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An Exploratory Analysis of Associations between Hansen's Disease, Schistosomiasis and Strongyloidiasis in Minas Gerais, Brazil

By

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Master of Public Health

Global Health

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By

Esther Amoakohene

B. A. Hunter College, 2013

Thesis Committee Chair: Jessica Fairley, MD

An abstract of

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Abstract

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By Esther Amoakohene

Background: Hansen's disease (Leprosy), schistosomiasis (Bilharzia) and strongyloidiasis are Neglected Tropical Diseases (NTDs) that are of significant public health concern in Brazil due to high endemicity, crippling effects and difficulty in early detection. The overall goals of this study were to explore the relationship between Hansen's disease (HD) and these helminths, to describe seroprevalence of these NTDs by analyzing data gathered utilizing a new testing method (Multiplex Bead Assay) and to garner information that might aid in the development of new strategies for NTD control.

Methods: Data from a case-control study conducted in Minas Gervais, Brazil was used for this analysis. Patients 3 years old and above were recruited and classified into HD positive, HD negative with no contact and HD with contact. Demographic data was collected via questionnaire, active HD cases were determined via slit skin smear tests and NTD antigens were detected via the multiplex serological testing. Chi square and regression analyses were conducted using SAS 9.4.

Results: Of the 221 participants involved in the analysis 78 were positive for HD, 87 participants were in control 1 and 67 were in control 2. Among the HD positive cases, 22 were classified as PB and 56 where MB. 34 participants tested positive for LID-1. Results from the analyses indicated that there was no statistically significant association between current HD status and helminth infection (schistosomiasis: control 1, cOR=1.02, 95%CI=0.55-1.87; control 2, cOR=0.76, 95%CI=0.39-1.47. strongyloidiasis. MBA results, utilizing NTD antigens, showed a negative association between HD antibodies (anti-LID-1) and schistosomiasis (cOR= 0.36, 95%CI=0.13-0.96) and a positive association between strongyloidiasis (cOR= 2.64, 95%CI=1.19-5.88). We also detected a higher number of schistosomiasis exposed individuals (n=66) compared to stool testing (n=12). Additionally, HD antibodies (LID-1) were detected in persons that did not have active HD.

Discussion: Results of this study encourage further exploration on the relationship between HD and schistosomiasis, the use of MBA on multiple helminth or early helminth infection detection, as well as follow up research on the risks of developing active HD after testing positive for LID-1 and other helminth antigens.

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INTRODUCTION

Hansen's Disease

Hansen's Disease (HD) (also known as leprosy) is a chronic, infectious disease caused by the bacteria *Mycobacterium leprae* and *Mycobacterium lepromatosis.* Symptoms of HD include skin lesions, muscle weakness and numbness (Saunderson, 2002). If not treated in a timely manner, HD can result in major health issues such as permanent nerve damage, crippling, paralysis, (Saunderson, 2002) and other deformities and disabilities (Nazario et al., 2017).

The exact mode of HD transmitted is unknown, but it is suspected to move from person to person via airborne transmission, i.e. coughing, sneezing, and the spread of other nasal fluids (Scollard et al., 2006). HD presents itself on a spectrum (de Sousa, Sotto, & Simões Quaresma, 2017). On one end is the tuberculoid (or paucibacillary) form and on the other is the lepromatous (or multibacillary) form (Serra et al., 2019). The paucibacillary (PB) form of HD is the mildest form of the disease and is defined as HD that has 5 or less lesions present or at least one damaged nerve (Nazario et al,2017) with little to no leprosy bacilli (bacteria) present in patients (Singh and Cole, 2011). The multibacillary form (MB), the more severe version of HD, is defined as leprosy with 5 or more skin lesions and/or one or more damaged nerves. Patient with MB have a large amount of the leprosy bacilli present in the host due to a weakened immune response against *M. leprae* (Virmond, Grzybowski, & Virmond, 2015). MB is also associated with 'leprosy reactions' (Nery et al., 2013), which are immunologic difficulties that result in deformity and disability in persons with HD. There are 2 types, Type 1 reactions (T1R or Reversible Reaction, RR) or Type 2 reactions (T2R or Erythema Nodosum Leprosum, ENL). They are seen in approximately 20 to 50 percent of leprosy patients. Resulting sequelae associated with MB are damage to the mucous membrane of the nose, mouth and tissue lining

openings such as the eyelids and can lead to possible blindness, nosebleeds (NIH U.S National Library of Medicine, 2019) and tissue decay (Singh & Cole, 2011). Between these two ends of the spectrum, the other intermediate forms of HD are known as the borderline tuberculoid, borderline-borderline and borderline lepromatous forms (de Sousa et al., 2017). Testing for HD is usually done via skin smears or skin biopsies but there is no 'gold standard' and it usually requires multiple tests to confirm a diagnosis (Lastória & Abreu, 2014).

There has been a steep decline in cases over the years (Martin, Gomez and Spies, 2017). Global estimates of HD incidence reduced from over 5 million infected people in 1985 (Martin, Gomez and Spies, 2017) to 211, 973 new cases discovered in 2015 (WHO Fact sheet, 2017). Over the last few years, the number of new cases has varied by very little. There were 232,857 new cases in 2012 (Walker, Lebas, Doni, Lockwood &Lambert, 2014) while in 2018 there were 208, 619 new cases of HD (WHO Fact Sheet, 2019). Despite this great improvement in HD control worldwide, with over 90% of disease being eliminated (Singh & Cole, 2011), the disease is still a cause for public health concern. Due to the inability to culture *M. leprae* (Alotaibi et al, 2016) and its relatively long incubation period (an average of 5 years) (WHO, 2018), there are still challenges involved in the early detection and interruption of transmission. Additionally, the role of environment on HD (Singh & Cole, 2017) and the exact transmission pathway are unknown (Phillips et al., 2017). This indicates a need for more research.

