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Hatem Mohamed

22nd April 2024

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Poor WASH, undernutrition, and food insecurity is associated with anti-PGL1 positivity, marker of leprosy infection, in Addis Ababa, Ethiopia

By

Hatem Mohamed
MPH

Hubert Department of Global Health

_____
Jessica K. Fairley, MD, MPH
Committee Chair

_____
Chad Robichaux, MPH
Committee member

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By

Hatem Mohamed
Bachelor of Medicine and Surgery (M.B.B.C.h)
Cairo University, Egypt
2014

Thesis Committee Chair: Jessica Fairley, MD, MPH

Thesis Committee member: Chad Robichaux, MPH

An abstract of
A thesis submitted to the faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Health
2024

Abstract

Poor WASH, undernutrition, and food insecurity is associated with anti-PGL1 positivity, marker of leprosy infection, in Addis Ababa, Ethiopia

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Given stagnant global leprosy cases, more needs to be done for elimination and halting local transmission. Leprosy has been associated with risk factors of those who are socioeconomically disadvantaged, including low education, unsafe access to WASH, and experiencing food insecurity. The use of point of care anti-PGL1, a specific IgM antibody to *M.leprae* infection, has been developed as a supplementary tool in diagnosis or identifying exposure and increased risk of developing leprosy. We conducted a cross-sectional study to determine the prevalence of anti-PGL1 seropositivity in surrounding communities of ALL-African Leprosy, Tuberculosis Rehabilitation Center (ALERT), a former leprosy hospital, and to compare them to seronegative. An interviewer-led structured questionnaire about sociodemographic, environmental, and nutritional factors was administered by trained health personnel in Amharic. Anthropometric measures were collected, and peripheral blood samples were drawn and tested against anti-PGL1 using point-of-care lateral flow (ML Flow). Three hundred and nine leprosy-free individuals were recruited from the clinics at ALERT hospital from May till December 2023. Of the 319 participants, 66% were females, 17% had no formal education, and 40% reported no source of income. The prevalence of anti-PGL1 seropositivity was 36.8% (n=118) with a mean age of 39(SD ±15), a mean BMI 23.1 (SD±3.4) and a mean mid upper arm circumference (MUAC) of 25.3 cm (SD±2.9) with a significant mean difference compared to seronegative. Our combined multivariable logistic regression for sociodemographic and environmental factors showed that PGL1 seropositive individuals had higher likelihood with owning agriculture land (aOR 2.95, 95% CI [1.22: 7.51]; p=0.019) and using unimproved bathing water source (aOR 3.85, 95% CI [1.57: 10.2]; p=0.004) compared to seronegative, controlling for age, sex, source of income, and education. The combined multivariable logistic regression for nutrition and sociodemographic factors showed that seropositive participants had lower MUAC (≤ 22 cm) (aOR 1.98, 95% CI [0.97:4.09], p=0.060) and reported a higher frequency of not eating for an entire day within the past year (aOR 1.77, 95% CI [0.95; 3.29]; p=0.071). In the integrated logistic regression model, seropositive participants demonstrated higher odds of owning agriculture land (aOR 2.85, 95% CI[1.16: 7.40]; p=0.025), utilizing unimproved water source for bathing (aOR 3.84, 95% CI[1.56: 10.1], p=0.005) and of younger age (31-45 years vs. above 45 years) (aOR 2.50, 95% CI[1.34: 4.75],p=0.004) compared to seronegative, controlling for sex, source of income and education. Our study identified an increased prevalence of PGL1-antibody among otherwise healthy community members that highlights the possibility of occult transmission of infection. Environmental and nutritional factors were shown to have a positive association with leprosy infection.

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Acknowledgements

I would like to express my sincere gratitude to Professor Jessica Fairley and Chad Robichaux for their invaluable guidance, unwavering support, and insightful feedback throughout the process of developing this work. Their expertise and encouragement have been instrumental in shaping my research and enhancing its quality. I extend my heartfelt appreciation to my wife, Salma, baby Adam, and to our extended family for their consistent support, understanding, and patience during this journey. Their love, encouragement, and belief in me have been a constant source of strength and motivation.

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Chapter I: Literature Review

Hansen's Disease, also known as leprosy, is caused by the *Mycobacterium leprae* pathogen, and less commonly by the almost identical *Mycobacterium lepromatosis*. This bacterium is an obligate intracellular organism that typically infects macrophages and Schwann cells within the nerves[1]. The primary mode of transmission is thought to be through prolonged contact with untreated individuals who have the disease, often occurring via respiratory droplets. However contact with environmental and armadillo reservoirs of infection is also thought to be a mode of transmission[2]. *M. leprae* predominantly targets the skin and peripheral nerves. If left untreated, it can result in permanent skin disfigurements and disabilities. The incubation period between exposure to the mycobacterium and the developing of disease is prolonged and may range between 2 years and up to 20 years before developing visible clinical symptoms[3]. It is noteworthy that 95% of individuals are thought to be resistant to leprosy[4]. This suggests that either the host's cell-mediated immune response successfully eliminates the infection, or the infection is effectively controlled and may become latent over time in the majority of those exposed [5]. The progression of disease is attributable to the potency of the cell mediated immune response and the release of pro-inflammatory or anti-inflammatory cytokines. Little is known what factors render people more susceptible to infection and develop clinical manifestations (clinical leprosy)[2].

Leprosy is regarded as one of the 20 neglected tropical diseases (NTDs) that the World Health Organization (WHO) has included in its 2030 roadmap to eliminate.[6] This roadmap builds

on the 2030 Sustainable Development Goals (SDGs) target 3.3, which entails ending the epidemic of multiple communicable diseases, including the NTDs.[7] The Global Leprosy Strategy 2021-2030 has set targets for leprosy elimination and interruption of transmission through 1) a 70% reduction of new cases from baseline set at 2020; 2) zero indigenous cases; 3) a 90% reduction in rate (per million) of children new cases; and 4) a 90% reduction in rate (per million) of population with grade 2 disabilities.[8] Since the introduction of multidrug therapy (MDT) in 1982 with a 6-month regimen (Rifampicin and Dapsone) for PB, and a 12-month triple therapy (Rifampicin, Dapsone and Clofazimine) for MB, several countries were successful in eliminating the disease and leprosy has been disregarded as a global public health threat[3]. However, new cases are still being detected globally, and in 2022, 174,000 new cases of leprosy were reported, which translates to 21.8 cases per million incidence detection rate, a 21% increase in incidence from 2021. Most of these cases were reported from Southeast Asia (72%) followed by the African region (12.6%).[9] Although substantial gains in leprosy control programs have occurred since the MDT introduction, diagnosis delay, disease associated stigma, and low access to health care have hindered global progress, especially in low- and middle-income countries.[10]

Diagnosis of leprosy depends predominantly on the main clinical symptoms with the help of histopathological skin biopsy (slit skin smears) to confirm diagnosis and classify cases according to Ridley-Jopling classification.[11] The three main signs are:1) loss of sensation in hypopigmented skin patch; 2) thickened or enlarged peripheral nerve with loss of sensation and/or weakness of the muscles supplied by nerve; 3) presence of acid fast bacilli in a slit-skin smear.[12] The slit skin smear results led to the classification into multibacillary (MB) that included polar lepromatous leprosy (LL), borderline lepromatous (BL) and mid-borderline (BB) where

bacterial index was more or equal to 2 at any site of smear. Paucibacillary included polar tuberculoid (TT), indeterminate (I) and borderline tuberculoid with bacterial index less than 2 at all sites.[11] Due to difficult technicalities required with the slit smear bacilli detection, another classification of leprosy was introduced by the WHO into three groups: 1) single lesion paucibacillary (one skin lesion); 2) paucibacillary leprosy(2-5 skin lesions); 3) multibacillary (more than five skin lesions).[12] This classification helps to determine the duration of multidrug therapy and avoid delays in the detection and the diagnosis of the cases.

Recognizing the challenges associated with clinically detecting leprosy, histopathological confirmation, and the inability of culturing *M. leprae* in vitro, serologic, and molecular testing through ELISA and PCR have been developed to assist in diagnosis. More recently, point-of-care lateral flow tests have also been introduced for this purpose.[13] Although PCR tests detecting the mycobacterium DNA is both more specific and sensitive than ELISA and lateral flow tests, it is not feasible to be used as a standard diagnostic test given most of leprosy cases are in low resource settings.[3] Phenolic glycolipid 1 (PGL1) which is present in the cell wall of the *M. leprae* have been extensively studied and determined as a species specific antigen that can offer more insights in proper classification of paucibacillary (PB) and multibacillary (MB) leprosy through the detection of anti-PGL1 antibodies(mainly IgM).[14] Penna et al. found in a systematic review that anti-PGL1 antibodies can be useful as a predictor for developing leprosy among healthy contacts, showing an three times higher odds of developing disease for those with positive anti-PGL1 result (3.11,95% CI [2.22-4.36]; I^2=26.6%).[15] Douglas et al. reported that household contacts who became positive by ELISA had a 7.65-fold-higher risk of developing MB or PB leprosy than contacts who were negative by ELISA.[16] In endemic areas, subclinical infection with

development of antibodies to PGL1 ranges between 1.7-35%.[17] Bührer-Sékula et al. described the development of a rapid lateral flow test (ML flow test) for the detection of IgM antibodies to PGL-I.[18] They found that the ML flow test, in comparison to ELISA, showed 91% of agreement, a sensitivity of 97.4% in correctly classifying multibacillary (MB) patients, and a specificity of 90.2% and suggested the use of the ML flow test for identification of contacts at higher risk of developing the disease in the future.

WASH, Environment and Leprosy Infection

According to WHO's report on the health burden of unsafe water supply, sanitation and hygiene (WASH), it has estimated that in 2019, 1.4 million deaths and the loss of 74 million disability-adjusted life years (DALY) were attributed to the use of unsafe WASH.[19] Anticipating and foreseeing such critical issues, numerous strategies and programs have been devised to monitor and advocate for safe WASH. Established in 1990, the WHO/UNICEF joint monitoring programme (JMP) on water supply, sanitation and hygiene produces updated reports on countries' WASH situation.[20] For each of these pillars, there are service ladders that entails their categorization. For water supply, it classifies it if delivered through an improved source (home tap, public standpipe, protected borehole, protected spring) as 1) Safely managed; 2) Basic; and 3) Limited given the availability on premises and the time needed to collect, in addition to 4) Unimproved (directly from dug well, spring water); and 5) Surface water. As for sanitation services, according to the facility type classified into improved and unimproved facility depending on the safe separation of human excreta from human contact. Improved facilities include flush toilets, pit latrines with slabs (or ventilated pit latrines), and composting toilets. The service ladder categorizes population using improved sources into 1) Safely managed; 2)

Basic; or 3) Limited, depending on presence of piped sewers, or treatment site of excreta (off-site vs on-site), and facility sharing with other households; 4) Unimproved facilities; or 5) Open defecation. Hygiene is categorized into Basic, limited, or No facility according to availability of handwashing facility with soap and/or water on premises.[20]

As the connection between neglected tropical diseases (NTDs) and WASH became increasingly acknowledged, the WHO developed the global strategy for Water, Sanitation and Hygiene (WASH), in 2015, recognizing its pivotal role in advancing progress on NTDs.[21] This paved the integration of improved WASH as one of the five main components in the global NTD road map. Furthermore, A renewed 2021-2030 strategy aimed to advance the cooperation and joint involvement between NTD and WASH stakeholders has been developed.[22] Within this framework, in the context of leprosy, alongside efforts in social inclusion, behavioral change and treatment, environmental interventions, particularly the provision of safe water and sanitation services, are recognized as an essential component in mitigating the disease burden.

