific understanding and develop treatments. Effectiveness of these treatments, elimination of leprosy in certain parts of the world, and eventual dissolution of leprosy colonies resulted in fewer and fewer healthcare providers with a comprehensive understanding of the disease. In non-endemic countries in particular, general practitioners and even dermatologists are unlikely to identify and assign a leprosy diagnosis in early stages. When the disease has progressed to the point of overt symptomatology, providers may not know how to formulate an effective treatment plan (3). Individuals with leprosy may further contribute to a delay in diagnosis due to the continued

stigma surrounding the disease, waiting to seek treatment until symptoms are severe and chronic disability is more likely (64).

Those who do seek care in a timely manner and receive a prompt diagnosis must also grapple with the potential consequences of drug therapy. Type 1 and type 2 immune reactions occur in approximately 30% of multibacillary patients during or after MDT. Steroids, the treatment of choice for modulating immune reactions, is often insufficient at staving off recurrence of immunoreaction with approximately 20-50% of cases experiencing a relapse. There are no good tests to predict the nerve damage these reactions may cause as the mechanisms involved in neural degeneration remain unclear. That said, around 15 million people have been treated with MDT to date, and an estimated 2 million people are free of disability as a result of these measures (64).

Much remains to be explored in the realm of leprosy. Genetic sequencing of *M leprae* occurred in 2001, opening the door to a plethora of new research avenues (64). Other opportunities to understand leprosy continue to occur in tandem with tuberculosis, another mycobacterium-causing disease. The physiology of these conditions is so similar that the Bacillus Calmette–Guérin (BCG) vaccine intended to confer protection against tuberculosis has actually been shown to incite an immune response against leprosy. Epidemiologic studies suggest that the presence of a BCG scar is in fact protective against leprosy, with an estimated vaccine effectiveness of approximately 52% among individuals at risk of infection (11). Many speculate that the administration of the BCG vaccine in infants in countries with endemic tuberculosis has contributed to the global decrease in leprosy prevalence (64). Others postulate that the BCG vaccine shifts the immune response to the less severe, tuberculoid end of the spectrum among vaccinated individuals exposed to leprosy (15). Unfortunately, concerns have

been raised that the creation of a more specific and protective vaccine against tuberculosis will replace the BCG vaccine, resulting in the loss of protection in individuals concurrently at risk of leprosy (64). As leprosy research garners less attention and financial investment than that of tuberculosis, this divergence would be of grave consequence to the advancement of leprosy control and prevention.

Interestingly, prior research has demonstrated that many of the proposed susceptibility factors in this paper, such as malnutrition and parasitic coinfection, reduce the ability of existing vaccines to produce the desired immune response. Malnourished individuals, for example, do not develop skin tests reactions to tuberculin after BCG vaccination as often or as robustly compared to their well-nourished counterparts (22). Advanced malnutrition has also been correlated with false negative tuberculin responses, suggesting that diagnostic measures are less effective within this demographic (17). Similar findings have been identified in individuals with concurrent helminth infection (31). The potential for these susceptibility factors to attenuate the effectiveness of prophylactic and diagnostic measures coupled with their ability to facilitate onset of disease has grave implications for the treatment of tuberculosis as well as for the treatment and control of other mycobacterial diseases, such as leprosy. This reality underscores the need for public health interventions that target the multifactorial etiologies of these diseases and preserve the efficacy of current prophylactic, diagnostic, and treatment measures.

Though progress in the realm of leprosy is likely to occur in tandem with tuberculosis, it is important to highlight the health consequences specific to leprosy including disability, poverty, stigma, and social isolation. In India, for example, an estimated 1 million individuals are chronically disabled as a result of past or present leprosy. Approximately 57% of these individuals experience undernutrition, with 10% suffering severely (14). Diffey and colleagues

found that individuals cured of leprosy with residual deformities were more undernourished than recovered leprosy patients without permanent physical disability. Undernutrition in this subgroup was also associated with decreased expenditures on food, increased unemployment, and loss of income (13). Others have equated lower food intake in recovered leprosy patients with decreased physical strength and reduced overall wellbeing (66).

Deeply rooted stigma as a result of misinformation, religious beliefs, and cultural norms continues to flame poorer health outcomes among individuals who are presently infected or have recovered from leprosy which in turn contributes to an increased risk of poverty (3). In a cyclical fashion, poverty has been linked to nutritional deficiencies, increased risk of parasitic exposure, and poor WASH conditions which were explored as susceptibility factors in this analysis (2, 19, 20, 36, 37). Thus, additional research in this arena not only has the power to improve the wellbeing of individuals with past or present leprosy infection, but also the potential to reduce the risk of leprosy onset among a particularly vulnerable population.

Perhaps of greatest importance, this study adds to the existing research implicating WASH as an environmental driver of leprosy (36, 37). The identification of this exposure as a probable factor in the transmission of *M. leprae* opens the door to a range of opportunities for public health intervention. Lack of toilet facilities, water treatment, and soap were all significantly related to infection in this analysis. Efforts to provide functional and sustainable toilet facilities and water filtration systems may be just a few of the ways in which to potentially interrupt the transmission cycle and reduce the burden of leprosy in endemic settings. Similar interventions may be possible in the context of undernutrition and parasitic coinfection. Going forward, these susceptibility factors and others should be studied in more depth so as to reduce the burden of leprosy and achieve a greater degree of control globally.

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