Schistosomiasis and Strongyloidiasis (*S. mansoni* and *S. stercoralis* Helminths)

Helminth is the general term for infectious, parasitic worms. They are classified as trematodes (blood flukes), cestodes and nematodes (Castro, 1996). Schistosomiasis (also known as Bilharzia) is a disease caused by a trematode helminth. The most common helminths associated with this disease are, *Schistosoma mansoni,* which causes intestinal schistosomiasis, *and*

Schistosoma haematobium, which causes urogenital schistosomiasis (Martins, Xavier, Masiero, Cordeiro, & Thyssen, 2015). These helminths use freshwater snails as a host. Infection occurs through contact with water sources contaminated with cercariae (S. *mansoni* larvae) (Mari et al., 2017). The cercariae enter the human host body through the skin, where they mature into schistosomes that lay eggs. The eggs are excreted from the host body through urine and feces, where they hatch into miracadia (ciliated larvae). These miracadia can also contaminate water sources, continuing the cycle of the schistosome. As of 2018, schistosomiasis had affected over 220 million people worldwide but less than half received treatment (Mari et al., 2017). Children between the ages of 5 to 17 have the highest risk of contracting the disease (Hajissa et al., 2018). Schistosomiasis is treatable with drugs such as praziquantel, however they work mostly on the adult worms and have little effect on younger worms or eggs (Vale et al., 2017), even though evidence indicates that morbidity is related to the presence of the eggs (Colley, Bustinduy, Secor, & King, 2014). The presence of the eggs result in inflammatory disease and can lead to acute or chronic schistosomiasis (Gray, Ross, Li, & McManus, 2011). Chronic schistosomiasis can lead to liver or kidney failure, ectopic pregnancies, bladder cancer, (WHO Fact sheet, 2019) and colorectal cancer (Hajissa et al., 2018).

The recommended method of diagnosing schistosomiasis is via stool sample using the Kato-Katz technique which is deemed the ‘gold standard’(Schistosomiasis & World Health, 2002), although there are other methods under investigation to improve sensitivity.

Strongyloidiasis is a disease caused by a nematode parasite. Though there are different species of the parasites, *Strongyloides stercoralis* is the one that can infect human beings (Ganesh & Cruz, 2011). *S. stercoralis* transmission can occur in 3 forms, hetero-infection, external autoinfection and internal autoinfection. With internal auto infection and external

autoinfection, *S. stercoralis* replicates itself in the host body and can, therefore, keep re-infecting the host body without the need for another exposure (Liu & Weller, 1993). This can result in chronic infections that can last for decades (Ericsson, Steffen, Siddiqui, & Berk, 2001). With hetero-infection transmission, *S. stercoralis* lay eggs in the intestinal mucosa of the host body, the eggs hatch into first-stage larvae (rhabditiform larvae) that are either released in feces, or develop directly into stage 2, stage 3 (filariform, or L3) or free living adult nematodes (Ericsson et al., 2001). Full adults can either reproduce sexually or female worms reproduce parthenogenetically. Infection occurs either through infection of eggs or skin contact (Buonfrate, Formenti, Perandin, & Bisoffi, 2015), ingestion or inhalation of the stage 3 larvae.(Ericsson et al., 2001). The infection can go unnoticed for decades. Symptoms include a rash known as larvae currens, diarrhea, abdominal discomfort and nausea (Ericsson et al., 2001). In cases of hyper-infection, or auto infection, mucosal pattern disruption, ulcers and paralytic ileus can occur. The ulceration can lead to bacterial and fungal infection. Secondary bacterial infection, due to the larvae's destruction, is one of the leading causes of death with patients who have hyper-infection syndrome (Ericsson et al., 2001). Strongyloidiasis affects 30 to 100 million people worldwide (CDC, Global Health, Division of Parasitic Disease, 2018). Strongyloidiasis is diagnosed by larvae detection in stool samples. Detection is quite difficult as the tests have low sensitivity (Buonfrate et al., 2015).

HD, schistosomiasis and strongyloidiasis are classified as Neglected Tropical Diseases (NTDs) and, by definition, affect poorer and more vulnerable populations in the world (Martins-Melo et al., 2018; Phillips et al., 2017). In Brazil, these three diseases are still significant public health concerns. The purpose of this study is to examine the relationship between HD, *S. mansoni* and *S. stercoralis* infections in hopes to improve knowledge on these ailments. We will

also explore seroprevalence of these conditions employing a novel multiplex beaded serological assay technique. This information could be used in the development of new methods of tackling these diseases in endemic areas. We hypothesize that there is a positive association between HD, *S. stercoralis* and *S. mansoni.* The study was conducted in Brazil due to its endemicity of HD (Amorim de Souza et al., 2018) and presence of *S. mansoni* and *S. stercoralis* parasites.

LITERATURE REVIEW

The literature review was conducted by searching PubMed for relevant studies. The key words and phrases searched for were “Hansen’s disease and soil transmitted helminths”, “leprosy and helminths”, “leprosy and parasitic worms”, and “Hansen’s disease and parasitic worms”. This was done to generalize and return more articles as specific searches for Hansen’ disease and *S. mansoni* or *S. stercoralis* generated no relevant results. Articles were chosen based on the relevance to the possible association between Hansen’s disease and soil transmitted helminths. This review also includes the effects of these health conditions in Brazil to provide more background information.

HD and Helminth Related Immune Response

The main reason for this study is to understand the relationship between helminths and HD. This is prompted by HD immune responses and their expression in the presence of helminth infections. There are two types of immune responses related to HD; the Th1 and Th2 responses. Resistance of the host body to HD is dependent on the type of immune response exhibited (Machado et al., 2015). A review of the relationship between these responses, and its relevance to our study, was conducted by de Sousa, Sotto and Quaresma (2017).

The Th1 response is the response required to combat HD. T helper cell 1 (Th1) produces Tumor Necrosis Factor-alpha (TNF-α) and interferon-gamma (IFN-γ) that bind to the M0 macrophages in the host body (Diniz, Magalhães, Pereira, Dietze, & Ribeiro-Rodrigues, 2010). These M0 macrophages transform into the M1 inflammatory macrophages (de Sousa et al., 2017) that are responsible for the production of cytokines and enzymes, such as induced nitric oxide synthase (iNOS). These cytokines and enzymes control the production of Nitric Oxide, the compound needed to generate the free radicals responsible for combating HD bacilli within the

body and is the reason this immune response is usually seen in those who have the PB form of HD. (de Sousa et al., 2017).

In the second type of response, the T helper cell 2 (Th2) triggers the production of M2 macrophages. These macrophages lead to the production of anti-inflammatory cytokines, such as interleukin 4 and 10 (IL-4, IL-10), growth factors (TGF-β and FGF b), and enzymes (Arginase 1 and IDO) that work in combination to induce an immunosuppressive response in a host body. This is important because these cells carry the CD163 receptor, which may assist in allowing bacilli to enter cells and, therefore, facilitate infection. The anti-inflammatory cytokines and growth factors present in the Th2 response reduce the effects of the macrophages needed to produce the free radicals that destroy the leprosy bacilli. This allows the bacilli to survive in the host body (de Sousa et al., 2017).