Despite leprosy's primary mode of transmission through respiratory droplets or close contact with a leprosy case, mainly in the household, evidence of viable *M. leprae* in non-human environment (soil, water, plants) indicating potential alternative routes of transmission.[23] Notably, *M. leprae* has been shown to remain viable in the environment for about 40 days under humid conditions.[24] Turankar et al., in West Bengal, found potentially viable M. leprae (through detection of 16S rRNA) in 28 (39.4%) out of 71 positive samples collected from the soil surrounding leprosy patients, which also displayed similar single nucleotide polymorphism(SNP) type (Type1) as that found in the patients' slit skin.[25] In India, Mohanty et al. collected environmental samples from the surroundings of 169 positive slit skin

smear patients and found 43 (25.4%) soil samples and 41 (24.2%) positive for viable leprosy bacilli.[26] Lavania et al. also found viable M. leprae in 28 of the 80 (35%) of the soil samples collected from villages of endemic area in Ghatampur, India.[27] This growing evidence of presence of viable *M.leprae* in the environment intrigued more researchers to uncover associations between environmental factors and leprosy disease.

A case control study in North Gondar, Ethiopia, found an association between leprosy cases and open defecation (OR= 2.81, 95% CI [1.40:5.61]), lack of water treatment (OR= 2.24, 95% CI [1.10:4.55]), lack of soap (OR= 2.19, 95% CI [1.16:4.15]).[28] An earlier study done in the same region by Emerson et al. found significant association between poor to moderate WASH access and Leprosy disease. Unimproved water source (OR = 4.22, 95% [CI 1.07:16.22]), lack of premises' water access (OR = 2.83, 95% CI [1.05:7.65]), lack of soap (OR = 2.61, 95% CI [1.06:6.42]), lack of handwashing (OR = 4.56, 95% CI [1.69:12.28]), and open defecation (OR = 4.32, 95% CI [1.67:11.18]) were significantly associated with leprosy.[29] Data from both studies combined showed that open defecation (aOR= 2.32, 95% CI [1.05:5.12]) and lack of soap (aOR= 2.53, 95% CI [1.17:5.47]) were significantly associated with leprosy. Kumar et al. found that people living in households with available sanitation facilities, in urban Agra India, had 28% lower adjusted odds of developing leprosy disease (OR= 0.72, 95% CI [0.53:0.97]).[30] In Brazil, a case control study showed that bathing in open water (OR= 1.79, 95% CI [1.18:2.70]), and household floor exposed to dirt (OR= 1.46, 95% CI [1.04:2.06]) were significantly associated with leprosy disease.[31] Although these studies highlight a possible link between environmental exposure and leprosy, the entry route is not clearly identified. Further research in this area is crucial to unravel this relationship and to inform preventative measures in the future.

Socioeconomic Status, Food insecurity and Leprosy

While unsafe WASH may be one way that leprosy disproportionately impacts impoverished, it has the long-standing association with socioeconomic disadvantaged and impoverished communities brings up the question of other mechanisms. This could encompass the living conditions within the household and the surrounding environment, as well as factors such as food insecurity.[32] Furthermore, Inadequate food intake leading to deficiencies in both macro and micronutrients has been linked to compromised immunity and hypothesized increased susceptibility to leprosy infection and the progression to clinical disease.[32]

A systematic review and meta-analysis examining studies assessing Body Mass Index (BMI) among leprosy patients and controls revealed a significant mean difference in BMI (-17.88 , 95% CI [-27.65:-8.12]; p=0.0003; I^2=100%).[33] Additionally, a case-control in Indonesia by Oktaria et al. identified a significant association between leprosy disease and anemia (OR = 4.01, 95% CI [2.10:7.64], p = 0.000).[34] In Gondar, Ethiopia, Anantharam et al, in the univariate analysis, found significant association between underweight (BMI less than 18.5) and low mid upper arm circumference (MUAC less than 21 cm) with leprosy disease (OR 9.25, 95% CI [2.77: 30.81]; p=0.003) and (OR 6.82, 95% CI [1.78:26.13]; p=0.0004) respectively, and in the multivariate analysis underweight status (less than 18.5) remained significant (aOR 10.32, 95% CI [1.79: 59.67]); p<0.05).[35] Two studies conducted in Bangladesh found a statistically significant association between food insecurity and leprosy disease (OR 1.79, 95% CI 1.06:3.02; p = 0.030)[36] and (OR 2.42, 95% CI 1.08:5.47; p = 0.034)[37] respectively. Similarly, a study in Brazil demonstrated that experiencing food shortage at any time in life increased the odds for leprosy disease (OR=1.65, 95% CI 1.11:2.42).[31] Educational status, a proxy indicator for socioeconomic

status, have been studied in relation to leprosy disease and low levels of education have been shown to have increased odds for leprosy disease (aOR 3.02, 95% CI[1.02:8.98])[28] and (aOR 5 1.87; 95% CI 1.29:2.74).[31]

Chapter II: Manuscript

Abstract

Given stagnant global leprosy cases, more needs to be done for elimination and halting local transmission. Leprosy has been associated with risk factors of those who are socioeconomically disadvantaged, including low education, unsafe access to WASH, and experiencing food insecurity. The use of point of care anti-PGL1, a specific IgM antibody to *M.leprae* infection, has been developed as a supplementary tool in diagnosis or identifying exposure and increased risk of developing leprosy. We conducted a cross-sectional study to determine the prevalence of anti-PGL1 seropositivity in surrounding communities of ALL-African Leprosy, Tuberculosis Rehabilitation Center (ALERT), a former leprosy hospital, and to compare them to seronegative. An interviewer-led structured questionnaire about sociodemographic, environmental, and nutritional factors was administered by trained health personnel in Amharic. Anthropometric measures were collected, and peripheral blood samples were drawn and tested against anti-PGL1 using point-of-care lateral flow (ML Flow). Three hundred and nine leprosy-free individuals were recruited from the clinics at ALERT hospital from May till December 2023. Of the 319 participants, 66% were females, 17% had no formal education, and 40% reported no source of income. The prevalence of anti-PGL1 seropositivity was 36.8% (n=118) with a mean age of 39(SD ±15), a mean BMI 23.1 (SD±3.4) and a mean mid upper arm circumference (MUAC) of 25.3 cm (SD±2.9) with a significant mean difference compared to seronegative. Our combined multivariable logistic regression for sociodemographic and environmental factors showed that PGL1 seropositive individuals had higher likelihood with owning agriculture land (aOR 2.95, 95% CI [1.22: 7.51]; p=0.019) and using unimproved bathing water source (aOR 3.85, 95% CI [1.57: 10.2]; p=0.004) compared to seronegative, controlling for age, sex, source of income, and education. The combined multivariable logistic regression for nutrition and sociodemographic factors showed that seropositive participants had lower MUAC (≤ 22 cm) (aOR 1.98, 95% CI [0.97:4.09], p=0.060) and reported a higher frequency of not eating for an entire day within the past year (aOR 1.77, 95% CI [0.95; 3.29]; p=0.071). In the integrated logistic regression model, seropositive participants demonstrated higher odds of owning agriculture land (aOR 2.85, 95% CI[1.16: 7.40]; p=0.025), utilizing unimproved water source for bathing (aOR 3.84, 95% CI[1.56: 10.1], p=0.005) and of younger age (31-45 years vs. above 45 years) (aOR 2.50, 95% CI[1.34: 4.75],p=0.004) compared to seronegative, controlling for sex, source of income and education. Our study identified an increased prevalence of PGL1-antibody among otherwise healthy community members that highlights the possibility of occult transmission of infection. Environmental and nutritional factors were shown to have a positive association with leprosy infection.

Introduction

Leprosy is one of three neglected tropical diseases (NTDs) that are aimed for elimination and interruption of transmission by the 2030 NTDs Road map.[6] The target in the Road map for leprosy includes zero locally acquired leprosy cases for 120 countries. With the aid of the multi-drug therapy (MDT), the global target of less than 1 case per 10,000 population in the registered prevalence, set by the World Health Assembly resolution WHA44.9 in 1991, has been achieved by multiple endemic countries.[38] However, over the past decade still around 200,000 new cases were reported annually worldwide, and in 2021, 174 087 new cases were reported globally, with the majority of cases in South-East Asian and African Regions.[9]

Ethiopia is regarded as one of the 23 global priorities for leprosy according to an index that takes into account the prevalence of disease, new cases detected and the proportion of these new cases who are children, women, or with grade 2 disability (G2D).[39] Over the past decade, Ethiopia has been reporting annually between 5000 - 3000 new cases of leprosy. In 2022, out of the 2966 new cases detected,79% were MB leprosy, 39% were women, 12% were children and 10% were with grade 2 disability.[9]

Leprosy is a chronic granulomatous condition caused by Mycobacterium Leprae. This pathogen primarily affects the skin and peripheral nerves, leading to disfigurement and functional impairments without adequate treatment.[2] While the main route of transmission is through respiratory droplets among contacts of individuals with leprosy, contact with environmental reservoirs or armadillos has also been regarded as a potential source of infection.[1,40]

M.leprea is a slow growing pathogen and cannot be cultivated in vitro and the diagnosis is mainly through clinical examination and confirmation occurs through histopathological examination from skin biopsy (slit-skin smear) from the lesions.[2] The criteria for diagnosing leprosy include: 1) one or more hypopigmented skin lesion with loss of sensation; 2) thickened peripheral nerves with loss of sensation and/or muscle weakness; or, 3) histopathological confirmation (slit-skin smear).[3] Although PCR detection of *M.leprae* DNA or detection of leprosy specific antibodies by Enzyme linked immunoassay (ELISA) are available, their use is limited to more specialized centers and not for routine diagnosis.[41] Consequently, the delays in diagnosis and treatment, aggravated by social stigma and isolation that the patients face, have posed as barriers to interrupt local disease transmission.

