



Research Paper

Opening a Can of Worms: Leprosy Reactions and Complicit Soil-Transmitted Helminths



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ABSTRACT

Background: >94% of new annual leprosy cases are diagnosed in populations co-endemic for soil-transmitted helminths (STH). STH can profoundly dysregulate host immune responses towards Th2 bias, which can be restored over time after deworming. We hypothesize that STH co-infection is associated with leprosy reaction (denoted as simply “reaction” herein) occurrence within a co-endemic population.

Methods: A cohort study was performed on a cohort of Nepalese leprosy patients across treatment and diagnostic classifications who were screened by routine fecal smear microscopy and multiplex quantitative PCR (qPCR) for *Ascaris lumbricoides* (Al), *Strongyloides stercoralis* (Ss), *Ancylostoma duodenale* (Ad) and *Necator americanus* (Na).

Results: Among 145 patients, 55% were positive for ≥ 1 STH (STH+): 34% Al+, 18% Ss+, 17% Ad+ and 5% Na+. Significant inverse STH and reaction relationships were evidenced by the bulk of cases: 63% reaction-negative were STH+ of total cases ($p = 0.030$) while 65% reaction-positive were STH− in new cases (96; $p = 0.023$). Strikingly, the majority of STH+ were reaction-negative, even when considering each species: 59% Al+, 60% Ss+, 62% Ad+ and 67% Na+ of new leprosy cases.

Conclusions: Absence of STH co-infection is associated with leprosy reaction at diagnosis within a co-endemic population. This is likely due to immune reconstitution effects after deworming or interruption of chronic STH-mediated immune dysregulation.

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1. Introduction

Over 94% of annual new leprosy cases originate from areas co-endemic for soil-transmitted helminths (STH), including all 16 countries reporting >1000 new cases annually (Fig. 1) (WHO, 2012; WHO, 2015). A growing body of evidence demonstrates that chronic STH infections wield profound systemic immune dysregulation towards a Th2 bias, proven relevant in chronic immunopathologies such as human immunodeficiency virus (HIV), tuberculosis (TB), malaria and allergy (Salgame et al., 2013; Coakley et al., 2016). Leprosy can present across the Th1–Th2 immunological spectrum, respectively classified as polar tuberculoid (TT) or polar lepromatous (LL) leprosy with borderline classifications between: borderline tuberculoid (BT), borderline borderline (BB) and borderline lepromatous (BL) leprosy (Scollard et al., 2006a). Chronic STH co-infections have been associated with

multibacillary (MB) as compared to paucibacillary (PB) leprosy, decreased Th1 and increased Th2 cytokines and likely facilitation *M. leprae* growth and disease progression (Diniz et al., 2010). Other STH co-infection immunopathologies have shown that antihelminthic treatment (deworming) can permit immune reconstitution over ≥ 2 –22 months likely depending on complex factors including complicit disease and (mal)nutrition as well as STH variables such as species, combination, burden and duration of infection (van den Biggelaar et al., 2004; Elias et al., 2008; Ivan et al., 2015).

The most damaging physical consequence of leprosy is permanent disability, primarily caused by neuropathy induced during dynamic and unpredictable Th1/Th2 complications called leprosy reactions (denoted as simply “reaction” herein) (Khadge et al., 2015; Corstjens et al., 2016). Due to persistent *Mycobacterium leprae* antigen, Reactions can variably persevere as a major clinical concern affecting up to 30–50% of patients either before, during or even years after multi-drug therapy (MDT) (Scollard et al., 2006a). Increased Th1 responses correlate with Type 1 Reaction (T1R), while patients with more dominant Th2 response histories (BL-LL) may develop a Type 2 Reaction (T2R) also known as Erythema Nodosum