The Th2 immune response is what combats helminths infections in a host (Allen & Maizels, 2011). However, it also suppresses Th1 response. That is, the presence of the Th2 lymphocyte mediated response obstructs the Th1 response, and therefore reduces the ability of the host to fight the HD bacilli. This would theoretically allow larger load of HD bacilli to be present in the host body and is the reason the Th2 response is present in hosts with the more severe and infectious MB HD. This implies that co-infections, or the presence of the helminths, can influence the immune response (Mabbott, 2018) and can result in an increase in HD bacilli.

HD and Helminth Relationships

Some early reports have alluded to a relationship between a reduction in cell-mediated immunity and the presence of helminths and other related cell-mediated immune complications in individuals positive for HD. Prost, Nebout and Rougemont (1979) conducted a study on the relationship between the lepromatous form of HD (MB) and onchocerciasis, a disease caused by

the helminth *Onchocerca vulva.* Their study compared HD rates in populations that had high levels of onchocerciasis and no levels of Onchocerciasis in the former Upper Volta region in West Africa (now known as Burkina Faso). They reviewed data from 26 districts that had onchocerciasis present (n=456,177) and 17 districts that did not have onchocerciasis present (n=2,366,034). Due to the diffrence in the sample sizes, the percentage of people with HD and random sampling were used compare prevalence of HD in the multiple regions.Their results indicated that there was a higher prevalence of MB HD in the areas that were endemic for *O. volvulus* compared to areas that were not endemic. The data used for this study were collected via the WHO Onchocerciasis Control Programe and the Upper Volta national medical field services.

Due to the Th1-Th2 relationship, Diniz et al (2001) conducted a retrospective study to determine if there was a possible relationship between intestinal parasites and MB HD in Brazil. Health records over a period of 7 years for 477 patients positive for HD were reviewed. A control cohort was designated by randomly selecting non-positive patients visiting the same health service providers as the HD positive patients. HD positive patients were then classified by the type of HD present: 115 classified as indeterminate, 191 as tuberculoid, 83 borderline and 88 classified as lepromatous. These patients were further grouped into MB or PB, where MB included the borderline and lepromatous form and PB included the indeterminate and tuberculoid forms of HD. The intestinal parasites involved in this study were nematodes and protozoa, and their presence was determined via stool sample. Results of this study indicated that patients with HD had a significantly higher frequency of stool samples that tested positive for intestinal helminths compared to patients that did not have HD (OR=2.99, 95%CI, 1.82–4.95). Results also indicated that frequency of helminth positive stool samples were higher in HD patients classified

as MB than those classified as PB (OR 1.81, 95%CI=1.17-2.79). The results were not statistically significant for the relationship between HD and intestinal protozoa involved in the study. A limitation for this study was that the patients at the clinic came from diverse neighborhoods and may have experienced different environmental exposures which could not be controlled for.

Diniz et al (2010) further explored the association between HD and positive helminth infection by conducting a prospective cohort study to determine if there was an increased risk of patients developing MB HD (as opposed to PB) with a helminth co-infection. 105 patients who had never received leprosy treatment were sampled in Espirito Santo state in Brazil and were grouped as either MB HD or PB HD. The control population was selected from the households of the HD positive patients and was matched on age and gender. Helminth infections were determined via stool sample, similar to their earlier study; however, this study utilized 3 different evaluation techniques, sedimentation, Kato-Katz and Baerman. This study also included assessment of intracellular cytokines to determine what type of immune response was present. Their results indicated that odds of helminth infections were significantly higher (OR= 10.88, 95%CI= 4.02–29.4) in people with the lepromatous form of HD (MB HD) compared with the other HD groups and the control. Frequency of helminth infections (how often they tested positive for a helminth infection) was higher in patients who had MB HD than the PB HD patients and the control population. The results also indicated that those with HD and a helminth co-infection produced less INF-γ, which is a necessary component of the Th1 immune response. They concluded that a possible pre-existing helminth infection may increase the risk of *M. leprae* infection and also a predisposition to MB HD, instead of PB HD. A noted strength is that the control group was derived from the households of the HD positive patients, who were potentially

exposed to the same risks as the cases. The helminths involved were determined by their presence in stool tests and *S. mansoni* was not included in this study, possibly due to a lack of detection. One limitation was that though 3 techniques were used to detect the presence of helminths, these methods are known to have low sensitivity (Hernandez-Chavarria & Avendaño, 2001; Lamberton, Kabatereine, Oguttu, Fenwick, & Webster, 2014).

Additional investigations on the relationship between helminths and the immune response was conducted by Oktaria et al (2016) and Hagge et al (2017). Oktaria et al (2016) conducted a cross sectional study to determine the presence of soil transmitted helminth (STH) infections in HD patients, their relationship to the type of HD present, and to T2R leprosy reaction. 81 HD patients (20 PB and 61 MB) between the ages of 18 and 60 who were about to begin WHO designated multidrug therapy were recruited from Cipto Mangunkusumo and Dr. Sitanlala hospitals in Indonesia. Helminth infection was determined by stool sample and HD was determined via skin smears. T2R reaction was determined by the presence of erythematous papules nodules or plaques, with arthralgia and other nerve related ailments. Results showed there were more patients with helminth infections present in the MB group than in the PB group (p-value=0.034) and also in the T2R than the non T2R (p-value=0.018). They concluded that there was a possible association between soil transmitted helminths and HD and believed that STHs play a role in a predisposition to MB rather than PB, supported by Diniz et al.'s work, and also that the presence of helminth may lead to the presence of T2R. One weakness of this was the low number of positive helminth infections (n=11) involved in the study, which makes it a little difficult to generalize the results.