Phenolic glycolipid 1 (PGL1), present in the cell wall of *M. leprae,* have been extensively studied and determined as a species specific antigen that can offer more insights in proper classification of paucibacillary (PB) and multibacillary (MB) leprosy through the detection of anti-PGL1 antibodies (IgM).[14] Furthermore, one study found that among healthy contacts with seropositive anti-PGL1 had a three fold odds of develop leprosy.[15] Another study even showed a 7.65-fold-higher risk of developing MB or PB leprosy than household contacts who were negative by ELISA.[16] In endemic areas, subclinical infection with development of antibodies to PGL1 ranges between 1.7-35%.[17] A rapid lateral flow test (ML flow test) was previously developed for the detection of IgM antibodies to PGL-I.[18] It was found that the ML flow test in comparison to ELISA showed 91% of agreement, sensitivity of 97.4% in correctly classifying multibacillary (MB) patients, and specificity of 90.2%, however for PB it had a lower sensitivity

at 40% and for household contacts 28.6%.[18] Nevertheless, ML flow test was suggested for the identification of contacts who at higher risk of developing the disease in the future.

Upon infection with *M.leprae*, based on the host-pathogen interaction, cell-mediated immune response and the release of anti-inflammatory or proinflammatory cytokines, patients could develop either multibacillary(MB) or paucibacillary(PB) form after an average incubation between 2-5 years and in some cases up to 20 years.[1,5] In addition, a proportion who gets infected may not show any clinical signs or symptoms and remain latently infected.[2] However, the exact factors that render people more susceptible to infection or progression to the disease remain poorly understood. Several studies investigated factors that could be associated with increase in susceptibility and the risk of infection. Poor access to water supply, sanitation and hygiene (WASH) has been shown to have an association with leprosy infection and disease.[28,29] Additionally, the WHO released a technical guidance to emphasize the intersection between clean and safe WASH and NTDs including leprosy.[21,22] Although, the exact role of unimproved water sources and sanitation facilities are not clearly established with leprosy diseases, studies have shown the shedding of *M.leprae* from infected individuals and these sources can act as a reservoir.[40] Furthermore, nutrition which have been shown to play an important role in immunity and especially cell mediated immunity in the case of leprosy, as the pathogen is an obligate intracellular, also have been studied to identify more risk factors associated with the disease.[32,33,35]

In the current cross-sectional study, we have used a point of care anti-PGL1 lateral flow kits to screen for the presence of the anti-PGL1 antibody among healthy community members

as a marker for latent leprosy infection in the outpatient clinics in the ALL-African Leprosy, Tuberculosis and Rehabilitation Training center (ALERT) in Addis Ababa, Ethiopia.

Materials and Methods

Ethical Approval

This study was approved by the Institutional Review Boards of Emory University and the AHRI/ALERT Ethics Review Board. Participants were informed about the study purpose, procedures, risks and benefits. They were informed that participation in the research study is voluntary and that they are free to decline to be in the study, or to withdraw from it at any point without any negative consequence.

Study area and population

An analytical cross-sectional study was conducted from May till December 2023 in Addis Ababa, Ethiopia, with a focus on Nifas Silk Lafto, Kolfe Keranyo and Gulele Sub-cities. Addis Ababa extends over 527 square kilometers and has a population estimate of 4 million people.[42] Participants were recruited from the ALL-African Leprosy, Tuberculosis and Rehabilitation Training center (ALERT),from non-leprosy outpatient clinics (surgery, ophthalmology and internal medicine), through a convenience sample.

Inclusion criteria:

1) No current or previous diagnosis with leprosy.
2) 18 years of age or above.
3) Provides informed consent.

Exclusion criteria:

1) Below 18 years of age.
2) Recent or previous diagnosis with leprosy disease.

Data collection

After signing an informed consent in the outpatient clinics, trained medical personnel collected the data, in Amharic, using structured questionnaire. In addition, peripheral blood sampling and anthropometric measures (weight, height, and mid upper arm circumference) were recorded.

The original questionnaire in English was translated into Amharic, back-translated, and validated prior to the commencement of the study. The questionnaire comprised three main blocks:1) Demographic and socioeconomic Characteristics; 2) Environmental and WASH survey; and 3) Food and nutrition survey.

The section on demographic and socioeconomic characteristics included questions regarding education level, income source, occupation type, household size, and the presence of children in the household. The environmental and WASH block incorporated questions adapted from WHO/UNICEF Joint Monitoring Programme for Water, Sanitation and Hygiene (JMP) household water and sanitation survey[43]. These questions covered source of water for drinking, bathing, and cooking, water treatment and type, time of water collection, types of used sanitation facility, sharing of facilities, and the presence of handwashing facility and soap, and lastly household floor (exposure to dirt), agricultural land ownership and possession of poultry or livestock in house/backyard. Water sources were categorized into improved and unimproved sources. Improved sources include home tap water; public tap / standpipe; bottled water; borehole; protected dug well; and rainwater collection are considered improved sources

otherwise unimproved (jerry can, surface water, tanker-truck, cart with small tank / drum, unprotected dug well, unprotected spring well). For sanitation, toilet facilities would be categorized as improved if they were any of the following types: flush/pour flush toilets to piped sewer systems or septic tanks; and pit latrines: ventilated improved pit (VIP) latrines, pit latrines with slabs; and composting toilets. Unimproved toilet facilities included: pit latrine without a slab or open pit; bucket; hanging toilet. Households with no facility or use of bush/field, are considered open defecation, and included with unimproved Finally, the nutrition block contained questions adapted from the US Department of Agriculture Household Food Security survey.[44]

Anti-PGL1 antibody was tested on the whole blood sample using the lateral flow point-of-care anti-PGL1 IgM (Bioclin) kit (Belo Horizonte, Brazil). Height and weight were measured, and body mass index (BMI) was calculated as kg/m^2. Underweight was defined as a BMI <18.5. Mid-upper arm circumference (MUAC) was measured with a MUAC band on the left arm at the mid-point between the tip of the shoulder and the tip of the elbow and recorded in centimeters. For adults, a low MUAC corresponding to being underweight or malnourished is defined as a MUAC ≤ 22 cm. Although a global cutoff for MUAC to correspond to malnourishment has not been standardized, a guide by the Food and Nutrition Assessment Technical Assistance (FANTA) project recommended the use of a cutoff range between ≤23-≤25.5 cm and for Ethiopia recommended a cutoff for ≤21 cm .[45] However when performing sensitivity(SENS), specificity(SPEC), positive predictive value(PPV) and negative predictive value(NPV) and diagnostic accuracy calculations, using OpenEpi,[46] for different cutoffs (21cm,21.5 cm,22 cm, 22.5 cm and 23 cm), a cutoff of ≤ 22 cm yielded better results(Annex

1,p.43) in correlation with underweight (BMI<18.5) with corresponding values SENS 52.38, SPEC 90.38, PPV 28.21 and NPV 96.34.

Data management and statistical analysis

Study data were collected and managed using REDCap electronic data capture tools hosted at Emory University.[47,48] REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies. Sample size: To determine the prevalence of anti-PGL1 antibody, a study sample of 300 was targeted.[49,50] Data exported from REDCap was then imported into R Studio.[51] Descriptive statistics, including frequencies, means, medians, standard deviations and interquartile ranges, were computed using base R functions as well as the gtsummary and tidyverse packages. Subsequently, we constructed three blocks comprising several related variables within a conceptual framework (Annex 2,p.44). For the outcome variable, anti-PGL1 seropositivity (dependent variable), univariate analysis was conducted using the glm function. Following this, stepwise multivariate analysis was done for each block to retain significant variables. Finally significant variables from each multivariate block-wise analysis were incorporated into the final integrated model. Additionally, an alternative method was explored where block interaction analysis was carried out, focusing on sociodemographic with either environmental or nutritional factors. Age and sex were controlled for in all logistic multivariable analyses.

Results

Demographic, socioeconomic, and clinical characteristics

In terms of total recruitment, 319 participants were enrolled in the study with 66% of participants, (***Error! Reference source not found.***), were females and 34% males. For age, 33% (n=105) were between 18 and 30 years of age, 29% (n=93) between 31-45 years and 38% (n=121) above 45 years. For education, 17% (n=54) of the participants had no schooling, 36% (n=116) at the level of middle school or less, and 47% (n=148) above middle school with only 60% (n=190) reported having a source of income. Average household size was 4 individuals (IQR 2) and 64% (n=205) reported having children. With regards to clinical characteristics, prevalence of anti-PGL1 seropositivity among the study participants was 36.9% (n=118) with a distribution, (Figure 1), between males and females at 10.3% and 26.6% respectively. Among anti-PGL1 seropositive and seronegative, (Table 2), mean age was 39(SD 15) and 44(SD 17), mean body mass index was 23.1 (SD 3.4) and 24 (SD 3.5) and mean mid-upper arm circumference was 25.3(SD 2.9) and 25.9 (SD 3.4) respectively. Two sample t-test of anthropometric measures, (Figure 2), revealed significant difference in mean BMI (-0.86, 95% CI [-1.6: -0.06]; p=0.034) and mean MUAC (-1.2,95% CI [-1.8: -0.46]; p=0.0012) between positive and negative anti-PGL1 participants respectively.

Environment and WASH Factors

The prevalence of improved sources, (Table 3), among 318 respondents, for drinking, bathing, and cooking were 95%, 92% and 93% respectively, and 34% treated their water with either chlorine, water filter or through boiling. Figure 3 shows the frequency of different water sources reported by type of usage. Although 91% of the participants used improved sanitation facilities, (Figure 4), 71% reported sharing the sanitation facility with other households, with an average 4 households per facility. Among households who have children (n=206), 63% of the

children were not toilet trained and 44 out of 123 respondents (35.7%) were disposing stool unsafely. Stool disposal was considered unsafe disposal if rinsed/placed into drain or ditch; thrown into garbage; buried; or left in the open. With regards to hygiene, majority reported having a handwashing facility, and only 3.5% reported not having available hand soap. Lastly, 37% of participants reported that the floor of the house they live in was exposed to dirt. Lastly, participants who reported owning agriculture land or poultry/livestock in their back yard/home were 8.8% (28/319) and 6.6% (21/319) respectively.

Nutrition and Food Access

Thirty-one percent reported, (Table 4), less frequent food shopping either once every 3 weeks or less often than every 3 weeks. Thirty four percent reported eating less proportion than they should have, 30% skipped meals and 20% of participants reported not eating for a whole day due to food shortage. A food statement was read to the participants "The food that my family bought just didn't last, and we didn't have money to get more" How often is this statement true in your household for the last 12 months?". Figure 5 shows the scaled responses of the participants. Those who responded, "Very often true";" Often true"; or "Sometimes true" were deemed as experienced food insecurity which was 46% of the total participants.