Hagge et al (2017) research was focused on the relationship between helminths and the T1R and T2R leprosy reactions, known in the text as 'reaction'. As these reactions are more

prominent in the MB form of HD, it was important to note if there was an association. Hagee et al (2017) theorized that the presence of STH is associated with leprosy reactions. They conducted an observational cohort study in Nepal with a sample of 145 leprosy positive patients. Stool samples were used to determine STH infection. The STHs in the study included *S. stercoralis, A. duodenale, A. lumbricoides* and *N. americanus.* Status of deworming was self-reported by the participants. A strength of this study was that samples were analyzed using a multiplex qPCR to diagnose helminths. Results indicated that being reaction positive (expressing a T1R or a T2R) was inversely associated with being STH positive. One limitation for this study was the lack of medical history for the patients, which is more common in low resource setting (Hagge et al., 2017). This resulted in the need to depend on self-reports on helminth treatment, which might not have been accurate. They also grouped T1R (more associated with Th1 response) with T2R, making it difficult to accurately interpret the outcomes. It also included those who received treatment and those who did not receive treatment for their HD.

HD, Schistosomiasis and Strongyloidiasis in Brazil

As previously mentioned, these three conditions are a concern in Brazil. Brazil is one of the six countries with the highest incidence rates of HD, coming second only to India (Alves de Oliveira Serra et al, 2019). In 2016, Brazil had 25, 218 cases of HD. This represents 12% of the global burden and 92% of the HD burden in Latin America (Amorim de Souza et al., 2018). HD can lead to permanent disabilities due to nerve damage even after treatment. This can result in employment difficulties. People are unable to work due to the disability or the stigma associated with having HD (Rafferty, 2005). The physical presentation of HD can also lead to social problems (Nazaro et al, 2017) such as ostracization by the community, especially in communities steeped strongly in religion (Nations, Lira, & Catrib, 2009). Due to the stigma, over 37.1% of the

HD positive women refused treatment (Nations et al., 2009). Though new measures have been set in place, detection rates of new cases of HD in Brazil have been declining (Amorim de Souza et al., 2018) possibly due to under diagnosing (Cunha et al., 2015).

Brazil also has an estimated 4-6 million people infected with schistosomiasis (Calasans, Souza, Melo, Madi, & Jeraldo, 2018) which is prevalent in 19 of Brazil's 27 member states (Nascimento et al., 2019). This makes Brazil the country with the highest transmission foci in Latin America (Calasans et al., 2018) and extreme cases and mortality still occur. A study was conducted by Nascimento et al (2019) in Brazil to identify the economic cost of schistosomiasis. This study was conducted using prevalence estimates of schistosomiasis in 2015. They concluded that schistosomiasis had a high economic burden of $41.7 million due to the loss in productivity and the direct and indirect costs to healthcare. Majority of this was due to indirect costs (costs not directly related to treatment), such as loss of productivity, (94.61%).

In addition, Brazil is classified as hyper endemic for strongyloidiasis (Paula & Costa-Cruz, 2011) with a prevalence of 13% (Arifin, Hanafiah, Ahmad, & Noordin, 2019) and a mortality rate of 87% in relation to hyper-infection (Cabral, Iñiguez, Moreno, Bóia, & Carvalho-Costa, 2015). These conditions lead to larger economic, social and psychological burdens to those infected and their families (Hajissa et al., 2018), a people that are already struggling with poverty. Due to these effects, new information is needed to address HD and these helminth infections. Improving the information on the relationship between HD and helminths may contribute to WHO goals of eradicating HD and other NTDs.

The studies above explore the relationships between helminths and HD with varying outcomes. However, results from the search indicates that studies on this topic are limited and are usually generalized to multiple helminths. There is very little data on *S. mansoni, S.*

stercoralis and HD. With HD and *S. mansoni's* relatively high prevalence and existing issues in Brazil, it reinforces the need to research and contribute more to this topic. It would be useful to study the relationships of individual helminths to include or eliminate an influence on HD, which this study will focus on. It is also noted that the studies utilized only stool samples, which have been shown to have lower sensitivity. This study would also use the opportunity to utilize data from multiplex bead assay (MBA) testing. This technique uses serum samples to detect the presence of pathogens and can detect past, present or subclinical infections (Won et al., 2017). This method of testing is unique, relative to other serology tests, because it is able to test for several pathogens simultaneously using the same serum sample with similar, if not better accuracy compared to ELISA testing (Moss et al., 2011). This would be more convenient, less labor intensive and less time consuming compared to the existing 'gold standard' tests (such as collecting stool samples for helminth infections) (Won et al., 2017). Therefore, MBA could be an asset in determining burden of disease in communities.

METHODS

The purpose of this study was to investigate the relationship between HD and helminths and was conducted in response to the limited data available on this relationship. It was based on data collected from a case control study conducted between June 2016 and December 2018 in partnership with the Universidade Federal de Juiz de Fora, in Minas Gerais, Brazil. The overall goals of the study were to explore a relationship between these helminths and HD, describe the seroprevalence of these often hidden NTDs in an endemic Brazilian community and garner information that might aid in the development of new NTD control strategies. The main helminths under investigation were *S. mansoni* and *S. stercoralis.* Other variables taken into consideration were age, race, gender, social economic status (SES), education and zone (community) and water activity. These variables were chosen due to their relevance to NTDs.

Sample Population

The population involved in the study were adults and children above the age of three living in Minas Gerais. The population was broken into the following categories:

1. New cases of Hansen's disease.
2. No presence of Hansen's disease but have close contact (such as living with a positive for HD), and
3. No presence of Hansen's disease and no close contact to it.

The cases for this study were people age 3 years and older, newly diagnosed with HD and had not received treatment. They were recruited from family health clinical posts and a HD reference center located in eastern Minas Gerais, Brazil. These family health clinical posts were located in Mantenha and Governador Valadares. Two controls were included to further explore any between exposure and no exposure to HD. Control 1 included patients age 3 and above who had

no HD but were in close contact with someone who was HD positive and was enrolled as a case in the study. Close contact was defined as living with or near a patient with HD and being in close proximity at least once a week. Control 2 included participants age 3 and above who had no HD and also no contact with people who had HD. Matching by community of origin and sex was conducted between cases and control 2 in order to control for possible confounding. The inclusion and exclusion criteria are summarized in Figure 1.

Figure 1

Inclusion and Exclusion Criteria

	Inclusion Criteria	**Exclusion Criteria**
Case	-Age 3 years and older -New diagnosis of HD within the previous 30 days -No prior treatment for HD -Confirmation of HD by skin smears and evaluation before or at first visit	-Children less than 3 years of age -Pregnancy -Indeterminate HD by pathology
Control 1	-Age 3 years and older -No current or previous diagnosis of HD -Close contact with a someone with HD enrolled in the study -match to cases based on age sex community of origin	-Children less than 3 years of age -Pregnancy -History of unknown skin or nerve disorders -Recent history of gastrointestinal ailments or weight loss of unknown cause
Control 2	-Age 3 years and older -No current or previous diagnosis of HD -match to cases based on age sex community of origin -No close contact with person positive for HD	-Children less than 3 years of age -Pregnancy -History of unknown skin or nerve disorders -Recent history of gastrointestinal ailments or weight loss of unknown cause -Close contact with a person positive for HD

The sample size was determined using helminth prevalence percentage. A prevalence of 15% was selected based on the known data on *S. mansoni* data provided for the region. Using a more conservative Odds Ratio of 3.5, compared to the OR of 4 garnered from available research on helminth co-infections, a prevalence of 38% was determined. The standard metrics with a power of 80% and a confidence interval of 95%. Coupled with a 10% dropout rate adjustment, it was determined that this study will require a population of at least 70 cases and 140 controls (70 for each control).

Instruments and Procedures

Participants for this study were recruited from clinics located in the municipalities mentioned previously. Recruitment was based on the patients' interest to participate in the study. After consent was obtained, a questionnaire was administered to all participants involved in the study. The questionnaire collected information pertaining to the variables under consideration (listed previously) and was administered during the initial visit. Age was collected in years, education was defined as the highest level of education obtained for adults and current level for school aged children, zone was classified as either urban or rural, SES was defined as 1x at or below the minimum wage and above the minimum wage and water activity was defined as regular performance of activities near water.

A dermatologist from the study team then conducted skin tests on the participants to determine the patients' HD status. Current HD infection was confirmed and classified by the following criteria; Skin lesions typical of HD, signs or evidence of nerve damage and bacilli positive slit skin smear tests. Patients that had a new, confirmed diagnosis of HD were designated as cases and those who did not have HD were controls. Slit skin smear tests were conducted on the newly diagnosed HD cases to aid the classification of either multibacillary, MB

or paucibacillary PB, HD. Control patients were examined to ensure they did not display any symptoms of active HD infection. They were then divided into exposure to HD and no exposure to HD. For the control containing participants that had no exposure to HD, the participants were matched to cases by age, gender and community.

Blood samples were drawn via venipuncture and centrifuged to obtain serum for the Multiplex Bead Assay (MBA) testing. Multiplex testing method was employed in order to study its ability to test for multiple pathogens. LID-1 protein was used to determine the presence of HD antibodies. LID-1 is a combination of the proteins ML0405 and ML2331, known for their high sensitivity in detecting HD (Amorim et al., 2016). The reaction of IgG and IgG4 antibodies to LID-1 was utilized to determine positive test results for HD. Antigens SEA and NIE were used to detect antibodies for *S. mansoni* and *S. stercoralis*, respectively. SEA is a soluble egg antigen extracted from homogenized *S. mansoni* egg cells (Meevissen, Yazdanbakhsh, & Hokke, 2012). NIE is a recombinant protein derived from the filariform larvae DNA (Ravi, Ramachandran, Thompson, Andersen, & Neva, 2002). These antigens were coupled to the beads and tested for validity prior to use on this MBA platform (O'Hearn et al., 2016). Detection of IgG4 antibodies against these antigens (LID-1, SEA, and NIE) were then measured by florescence on the MBA platform by colleagues at the Centers for Disease Control (CDC) in Atlanta, GA.

Stool samples were collected for diagnosis and to determine the intensity of *S. mansoni* infection. Intensity of infection was characterized by the number of *S. mansoni* eggs present in the stool sample using WHO guidelines. Heavy helminth infection was equal to or greater than 400 eggs per gram (epg) of stool, moderate infection was 100-399 epg and light infection was 1-99 epg. Participants were provided with stool collection kits in order to provide self-collected

samples over 3 successive days. The egg quantity in the stool sample was determined using the Kato Katz method (Katz, Chaves, & Pellegrino, 1972)

Ethics

Approval was obtained from the IRB of both Emory University and the Universidade Federal de Juiz de Fora. No invasive procedures were conducted beyond venipuncture. Consent was obtained from patients and parents of patients below 3 years of age. Assent was obtained from children over the age of 6.

Statistical Methods

This analysis was conducted utilizing a subset of the sample population, focusing on those who consented to multiplex testing, and did not observe the matching criteria. Therefore, matching methods of analysis were not used. Data were analyzed using SAS 9.4 (Carey, NC). Odds Ratios (ORs) were calculated using Chi square to analyze the relationship between HD and helminth infection. A Fisher's exact test was used to determine a relationship between the classification of Hansen's disease (PB or MB), *S. stercoralis*, and *S. mansoni.* infections. Linear regression was used to analyze the relationship between the type of HD (multibacillary, MB and paucibacillary, PB) and the intensity of *S. mansoni* infection. Logistic regression was used to determine any associations between the presence of LID-1 (the HD antibodies) and the demographic covariates identified and to explore any relationship between the helminths and those covariates in the study. An alpha value threshold of 0.05 was utilized to determine if p-values were statistically significant.

RESULTS

Table1: *Demographics of Participants*

Variable	Case	Control 1	Control 2	Total
	N (%)	N (%)	N (%)	N (%)
Age				
3 to 15	9 (11.5)	16 (18.4)	10 (15.2)	35 (12.2)
15 to 30	13 (16.7)	21 (24.1)	9 (13.6)	43 (18.6)
30 to 50	22 (28.2)	28 (32.2)	20 (30.3)	70 (30.3)
50 +	34 (43.6	22 (25.3)	27 (40.9)	83 (35.9)
Gender				
Male	43 (55.1)	33 (37.9)	36 (53.7)	112 (48.3)
Female	35 (44.9)	54 (62.1)	30 (44.8)	119 (51.3)
Unknown	0 (0.0)	0 (0.0)	1 (1.5)	1 (0.4)
Race				
White	13 (16.7)	24 (27.6)	11 (16.4)	48 (20.7)
Black	14 (18.0)	13 (14.9)	13 (19.4)	40 (17.2)
Asian	0 (0.0)	2 (2.3)	1 (1.5)	3 (1.3)
Mixed	38 (48.7)	43 (49.4)	41 (61.2)	122 (52.6)
Native	1 (1.3)	0 (0.0)	0 (0.0)	1 (0.4)
Unknown	12 (15.4)	5 (5.8)	1 (0.4)	18 (7.8)
Zone				
Urban	37 (47.4)	50 (57.5)	30 (44.8)	117 (50.4)
Rural	36 (46.1)	37 (42.5)	35 (52.2%	108 (46.6)
Unknown	5 (6.4)	0 (0.0)	2 (3.0)	7 (3.0)
Education				
University	1 (1.3)	6 (6.9)	4 (6.0)	11 (4.72)
High School	10 (12.8)	19 (21.8)	10 (14.9)	39 (16.8)
Elementary	56 (71.8)	53 (60.9)	44 (65.7)	153 (66.0)
Never attended	11 (14.1)	8 (9.2)	7 (10.5)	26 (11.2)
Unknown	0 (0.0)	1 (1.2)	2 (3.0)	3 (1.3)
SES				
Above minimum	46 (59.0)	61 (70.1)	40 (59.7)	147 (63.4)
Below minimum	30 (38.5)	25 (28.7)	25 (37.3)	80 (34.5)
Unknown	(2.6)	1 (1.2)	2 (3.0)	5 (2.2)
Water Activity				
Yes	23 (29.5)	15 (17.2)	15 (22.4)	53 (22.8)
No	52 (66.7)	71 (9.6)	51 (76.1)	174 (75.0)
Unknown	3 (3.9)	1 (1.2)	1 (1.5)	5 (2.16)