Univariate, Block-wise and Integrated analyses

Univariate analysis of sociodemographic factors, (Table 1), revealed that ages between 18-30 and 31-45 years had a significant association with seropositivity (OR 2.28, 95% CI [1.30: 4.03]; p=0.004) and (OR 2.61, 95% CI [1.47: 4.69]; p=0.001) respectively. Also, having children in household (OR 1.83; 95% CI [1.12: 3.03]; p=0.017) also showed significant association with leprosy infection. Gender (female) and no schooling showed positive association but

insignificant. As for the environmental factors, (Table 3), using unimproved water sources for cooking (OR 2.35; 95% CI [1.00: 5.69]; p=0.051) or bathing (OR 4.04; 95% CI [1.73: 10.2]; p=0.002) were associated with seropositivity. Unimproved sanitation facility OR 2.27; 95% CI [1.05: 4.98]; p=0.037) and sharing the sanitation facility (OR 1.69,95% CI [1.01- 2.87]; p=0.049) were as well significantly associated with anti-PGL1 seropositivity (latent leprosy infection). In addition, having children not toilet trained (OR 1.87,95% CI [1.04; 3.40]; p=0.038), house floor was exposed dirt (OR 2.03,95% CI [1.27: 3.28]; p=0.003), and owning agriculture land (OR 2.94, 95% CI [1.34: 6.69]; p=0.008) showed significant association. Drinking water from unimproved source, no handwashing, lack or infrequent use of hand soap, and owning poultry/livestock in backyard or home showed positive association but were insignificant. Lastly for nutritional and food security factors, (Table 4), low MUAC (≤ 22cm) (OR 2.43,95% CI [1.23: 4.85]; p=0.011) and not eating for a whole day due to food shortage (OR 2.39; 95% CI [1.37: 4.20]; p=0.002) were significantly associated with leprosy latent infection. Underweight and other food shortage variables showed positive association but non-significant.

Block-wise multivariable logistic regression analysis, (Table 5), for the sociodemographic factors, younger age groups 18-30 years (aOR 2.37, 95%CI [1.29: 4.43]; p=0.006) and 31-45 years (aOR 2.53, 95% CI [1.38: 4.70]; p=0.003), and no schooling (aOR 1.94, 95% CI[0.95:4.03],p=0.071) had positive associations. Having children in household showed a positive association but insignificant. For the environmental factors, (Table 5), controlling for age and sex, house floor exposed dirt (aOR 1.94, 95% CI [1.04: 3.63]; p=0.039) and bathing from an unimproved water source (aOR 4.27, 95% CI [1.47: 14.3]; p=0.011) remained significant while unimproved sanitation facility, sharing facility and owning agriculture land, although still

positive associations were no longer significant. Lastly for nutritional factors, low MUAC (≤22 cm) and not eating for a whole day remained significant associated with leprosy infection (aOR 2.13 ,95% CI [1.05-4.37]; p=0.037) and (aOR 1.98, 95%CI [1.09 -3.60]; p=0.024) controlling for age and sex.

Multivariable logistic regression of combined sociodemographic factors with either environmental or nutritional covariates (Table 6;Figure 6;Figure 7). For the former, owning agriculture land (aOR 2.95, 95% CI [1.22: 7.51]; p=0.019), unimproved bathing water source (aOR 3.85, 95% CI [1.57: 10.2]; p=0.004), had significant association with leprosy infection, controlling for age, sex, source of income and education. House floor exposed to dirt showed a near positive significant association aOR 1.64, 95% CI [0.97: 2.77]; p=0.065). While for nutrition and sociodemographic model, having low MUAC (≥ 22 cm) and not eating for a whole day in the past year showed positive association (aOR 1.98, 95% CI [0.97:4.09], p=0.060) and (aOR 1.77, 95% CI[0.95; 3.29];p=0.071) respectively. In both models, age groups of 45 years or younger had positive significant association with anti-PGL1 seropositivity as shown in Table 6.

In the integrated multivariable logistic regression model, (Table 7; Figure 8), including significant variables from block-wise and combined multivariable models, owning agriculture land (aOR 2.85, 95% CI[1.16: 7.40]; p=0.025), bathing from an unimproved source(aOR 3.84, 95% CI[1.56: 10.1], p=0.005) and age group 31-45 years in comparison to those above 45 years (aOR 2.50, 95% CI[1.34: 4.75],p=0.004) remained significant controlling for sex, source of income and education. House floor exposed to dirt, low MUAC and not eating for a whole day showed also positive association but lost the significance.

Discussion

We hypothesized in our study that given the annual increased incidence of leprosy cases in Ethiopia,[9] there would be hidden or ongoing undetected transmission of leprosy that is unidentified and is contributing to the local transmission and the incidence of new leprosy cases. In addition, risk factors such as environmental exposure including unimproved WASH,[23,28,29] inadequate nutrition,[32,35,36] and sociodemographic factors have been previously associated with leprosy disease.[31,52] We hypothesized that these same risks likewise have a similar effect on leprosy infection.

This is a pilot study using field-friendly anti-PGL1 lateral flow (ML Flow) in an effort to complement active surveillance of leprosy infection and control in Addis Ababa, Ethiopia.[18] Penna et al. found, in a systematic review on anti-PGL1, a three times higher odds for healthy contacts with positive anti-PGL1 result (OR 3.11,95%CI [2.22-4.36]; I^2=26.6%) to develop disease.[15] Douglas et al reported that household contacts who became positive by ELISA had a 7.65-fold higher risk of developing MB or PB leprosy than contacts who were negative by ELISA[16]. In endemic areas, subclinical infection with the development of antibodies to PGL1 ranges between 1.7-35%[17]. Another study recommended that those deemed positive for anti-PGL1 should be monitored, followed up, and considered for post-exposure prophylaxis by single-dose rifampicin (PEP-SDR).[53,54] In addition, PEP-SDR is recommended by the WHO's guidelines for prophylaxis to contacts of leprosy cases.[41] In Ethiopia's national guidelines for leprosy, issued in 2017, healthcare workers are urged to prioritize active surveillance and the identification of cases, along with tracing their contacts.[55] However, it's noteworthy that it does

not incorporate PEP-SDR for contacts. Instead, the emphasis is placed on educating contacts about early symptoms of disease.

Out of the recruited 319 study participants, the prevalence of anti-PGL1 seropositivity was 37%(n=118) and prevalent among younger adults with a median age of 35 years (IQR 26,47]). This identified anit-PGL1 prevalence is higher than the previously reported by other studies.[18,56-60] Since the participants were not fully screened for the disease, it is possible that some had leprosy themselves, thereby, misclassifying some seropositive individuals as exposed / latently infected instead of disease. In retrospect, we also did not inquire if the participants had any contact with an individual with leprosy. While it would not affect the classification of participants, it would have given us a better idea of the amount of known disease / contact in the communities. While not every anti-PGL1 seropositive participant will progress to the disease,[15] it could highlight an ongoing transmission among the community. Historically ALERT center was a previous leprosarium where a great influx of leprosy patients and their families settled in communities surrounding the hospital[61] , and coupled with the long incubation period could explain that ongoing transmission and increased prevalence of latent infection.

Of the 118 seropositive participants, females had a higher prevalence (72%), which is higher than findings of a study in northwestern São Paulo, Brazil, where 52.3% of seropositive household contacts were females.[62] Also, the reported incident cases in Ethiopia in 2022 showed that more than 60% of the newly diagnosed cases were males.[9] Despite our study population having a higher female proportion (66%), it could be explained that as leprosy is a stigmatizing disease, especially to women, so maybe under-reporting or a decreased health-seeking behavior is affecting the reported gender distribution of the disease. Still, as anti-PGL1

detects IgM antibodies, and it has been found that in general IgM levels are higher among females and those of younger age.[63] Another interesting finding that those who owned agricultural land, although a small proportion of the study participants (8.8%), had higher odds (aOR 2.85,95%CI[1.16-7.40];p=0.0025) of being seropositive. Leprosy was shown to be prevalent among laborers and farmers in a study done in India.[64] In our study, only 2 participants of the 28 who owned agricultural land reported working as farmers, so there is no clear explanation of why this significant association arose. Previous studies have detected viable *M.leprae* in the soil and could pose an alternative route of transmission, therefore, it is possible that those with land are more exposed to environmental reservoirs of *M. leprae*.[26,27,40] However, this pathway of infection have not been established.

The majority of our participants, according to the UNICEF/WHO Joint Monitoring Programme on WASH categorization,[65] used improved water sources, (Figure 3), for drinking (95%), cooking (93%) or bathing (92%). This was not unexpected as we conducted the study in the capital city of Ethiopia and substantial gains, at least in urban settings, have been accomplished in the WASH infrastructure.[66] Using unimproved water source for bathing showed a significant association with leprosy infection and anti-PGL1 seropositivity (aOR 3.82, 95%CI[1.56, 10.1],p=0.005). This was similar to the findings from a study in Gondar, Ethiopia, that reported using unimproved water source was significantly associated with leprosy disease (OR = 4.22, 95% CI [1.07, 16.22]).[29] The role of unimproved water source in leprosy infection or disease has not been clearly recognized, despite the fact that *M.leprae* has been found in free-living amebae and speculated to play a role in transmission.[25] In our combined block analysis, when we only combined sociodemographic factors with either environmental or nutrition

factors, we found positive association between leprosy infection and house floor exposed dirt, this was similarly stated in a study that showed muddy/sand floor was associated with leprosy disease.[31] Again this gives more weight on the possibility of an alternative route of infection and the role of viable *M.leprae* in the environment.

Furthermore, not eating for a whole day or low MUAC (less than or equal 22 cm), in our combined analysis, were positively associated with leprosy infection. Similar findings were reported in the study in north Gondar where high prevalence of low MUAC and underweight were detected among leprosy cases.[35] The association between low food intake or food insecurity has been identified as a risk for leprosy disease in studies conducted in Ethiopia,[35] Indonesia,[34] Bangladesh,[37]and Brazil.[31] Inadequate food intake leading to deficiencies in macro and micronutrients has been linked to compromised immunity, increased susceptibility to leprosy infection, and the progression to clinical disease.[32] One of the strengths of this study is that we have identified individuals who are latently infected (pre-diseased) and compared their nutritional status to those who are seronegative. This approach is more compelling than comparing nutritional markers of cases to controls since it becomes harder to determine whether the nutritional deficiency increased susceptibility to disease progression or if the disease progression resulted in nutritional deficiency.

Limitations

The study has some limitations, firstly owing to being a cross-sectional study where we assessed the exposure and the outcome at the same point in time, we cannot determine temporality and the sequence of events. Also, anti-PGL1 lateral flow has a low sensitivity to diagnose PB leprosy at 40%. And since there are no biomarkers for latent disease, we do not

know the true sensitivity and specificity of PGL1 IgM for infection. Therefore, it is possible that we have even missed some individuals in this group and /or had some false positives. It would have been interesting to have data on household contacts and BCG to better assess factors related to infection given the fact that other studies have illustrated some protective effect of BCG vaccine. Infection among children correlates with increased local transmission, however as a pilot study we did not recruit any children. Addis Ababa is an urban city with better infrastructure than rural areas so the proportion using improved WASH sources were higher, and limited the ability to assess this association. Participant recruitment was through convenience sampling although having a large sample size balances it out, still limits generalizability. WASH questions were asked and not observed so both risk of recall bias and social desirability is present. Food security questions were adapted from the USDA household food security questionnaire which may not have captured all the aspects of food shortage and insecurity.