Study Demographics

We enrolled 232 participants for this study with a mean age of 40.3 years, median 41 and standard deviation of 20.7. 66 patients (mean age of 38.4) tested positive for *S. mansoni* antigens and 44 tested patients (mean age of 38.4) tested positive for *S. stercoralis* antigens. Seroprevalence for *S. stercoralis* and S. *mansoni* in this study population were 19.9% and 29.9% respectively. For schistosomiasis, ova and parasites by Kato Katz were found in 12 of 214 stool samples tested (5.6%). A univariate analysis was used to summarize demographic information (table 1). In our sample, 78 (33.8%) were enrolled as HD positive cases, 87 (37.7%) as control 1 and 67 (28.6%) as control 2. Data collected from 221 participants were used in the analysis. Controls 1 and 2 were compared to the cases separately in order to determine if there were any possible differences in people that were exposed versus not exposed to HD

HD status and Helminth Infection

Distribution of helminth infection among the 3 groups did not vary significantly with control 2 having slightly lower frequencies of the helminth infection than control 1 and the cases. Results are summarized in table 2. Helminths were analyzed together then separately. Chi square results indicated that there was no statistically significant association between the combined presence of either helminth and HD in control 1 (cOR=1.02, 95% CI=0.55-1.87) and in control 2 (cOR=0.76, 95%CI= 0.39-1.47). There were also no statistically significant associations found between HD and *S. stercoralis* specifically in both control 1 (cOR=1.01, 95%CI=0.47-2.17) and control 2 (cOR 1.54, 95%CI=0.65-3.70) and between HD and *S. mansoni* specifically in both control 1 (cOR=1.07, 95%CI=0.54-2.101) and control 2 (cOR=1.20, 95% CI=0.58-2.50).

Table 2: *Chi Square Analysis of Helminth with HD as Outcome*

	Case, n (%)	**Control 1, n (%)**	**cOR, 95% CI (case vs control1)**	**Control 2, n (%)**	**cOR, 95% CI (case vs control 2)**
Both Helminths	36 (45.6)	40 (46.0)	1.02, 0.55-1.87	26 (38.8)	0.76, 0.39-1.47
S. stercoralis infection	16 (21.9)	18 (21.7)	1.01, 0.47-2.17	10 (15.4)	1.54, 0.64-3.70
S. mansoni infection	23 (31.5)	25 (30.1)	1.07, 0.54-2.11	18 (27.7)	1.20, 0.58-2.50

cOR = crude odds ratio CI=confidence interval

LID-1 and Helminth Infection

Next, the relationship between LID-1 and helminths was evaluated. In this analysis, HD patients were not separated into separate controls. No association was determined between the combined helminths and HD (OR=1.04 95%CI=0.50-2.17). However, statistically significant associations were determined between LID-1 and *S. stercoralis* (OR=2.642, 95%CI=1.188-5.88) and *S. mansoni* (OR=0.36, 95%CI=0.13-0.97) when analyzed separately (table 3).

Table 3: *Chi Square analysis: S. stercoralis, S. mansoni with LID-1 as Outcome*

	LID-1 positive N (%)	**OR**	**95%CI**
Any Helminth	16 (47.1)	1.04	0.50-2.17
S. stercoralis	12 (35.3)	**2.64**	**1.19-5.88**
S. mansoni	5 (14.7)	**0.36**	**0.13-0.97**

OR=Odds Ratio Significant results are indicated in bold text

Classification of HD and Helminth Infection

To assess the relationship between the type of HD and the helminth infection, the 78 patients who were positive for HD were classified as either PB or MB. 22 (28.2%) participants were determined to have PB HD and 56 (71.8%) had MB HD. The distribution of *S. stercoralis* and *S. mansoni* among cases was evaluated. The percentage of patients testing positive for *S. stercoralis*

was higher in those with MB HD (n=13, 24.5%) than in those with PB HD (n=3, 15.0%). The inverse occurred for patients positive for *S. mansoni,* with a higher percentage occurring in those with PB HD (n=15, 40%) than in MB HD (28.3%). A Fisher's exact test indicated that there was no statistically significant association between the classification of HD and *S. mansoni* infection (cOR=0.59, 95%CI=0.20-1.73) or *S. stercoralis* infection (OR=1.8, 95%CI=0.46-7.30). Results are summarized in table 4.

Table 4: *Fisher's Exact Test Analysis: Classification of HD and Helminth*

	PB	**MB**	**(MB vs PB)**	
	N (%)	**N (%)**	**OR**	**95%CI**
S. stercoralis	3 (15.0)	13 (24.5)	1.84	0.46-7.30
S. mansoni	8 (40.0)	15 (28.3)	0.59	0.20-1.73

Classification of HD and intensity of *S. mansoni* infection

A two-sample t test was used to explore the relationship between classification of HD and intensity of *S. mansoni* infection as measured by the Kato Katz (Table 6). Twelve stool samples tested positive for *S. mansoni* ova, of which 7 were also positive for HD. Out of the 7 HD patients with positive stool tests, 4 patients were determined to have medium infection and 4 had light infection. Although the mean egg count was higher in PB than MB (Table 5), results of the t test indicate that this difference was not statistically significant (t score 1.61, p-value =0.1128).