Conclusion

Our study highlights the complex interplay between sociodemographic, environmental, and nutritional factors associated with leprosy transmission, and points to both environment and nutritional factors as potential risk factors. To be on track with the global leprosy strategy and halt the local transmission, a multidisciplinary approach that includes active surveillance, WASH and environmental intervention, and nutritional support to population at risk and endemic countries. Longitudinal studies can also better describe these factors as true risk factors or not. Further research is required to support the use of the anti-PGL1 (ML Flow) to complement contact tracing and identification of early infections in the community.

Public Health Implications

The study conducted in Addis Ababa sheds light on the public health implications of leprosy, an often-neglected tropical disease, within the urban context of the capital city. Understanding the epidemiological background and the risk factors associated with leprosy transmission and disease is crucial for developing targeted interventions to mitigate its impact on population health. The study reveals the presence of hidden or undetected leprosy transmission in Addis Ababa, emphasizing the importance of active surveillance and early detection strategies. Implementing field-friendly screening tools, such as anti-PGL1 lateral flow tests, can facilitate the identification of high-risk groups and prompt referral for clinical examination and prophylaxis with post-exposure prophylaxis with single-dose rifampin as recommended by the WHO. By identifying seropositive community members and possible tracing back to unidentified leprosy cases, this can aid public health entities to identify and halt the transmission chains and prevent further spread of the disease. Leprosy is a complex disease and requires integrated interventions. The disease is linked to sociodemographic, environmental, and nutritional factors. Low socioeconomic status, lack of access to improved water sources, and food insecurity are identified as significant factors associated with leprosy infection. Targeted interventions aimed at improving WASH infrastructure, enhancing nutritional support, and addressing poverty-related barriers to healthcare access are essential for reducing leprosy incidence. Strengthening collaboration between healthcare providers, community organizations, and government agencies is crucial for implementing comprehensive interventions that target both disease transmission and underlying determinants of health. By

adopting a multi-sectoral approach to leprosy control, Ethiopia can achieve sustainable improvements in population health and well-being.

In conclusion, the study underscores the importance of addressing hidden leprosy transmission and socioeconomic determinants to effectively control the disease in Addis Ababa. By implementing active surveillance strategies, targeting vulnerable populations, and adopting a holistic approach to public health interventions, policymakers and stakeholders can work towards achieving the goal of eliminating leprosy as a public health problem in the capital city.

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Tables and Figures

Table 1. Description of Sociodemographic covariates and groups were compared using chi-square test to determine significant differences. P-value < 0.05 was determined significant.

	Descriptive by anti-PGL1 Result			Univariate Analysis		
Covariates	**Overall**, N = 319[1]	**Positive**, N = 118	**Negative**, N = 201	**cOR**[2]	**95% CI**[2]	**p-value**
Age, n (%)						
Above 45 years	121 (38%)	30 (25%)	91 (45%)	—	—	
18-30 years	105 (33%)	45 (38%)	60 (30%)	2.28	1.30, 4.03	**0.004**
31-45 years	93 (29%)	43 (36%)	50 (25%)	2.61	1.47, 4.69	**0.001**
Sex, n (%)						
Male	110 (34%)	33 (28%)	77 (38%)	—	—	
Female	209 (66%)	85 (72%)	124 (62%)	1.60	0.98, 2.64	0.062
Education, n (%)						
Above Middle School	148 (47%)	52 (44%)	96 (48%)	—	—	
Middle School or less	116 (36%)	41 (35%)	75 (38%)	1.01	0.61, 1.68	>0.9
No schooling	54 (17%)	25 (21%)	29 (15%)	1.59	0.84, 3.00	0.15
Unknown	1	0	1			
Source of income, n (%)						
Yes	190 (60%)	72 (61%)	118 (59%)	—	—	
No	128 (40%)	46 (39%)	82 (41%)	0.92	0.58, 1.46	0.7
Unknown	1	0	1			
Income level, n/N (%)						
> 3000 Birr	77/186 (41%)	29/70 (41%)	48/116 (41%)	—	—	
≤ 3000 Birr	109/186 (59%)	41/70 (59%)	68/116 (59%)	1.00	0.55, 1.83	>0.9
Unknown	133	48	85			
Occupation, n (%)						
No Occupation	136 (43%)	50 (42%)	86 (43%)	0.00		>0.9
Private business	101 (32%)	39 (33%)	62 (31%)	0.00		>0.9
Civil servant	62 (19%)	23 (19%)	39 (19%)	0.00		>0.9
Pensioned	18 (5.6%)	4 (3.4%)	14 (7.0%)	0.00		>0.9
Farmer	2 (0.6%)	2 (1.7%)	0 (0%)	—	—	
Size of household				1.04	0.92, 1.17	0.5
Median (IQR)	4 (3, 5)	4 (3, 5)	4 (3, 5)			
Unknown	2	1	1			
Children in household, n (%)						
No	113 (36%)	32 (27%)	81 (41%)	—	—	
Yes	205 (64%)	86 (73%)	119 (60%)	1.83	1.12, 3.03	**0.017**
Unknown	1	0	1			

[1]n (%); n/N (%)
[2]cOR = Crude Odds Ratio, CI = Confidence Interval

Table 2. Distribution of age, BMI, and mid upper arm circumference according to anti-PGL1 seropositivity. Mean difference and T-test were calculated to determine significant difference between groups. P-values< 0.05 were significant.

	Anti-PGL1 Results				
Covariates	**Positive**, N = 118	**Negative**, N = 201	**Difference**[1]	**95% CI**[12]	**p-value**[1]
Age in years, Mean (SD)	39(15)	44(17)	-5.7	-9.3, -2.1	**0.002**
Body Mass Index (Kg/m^2), Mean (SD)	23.1(3.4)	24.0(3.5)	-0.86	-1.6, -0.06	**0.034**
Mid upper arm circ.(cm), Mean (SD)	25.3(2.9)	26.4(3.2)	-1.2	-1.8, -0.46	**0.001**

[1]Welch Two Sample t-test
[2]CI = Confidence Interval

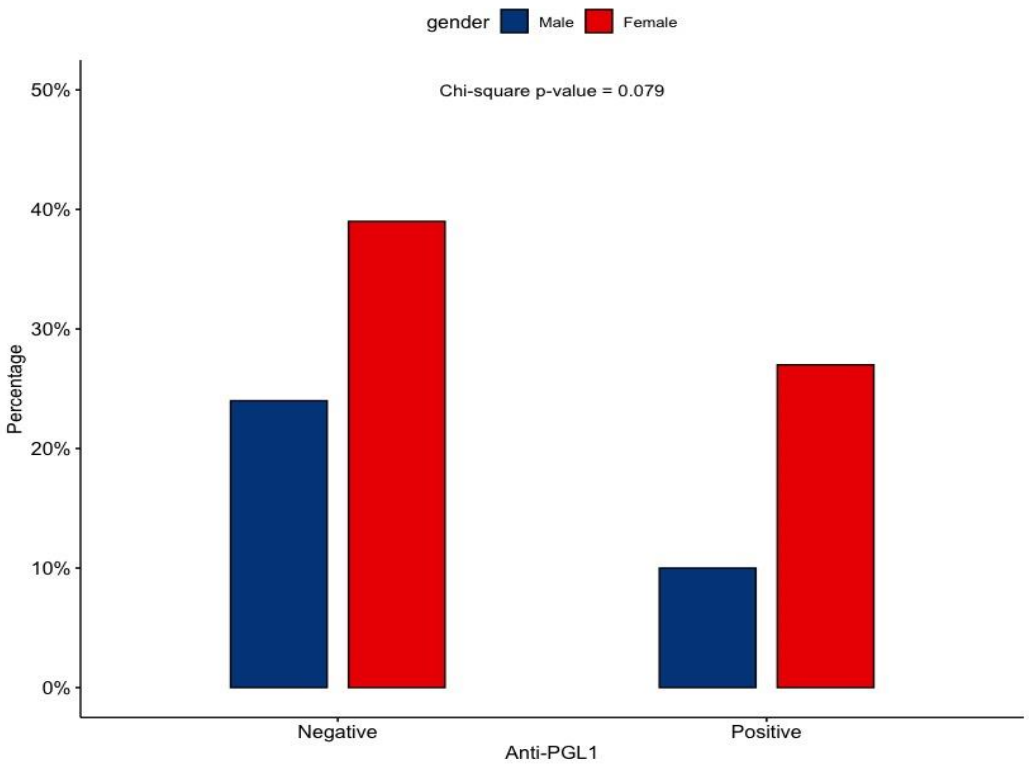


Figure 1.Barplot of distribution of study population proportion of anti-PGL1 results by sex. Chi-square test with a significance level < 0.05.

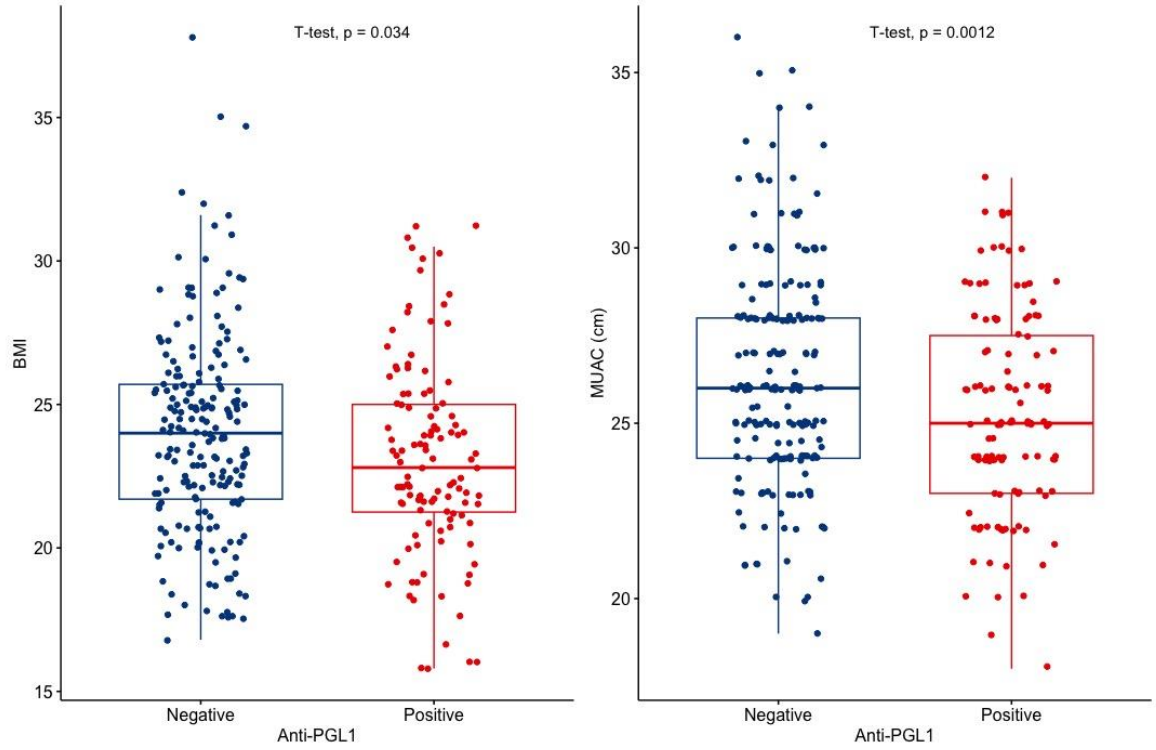


Figure 2.Boxplot of Distribution of Body Mass Index and Mid Upper Arm Circumference According to anti-PGL1 (ML Flow) Results. Boxplot boundaries depicits 1st quartile, median and 3rd quartile values. T-test for difference in means were statistically significant.