Table 5: T Test: Classification of HD and S. mansoni Egg Count

Group	**N**	**Mean number of *S. mansoni* eggs in stool**	**Standard Deviation**	***t***	**P value**
Hansen's disease	**7**	9.1	69.7	1.61	0.11
Paucibacillary	4	15.4	39.6		
Multibacillary	3	3.5	23.3		

Other Findings

To take full advantage of the results from the multiplex (MBA) data, we also explored potential associations between LID-1 and the other demographic variables in the study using a multivariable logistic regression (Table 6).

Table 6: *Regression Analysis of LID-1 and Demographics with*

	LID-1 Positive N (%)	**aOR**	**95%CI**
Helminth			
*Yes	16 (47.1)		
No	18 (52.9)	0.91	0.35-2.38
Age			
*3 to 15	3 (8.8)		
15 to 30	7 (20.6)	3.07	0.48-19.86
30 to 50	8 (23.5)	2.19	0.38-12.58
50 +	16 47.1)	2.06	0.36-11.89
Gender			
* Male	24 (70.6)		
Female	10 (70.6)	**0.33**	**0.12-0.86**
Race			
*White	8 (26.7)		
Black	10 (33.3)	1.05	0.31-3.51
Mixed	12 (40.0)	0.49	0.17-1.43
Zone			
Urban	23 (69.7)	2.23	0.85-5.83
* Rural	10 (30.3)		
Education			
High School	4 (11.8)	0.469	0.073-3.031
Elementary	25 (73.5)	0.64	0.16-2.53
*Never attended	5 (14.7%		
SES			
Above minimum	22 (66.7)	1.087	0.424-2.784
*Below minimum	11 (33.3)		
Water Activity			
*Yes	6 (18.8)		
No	26 (81.2)	0.81	0.27-2.46

**Reference variable* *Statistically significant results are in bold test*.

Of the 34 patients that tested positive for LID-1, 22 (64.7%) were HD positive and 12 (35.3%) were HD negative, with 1 patient having no contact with HD. Adjusting for other covariates, there was a statistically significant association between LID-1 and gender, with female participants being less likely to test positive for LID1 than male participants. (aOR=0.33, 95%CI=0.12-0.86) .

Finally, a multivariable logistic regression was conducted on the helminths and the demographic variables involved to determine any significant associations (Table 7). There was no association between *S. stercoralis* and the demographics but there was a significant association between *S. mansoni* and the zone the participants lived in, with people living in urban areas less likely to test positive for schistosomiasis than people living in rural areas (aOR= 0.38, 95%CI= 0.19-0.79).

Table 7*: Regression Analysis of Demographic Variables with Helminth Infection as Outcome*

	S. stercoralis			*S. mansoni*		
	p-value	**aOR**	**95%CI**	**p-value**	**aOR**	**95%CI**
Other Helminth						
*Yes	36 (81.8)			58 (87.9)		
No	8 (18.2)	0.47	0.18-1.19	8 (12.1)	2.23	0.86-5.78
Age						
*3 to 15	3 6.8)			13 (19.7)		
15 to 30	1 (2.3)	0.25	0.02-3.32	12 (18.2)	1.08	0.31-3.78
30 to 50	14 (31.8)	2.66	0.63-11.37	20 (30.30)	1.08	0.38-3.05
50 +	26 (59.1)	3.96	0.92-17.00	21 (31.8)	0.87	0.28-2.74
Gender						
* Male	26 (59.1)			36 (54.6)		
Female	18 (40.9)	0.56	0.24-1.30	30 (45.4)	0.84	0.42-1.70
Race						
*White	12 (28.6)			16 (25.0)		
Black	10 23.8)	0.59	0.18-1.92	15 (23.4)	1.21	0.42-3.48
Asian	2 (4.8)	10.5	0.35-320.03	0 (0.0)	<0.001	<0.001->99
Mixed	18 (42.9)	0.43	0.16-1.14	32 (50.0)	0.61	0.27-1.42
[a]Native	0 (0.0)			1 (1.56)		
Zone						
Urban	25 (56.8)	0.82	0.35-1.93	21 (31.8)	**0.38**	**0.19-0.79**
*Rural	19 (43.2)			45 (68.2)		
Education						
University	1 (2.3)	0.53	0.04-6.60	7 (10.6)	**7.31**	**0.99-53.7**
High School	5 (11.4)	0.57	0.09-3.49	5 (7.6)	0.71	0.13-3.90
Elementary	29 (65.9)	0.78	0.25-2.46	50 (75.8)	2.02	0.56-7.22
*Never attended	9 (20.5)			4 (6.4)	0.68	0.31-1.49
SES						
*Above minimum	28 (65.1)			39 (60.0)		
Below minimum	15 (34.9)	1.00	0.41-2.44	26 (40.0)	0.96	0.45-2.03
Water Activity						
*Yes	11 (25.0%)			25 (37.9%)		
No	33 (75.0%)	0.60	0.23-1.54	41 (62.1%)	0.68	0.31-1.49

**reference variable Significant values are in bold text*

DISCUSSION

In a study exploring the relationship between helminths and HD, we did not find any statistically significant relationships between active HD, *S. stercoralis* and *S. mansoni*; however, we did find a statistically significant relationship between HD antigen LID-1 and the helminths.

We observed similar proportions of *S. stercoralis* and *S. mansoni* in patients with HD and patients in close contact with HD and a slightly lower proportion in those with no contact with HD. The similarities in proportion between cases and exposed control could be related to the fact that the exposed control was in close contact with HD patients, usually a family member, and would more likely experience similar environmental exposures. In relation to classification of HD, although not statistically significant, the distribution of *S. mansoni* and *S. stercoralis* differed. A higher percentage of *S. stercoralis* infection was seen in patients with MB than in PB. The opposite was seen for *S. mansoni* infection, with more positive cases found in the PB group than in the MB group.-Nevertheless, there was no significant association between the classification of HD and helminth infection (*S. stercoralis*, cOR=1.84, 95%CI=0.46-7.30) and (*S. mansoni*, cOR=0.59, 95%CI=0.20-1.74). This is possibly because schistosomiasis is usually seen in younger people, who are more likely to have PB, while strongyloidiasis is usually seen in the older population (Naves & Costa-Cruz, 2013), where MB is more likely to be seen (Nobre et al., 2017). This is supported in our data by a lower mean age for those with *S. mansoni* compared to those with *S. stercoralis* (38.4yrs vs 51.6yrs) in this study population.