Table 3. Description of Environmental covariates according to anti-PGL1 results and groups were compared using chi-square test to determine significant differences. P-value < 0.05 was determined significant..

	Descriptive by anti-PGL1 Result			Univariate Analysis		
Covariates	**Overall**, N = 319[1]	**Positive**, N = 118	**Negative**, N = 201	**cOR**[2]	**95% CI**[2]	**p-value**
Drinking Water, n (%)						
Improved	303 (95%)	110 (93%)	193 (97%)	—	—	
Unimproved	15 (4.7%)	8 (6.8%)	7 (3.5%)	2.01	0.70, 5.86	0.2
Bathing Water, n (%)						
Improved	293 (92%)	101 (86%)	192 (96%)	—	—	
Unimproved	25 (7.9%)	17 (14%)	8 (4.0%)	4.04	1.73, 10.2	**0.002**
Cooking Water, n (%)						
Improved	295 (93%)	105 (89%)	190 (95%)	—	—	
Unimproved	23 (7.2%)	13 (11%)	10 (5.0%)	2.35	1.00, 5.69	0.051
Water Treatment, n (%)						
Yes	110 (34%)	38 (32%)	72 (36%)	—	—	
No	209 (66%)	80 (68%)	129 (64%)	1.18	0.73, 1.91	0.5
Time to collect water				1.01	0.98, 1.03	0.6
Median (IQR)	3 (1, 5)	3 (1, 5)	2 (1, 5)			
Sanitation Facility, n (%)						
Improved	290 (91%)	102 (86%)	188 (94%)	—	—	
Unimproved	29 (9.1%)	16 (14%)	13 (6.5%)	2.27	1.05, 4.98	**0.037**
Sharing sanitation facility, n (%)						
No	94 (29%)	27 (23%)	67 (33%)	—	—	
Yes	225 (71%)	91 (77%)	134 (67%)	1.69	1.01, 2.87	**0.049**
No. of households sharing facility						
Median (IQR)	5 (4, 8)	5 (4, 9)	6 (3, 8)	1.03	1.00, 1.08	0.092
Children not toilet trained, n (%)						
No	77 (37%)	25 (29%)	52 (43%)	—	—	
Yes	129 (63%)	61 (71%)	68 (57%)	1.87	1.04, 3.40	**0.038**
Stool disposal (Children), n (%)						
Safe	79 (64%)	38 (64%)	41 (64%)	—	—	
Unsafe	44 (36%)	21 (36%)	23 (36%)	0.99	0.47, 2.06	>0.9
Handwashing facility, n (%)						
Jug and bucket	220 (70%)	84 (72%)	136 (68%)	1.24	0.42, 4.08	0.7
By latrine/toilet	79 (25%)	26 (22%)	53 (27%)	0.98	0.31, 3.42	>0.9
In kitchen	15 (4.7%)	5 (4.3%)	10 (5.0%)	—	—	
No handwashing	2 (0.6%)	1 (0.9%)	1 (0.5%)	2.00	0.07, 58.3	0.6
Availability of hand soap, n (%)						
Yes	304 (97%)	110 (95%)	194 (97%)	—	—	
No	11 (3.5%)	6 (5.2%)	5 (2.5%)	2.12	0.62, 7.49	0.2
Soap use, n (%)						
Frequent	229 (72%)	83 (70%)	146 (73%)	—	—	
Infrequent	90 (28%)	35 (30%)	55 (27%)	1.12	0.67, 1.84	0.7
House floor exposed to dirt, n (%)						
No	194 (63%)	61 (52%)	133 (69%)	—	—	
Yes	116 (37%)	56 (48%)	60 (31%)	2.03	1.27, 3.28	**0.003**
Own agriculture land, n (%)						
No	290 (91%)	100 (85%)	190 (95%)	—	—	
Yes	28 (8.8%)	17 (15%)	11 (5.5%)	2.94	1.34, 6.69	**0.008**
Own poultry/livestock in home or yard, n (%)						
No	298 (93%)	107 (91%)	191 (95%)	—	—	
Yes	21 (6.6%)	11 (9.3%)	10 (5.0%)	1.96	0.80, 4.86	0.14

[1]n (%)
[2]cOR = Crude Odds Ratio, CI = Confidence Interval

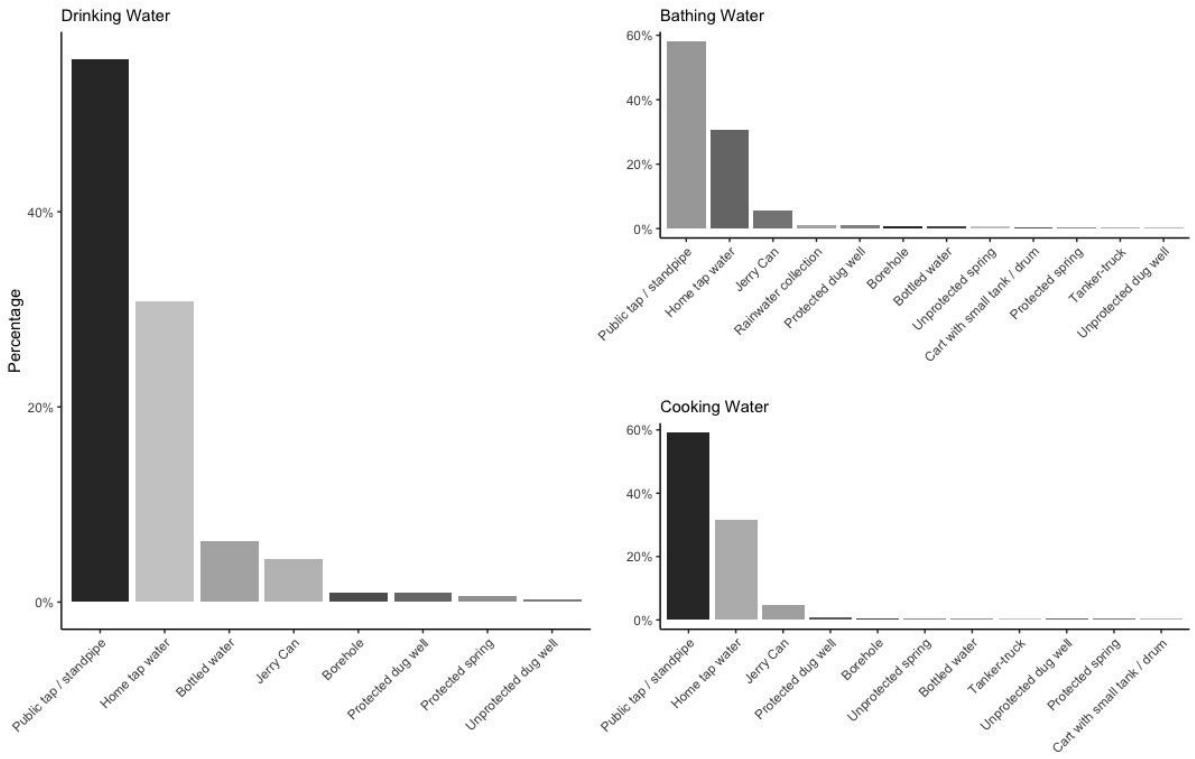


Figure 3. Barplot of reported water sources by the type of usage whether drinking, cooking, or bathing.

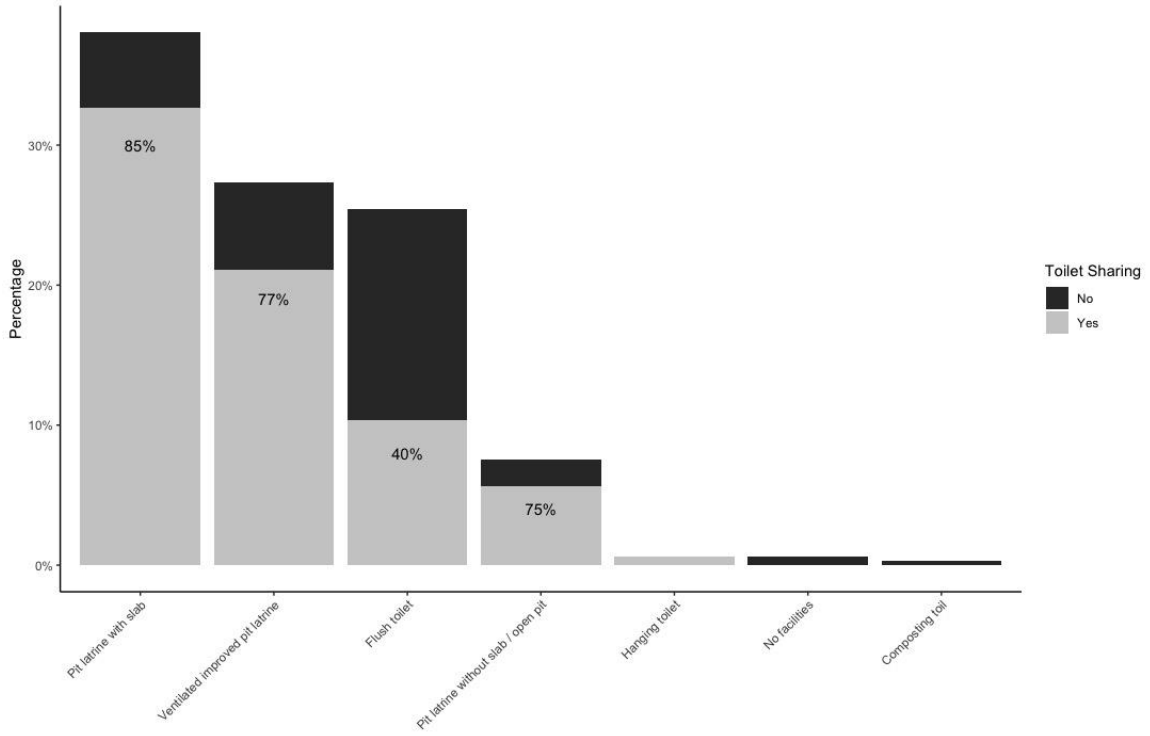


Figure 4.Barplot of type of sanitation facilities reported and percentage of facility sharing between households.