We observed that the mean number of *S. mansoni* eggs was higher in those with PB (15.4 epg) than in MB (3.5 epg), however, we found no statistically significant association between the classification of HD and intensity of *S. mansoni* infection (p-value=0.12). This could be due to the limited sample size for this analysis; only 7 HD patients tested positive for *S. mansoni* ova.

The low *S. mansoni* positive rate may be due to the accuracy of stool tests. Research has shown that though the Kato-Katz method is recommended by WHO for *S. mansoni* testing, it has low sensitivity (Silva-Moraes et al., 2019). Therefore, it is possible that the intensity of infection may not have been accurately captured for the study, especially for lower intensity infections. This poor test and the small sample size limited the interpretation of these results. While this study does not find a statistically significant relationship between helminth infection (as measured by sero-reactivity to helminth antigens) and current HD status, past research conducted indicates there is an association, with MB HD patients more likely to be positive for helminth infections than PB HD patients (Diniz et al., 2010; Oktaria et al., 2016). One explanation could be that we used serology as a measure of helminth infection and, therefore, could represent a prior infection.

Our results differed when looking at LID-1 positivity as opposed to the clinical diagnosis of HD. As previously mentioned, MBA tests for the presence of antibodies to pathogens in serum. Though serological testing is relatively sensitive, it is limited in identifying active HD cases because of the anti-LID-1's (antibody detected) reduced sensitivity to detect active HD (Amorim et al., 2016). Because of this low sensitivity among active HD cases, many clinically diagnosed cases in our study population were anti-LID-1 negative. Coupled with several control 1 contacts and 1 control 2 being anti-LID-1 positive., different results were attained from MBA testing relative to the clinical definition of cases. While difficult to interpret, given these complexities with this serological test, results indicated there was no statistically significant association between LID-1 and the helminths combined. However, there was a statistically significant association when the helminths were analyzed separately (*S. stercoralis,* cOR=2.64, 95% CI= 1.20-5.88; *S. mansoni* cOR=*0.36*, 95%CI=0.13-0.97). With an OR of 2.64, the positive association between *S. stercoralis* and the presence of LID-1 antibodies is consistent with

previous research conducted by Diniz et al (2010) that also found a positive association between helminths (including *S. stercoralis*) and lepromatous leprosy (MB), possibly related to the Th2 response the helminths elicit. Interestingly, with an OR of 0.36, *S. mansoni* had a negative association with HD. This is not supported by research including other helminths but, since there is almost no information on the relationship between schistosomiasis and HD, it is difficult to interpret these results. We were also unable to determine a possible association between LID-1 and intensity of infection as there were no patients that tested positive for both LID-1 antigen and the stool tests.

Besides the possible discovery of an HD-helminth relationship, the multiplex was also utilized to describe seroprevalence of these NTDs. Thirty-four patients tested positive for LID-1 and were distributed among patients who had HD 22 (64.7%) and those who did not, with 11(32.4%) in the exposed group and 1 (2.94%) in the non-exposed group. As previously discussed, due to the nature of MBA testing, it was difficult to determine the prevalence of active HD for this population. All cases that tested positive for LID-1 had MB HD. These findings were also consistent with existing literature. Studies indicate that anti LID-1 antibodies are detected in patients with HD, have been exposed to HD or have a latent infection as well as being more present in MB than PB (Amorim et al., 2016). We noted some statistical significance results between the LID-1 and gender. They indicated that women were less likely to be positive for LID-1 than men (cOR=0.33, 95%CI=0.12-0.86). Prevalence of the helminths in this population were 19.9% for *S. mansoni* and 29.9% for *S. stercoralis*, which were slightly higher than the 15% or lower established for the area. We noted a statistically significant relationship between *S. mansoni* and zone, with those in urban areas less likely to test positive compared to rural areas

(cOR=0.38, 95%CI=0.19-0.79), This is logical due the rural area's proximity to bodies of water with *S. mansoni* present.

Though not part of the main analysis, we compared we noted that 66 (29.9%) patients were determined to have *S. mansoni* antibodies utilizing the multiplex. Employing just the stool test, an egg count could be determined in only 12 of the 214 patients tested (5.6%). While serological tests are usually positive for current and past infections, MBA uses SEA, which is more indicative of a fairly recent or current infection. The MBA test may help improve detection of schistosomiasis, especially low level infection, which usually cannot be determined using stool tests (Grenfell et al., 2013). Therefore, this type of testing may be more useful in *S. mansoni* testing relative to the current methods.

CONCLUSION

While we were unable to determine an association between current HD and helminth infections, the higher percentage of *S. mansoni* infection in patients with PB, the negative association with LID-1 antigen and the higher mean egg count in PB HD should prompt further exploration to understand the relationship between schistosomiasis and HD.

Similarly, while we acknowledge that the characteristics of serological testing makes the interpretation of the LID-1 HD-helminths associations harder to interpret, the associations determined using LID-1 as a marker of HD indicates that there is a need to explore the MBA method with HD and other NTD detection. Its ability to also detect antibodies in those that do not exhibit symptoms should also be explored as it may assist in detection, especially for early and low levels of infection, and help determine community prevalence of these NTDs.

IMPLICATIONS

Results summarized above indicate that there is a need to continue the research on HD and other NTD associations and interactions. One proposed study would be a longitudinal study comparing patients who did not have active HD but tested positive for LID-1 antigen and at least one helminth infection to patients who only tested positive for LID-1 antigen to determine if there is a difference in risk of developing active HD.

Apart from NTD relationships, the MBA's ability to test for multiple infections at the same time should also be further investigated. This may open many new doors for NTD detection. Its use can be expanded to incorporate other NTDs with minimal extra efforts allowing testing of multiple NTDS in the same population. Further studies on the detection of at-risk populations should also be conducted. This information could potentially be used to improve existing methods of tackling a disease. For example, being able to test for multiple infections at once may help determine at-risk populations or detect infections for different diseases at the same time. This would be quite useful in determining who may receive treatment and other resources, thereby improve resource allocation and efficiency. Determining a-risk populations could also help further understand, and possibly lead to, improved prophylactic attempts to control infections.

In conclusion while we were unable to determine an association between active HD and the helminths under review, we were able to determine an association between HD antigens (LID-1) and the helminths, with our results prompting the need for further exploration of a relationship between schistosomiasis and HD as well as research on multiplex usage.

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