Table 4. Description of Nutritional and Food Access covariates according to anti-PGL1 results, and groups were compared using chi-square tests to determine significant differences. P-values < 0.05 are determined significant.

	Descriptive by anti-PGL1 Results			Univariate Analysis		
Covariates	**Overall**, N = 319[1]	**Negative**, N = 201	**Positive**, N = 118	**cOR**[2]	**95% CI**[2]	**p-value**
Body Mass Index, n (%)						
Normal	194 (61%)	118 (59%)	76 (66%)	—	—	
Overweight-Obese	101 (32%)	71 (35%)	30 (26%)	0.66	0.39, 1.09	0.11
Underweight	21 (6.6%)	12 (6.0%)	9 (7.8%)	1.16	0.46, 2.88	0.7
Mid upper arm circ., n (%)						
Normal (> 22 cm)	276 (88%)	180 (91%)	96 (81%)	—	—	
Low (≤ 22 cm)	39 (12%)	17 (8.6%)	22 (19%)	2.43	1.23, 4.85	**0.011**
Food shopping frequency, n (%)						
Frequent	211 (69%)	139 (71%)	72 (64%)	—	—	
Less frequent	97 (31%)	56 (29%)	41 (36%)	1.41	0.86, 2.31	0.2
Eating less than should in the past year, n (%)						
No	210 (66%)	140 (70%)	70 (60%)	—	—	
Yes	107 (34%)	60 (30%)	47 (40%)	1.57	0.97, 2.53	0.065
Not eating for a whole day (no enough food), n (%)						
No	255 (80%)	171 (86%)	84 (71%)	—	—	
Yes	63 (20%)	29 (15%)	34 (29%)	2.39	1.37, 4.20	**0.002**
Skipped meals (not enough food) in the past year, n (%)						
No	222 (70%)	147 (73%)	75 (64%)	—	—	
Yes	96 (30%)	54 (27%)	42 (36%)	1.52	0.93, 2.49	0.092
Food insecurity statement, n (%)						
Didn't Experience	171 (54%)	115 (58%)	56 (48%)	—	—	
Experienced	144 (46%)	83 (42%)	61 (52%)	1.51	0.95, 2.39	0.079

[1]n (%)
[2]cOR = Crude Odds Ratio, CI = Confidence Interval

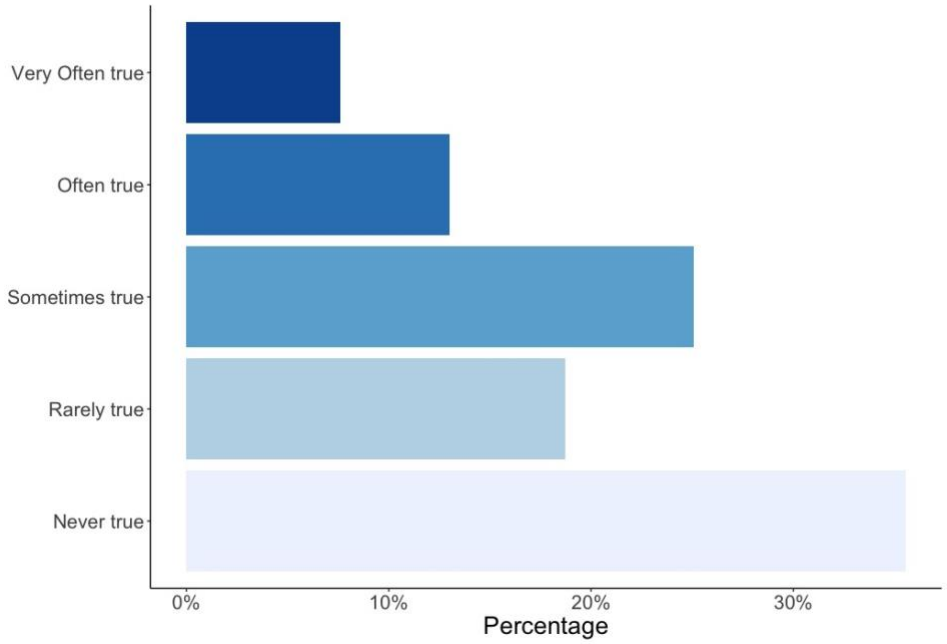


Figure 5. Responses to the Food Statement" The food that my family bought just didn't last, and we didn't have money to get more" How often is this statement true in your household for the last 12 months?"

Table 5. Block-wise multivariable logistic regression of positive anti-PGL1 and different block factors. P values< 0.05 are determined significant

	Sociodemographic			Environmental			Nutrition		
Covariates	**aOR[1]**	**95% CI[1]**	**p-value**	**aOR[1]**	**95% CI[1]**	**p-value**	**aOR[1]**	**95% CI[1]**	**p-value**
Age									
Above 45 years	—	—		—	—		—	—	
18-30 years	2.37	1.29, 4.43	**0.006**	1.95	0.84, 4.65	0.12	1.77	0.98, 3.21	0.060
31-45 years	2.53	1.38, 4.70	**0.003**	2.08	0.91, 4.92	0.087	2.38	1.31, 4.36	**0.004**
Sex									
Male	—	—		—	—		—	—	
Female	1.41	0.83, 2.43	0.2	1.61	0.82, 3.23	0.2	1.37	0.82, 2.32	0.2
Education									
Above Middle School	—	—							
Middle School or less	1.08	0.63, 1.85	0.8						
No schooling	1.94	0.95, 4.03	0.071						
Source of income									
Yes	—	—							
No	0.77	0.46, 1.27	0.3						
Children in household									
No	—	—							
Yes	1.43	0.85, 2.44	0.2						
Bathing Water									
Improved				—	—				
Unimproved				4.27	1.47, 14.3	**0.011**			
Sanitation Facility									
Improved				—	—				
Unimproved				2.16	0.71, 7.02	0.2			
Sharing sanitation facility									
No				—	—				
Yes				1.34	0.62, 2.93	0.5			
Children not toilet trained									
No				—	—				
Yes				0.94	0.45, 1.93	0.9			
House floor exposed dirt									
No				—	—				
Yes				1.94	1.04, 3.66	**0.039**			
Own agriculture land									
No				—	—				
Yes				1.64	0.48, 5.73	0.4			
Own poultry/livestock in home or yard									
No				—	—				
Yes				1.12	0.29, 4.14	0.9			
Mid upper arm circ.									
Normal (> 22 cm)							—	—	
Low (≤ 22 cm)							2.13	1.05, 4.37	**0.037**
Not eating for a whole day (not enough food)									
No							—	—	
Yes							1.98	1.09, 3.60	**0.024**

[1]aOR = Adjusted Odds Ratio, CI = Confidence Interval

Table 6. Combined multivariable logistic regression of positive anti-PGL1 and sociodemographic factors with either environmental or nutritional factors. P-values < 0.05 are determined significant.

	Sociodemographic & Environmental			Sociodemographic & Nutrition		
Covariates	**aOR[1]**	**95% CI[1]**	**p-value**	**aOR[1]**	**95% CI[1]**	**p-value**
Age						
Above 45 years	—	—		—	—	
18-30 years	2.04	1.07, 3.93	**0.032**	2.08	1.11, 3.96	**0.024**
31-45 years	2.52	1.32, 4.88	**0.005**	2.59	1.41, 4.84	**0.002**
Sex						
Male	—	—		—	—	
Female	1.43	0.82, 2.52	0.2	1.32	0.77, 2.28	0.3
Source of Income						
Yes	—	—		—	—	
No	0.81	0.47, 1.38	0.4	0.88	0.52, 1.48	0.6
Education						
Above Middle School	—	—		—	—	
Middle School or less	0.99	0.56, 1.73	>0.9	1.03	0.60, 1.77	>0.9
No schooling	1.55	0.72, 3.36	0.3	1.77	0.84, 3.77	0.13
Own agriculture land						
No	—	—				
Yes	2.95	1.22, 7.51	**0.019**			
Children in household						
No	—	—				
Yes	1.29	0.75, 2.23	0.4			
Bathing Water						
Improved	—	—				
Unimproved	3.85	1.57, 10.2	**0.004**			
House floor exposed to dirt						
No	—	—				
Yes	1.64	0.97, 2.77	0.065			
Not eating for a whole day (not enough food)						
No				—	—	
Yes				1.77	0.95, 3.29	0.071
Mid upper arm circ.						
Normal (> 22 cm)				—	—	
Low (≤ 22 cm)				1.98	0.97, 4.09	0.060

[1]aOR = Adjusted Odds Ratio, CI = Confidence Interval

Table 7. Integrated multivariable logistic regression of positive anti-PGL1 with sociodemographic, environmental, and nutritional factors. P-values < 0.05 are determined significant.

Covariates	OR[1]	95% CI[1]	p-value
Age			
Above 45 years	—	—	
18-30 years	1.84	0.95, 3.61	0.071
31-45 years	2.61	1.37, 5.04	**0.004**
Sex			
Male	—	—	
Female	1.34	0.77, 2.37	0.3
Source of income			
Yes	—	—	
No	0.91	0.52, 1.59	0.7
Education			
Above Middle School	—	—	
Middle School or less	0.97	0.55, 1.71	>0.9
No schooling	1.52	0.69, 3.35	0.3
Own agriculture land			
No	—	—	
Yes	2.85	1.16, 7.40	**0.025**
Bathing Water			
Improved	—	—	
Unimproved	3.82	1.56, 10.1	**0.005**
House floor exposed dirt			
No	—	—	
Yes	1.45	0.84, 2.50	0.2
Mid upper arm circ.			
Normal (> 22 cm)	—	—	
Low (≤ 22 cm)	1.52	0.71, 3.27	0.3
Not eating for a whole day (not enough food)			
No	—	—	
Yes	1.65	0.85, 3.21	0.14

[1]OR = Odds Ratio, CI = Confidence Interval

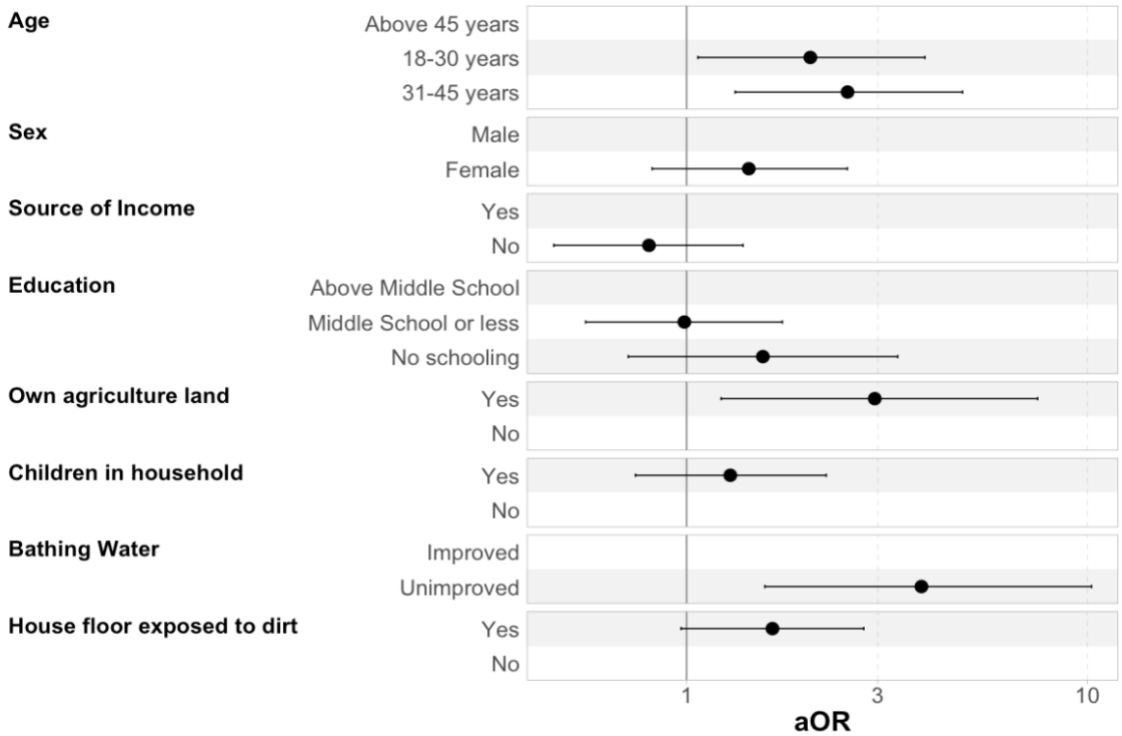


Figure 6. Adjusted odds ratio and 95% confidence interval of multivariable logistic regression of positive anti-PGL1 with combined sociodemographic and environmental factors.

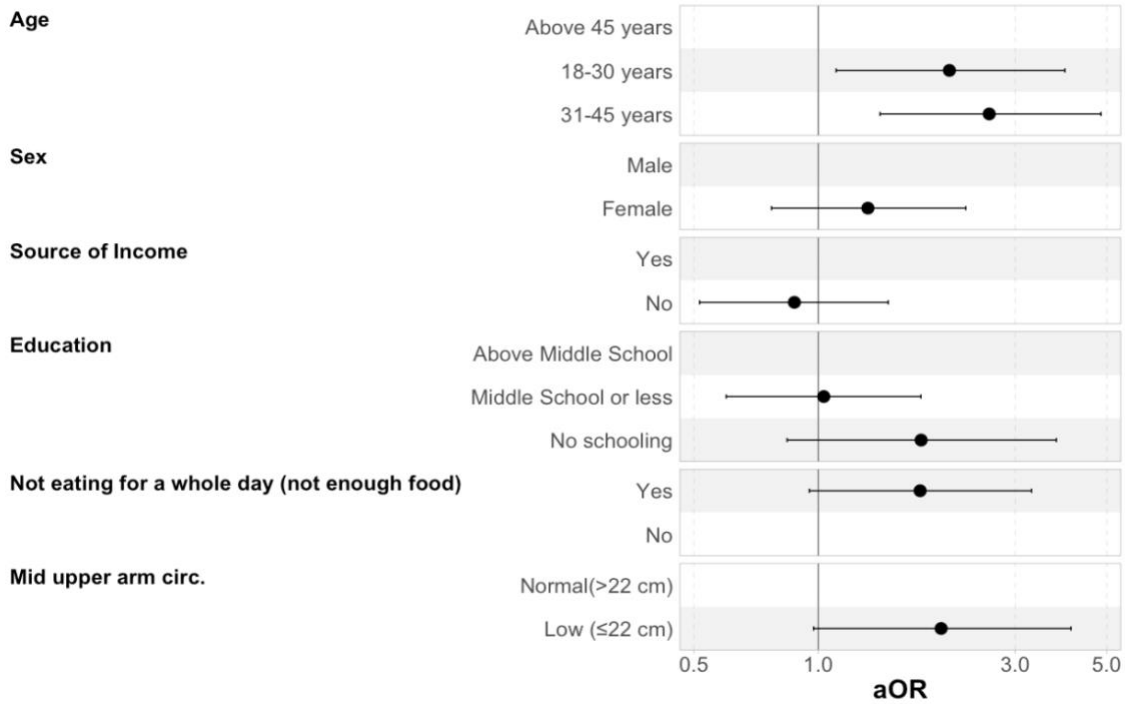


Figure 7. Adjusted odds ratio and 95% confidence interval of multivariable logistic regression of positive anti-PGL1 with combined sociodemographic and nutritional factors.

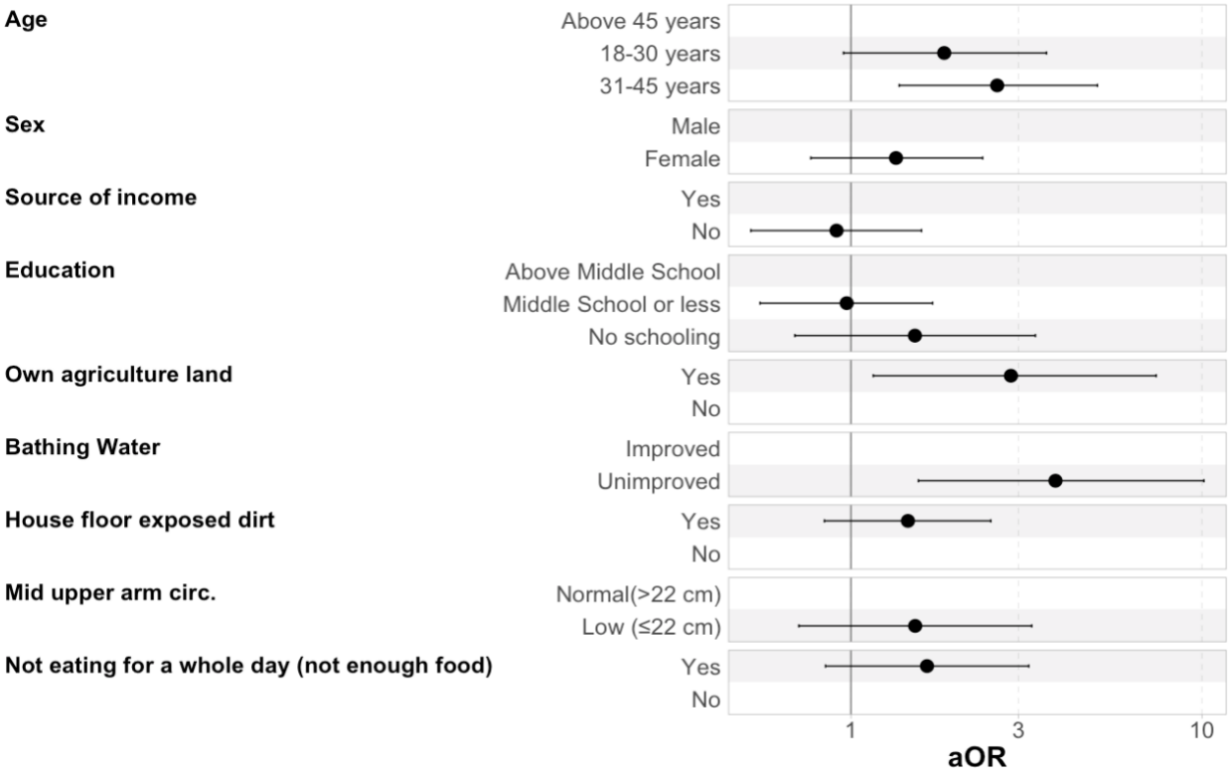


Figure 8.Adjusted odds ratio and 95% confidence interval of integrated multivariable logistic regression of positive anti-PGL1 with sociodemographic, environmental, and nutritional factors.

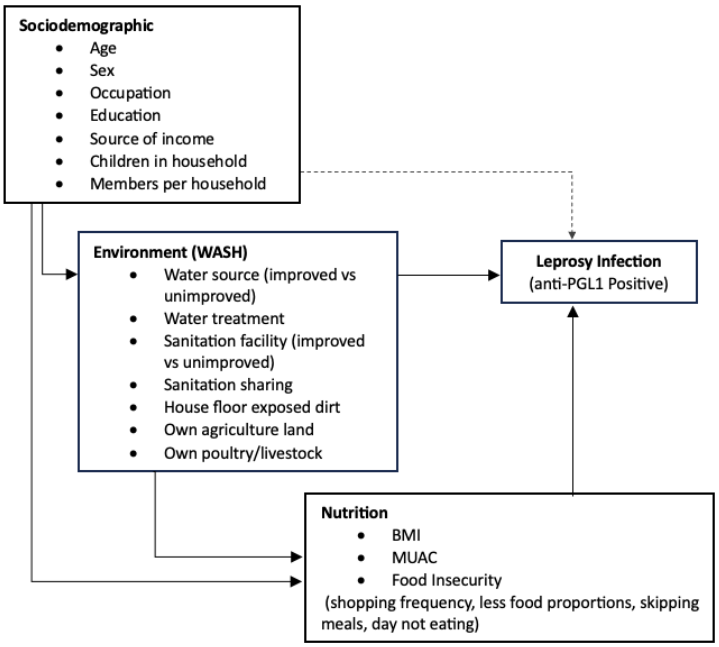
Annex 1

Supplementary Table 1.Calculation of sensitivity, specificity, positive predictive values, negative predictive values, and diagnostic accuracy for different MUAC (cm) cut-off points against underweight BMI (<18.5) to determine an appropriate low MUAC level.

MUAC Cutoff Point	Sensitivity (95%CI)	Specificity (95%CI)	Positive Predictive Value (95%CI)	Negative Predictive Value (95%CI)	Diagnostic Accuracy (95%CI)
21 cm	28.57% (13.81, 49.96)	95.53% (92.51, 97.37)	31.58% (15.36, 53.99)	94.88% (91.73, 96.87)	91.03% (87.34, 93.72)
21.5 cm	33.33% (17.19, 54.63)	95.53% (92.51, 97.37)	35% (18.12, 56.71)	95.21% (92.11, 97.12)	91.35% (87.7, 93.98)
22 cm	52.38% (32.37, 71.66)	90.38% (86.44, 93.26)	28.21% (16.54, 43.78)	96.34% (93.39, 98)	87.82% (83.72, 91)
22.5 cm	52.38% (32.37, 71.66)	89.35% (85.28, 92.39)	26.19% (15.3, 41.07)	96.3% (93.32, 97.98)	86.86% (82.66, 90.16)
23 cm	66.67% (45.37, 82.81)	83.16% (78.44, 87.02)	22.22% (13.72, 33.91)	97.19% (94.31, 98.63)	82.05% (77.41, 85.91)

Results from OpenEpi, Version 3, open-source calculator--Diagnostic Test

Annex 2



Supplementary figure 1. Conceptual Framework of sociodemographic, environmental, and nutritional factors with leprosy infection