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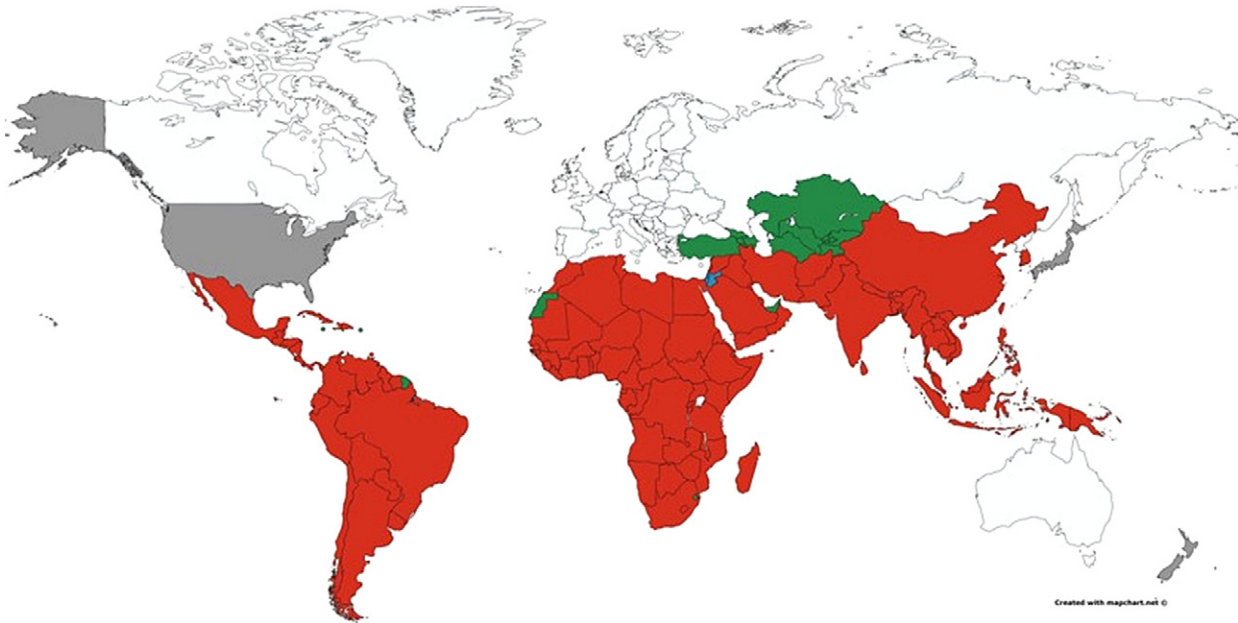


Fig. 1. Global map of reported STH and leprosy. STH and leprosy exist primarily within populations living with significant and sustained sanitation, housing and poverty issues. While the World Health Organization posts regular bulletins, individually updated data is not always submitted; therefore, the most recent reported data from 2009 to 2015 is shown for each nation (WHO, 2012; WHO, 2015; Pullan et al., 2014; WHO, 2009). Red indicates STH and leprosy are both reported. Green indicates STH reported but leprosy data unreported or unavailable. Blue indicates STH reported but no leprosy cases reported. Gray indicates no endemic STH with few leprosy cases reported. White indicates neither leprosy nor STH are endemic.

Leprosus (ENL). Therapeutic interventions linked with immune reconstitution inflammatory syndrome (IRIS) after prolonged immunosuppression have been associated with reaction, including HIV highly active antiretroviral therapy (HAART) or cessation of extended TNF- α interceptor therapy (Scollard et al., 2006b; Deps & Lockwood, 2008). Therefore, it is reasonable that immune reconstitution after removal or interruption of chronic STH co-infection could be a previously unsuspected yet indigenous trigger for reaction development.

We hypothesized that STH co-infection status is associated with the occurrence of reaction. In this observational cohort study, we employed routine microscopy and multiplex quantitative real time PCR (qPCR) to screen 145 Nepalese leprosy patients for endemic STH. Results indicate that reaction status is significantly and inversely associated with STH.

2. Materials and Methods

Fecal samples alongside clinical information were collected from 145 Nepalese leprosy patients collected by convenience sampling as they attended Anandaban Hospital (Lalitpur) or satellite clinics (Patan,

Butwal and Chandranigahapur) from December 2011 to September 2013. Participants included: newly diagnosed, undergoing MDT, new reaction, relapse or defaulter at MDT restart and released from treatment (RFT) after completion of MDT. Comprehensive leprosy diagnosis included clinical and physiotherapist evaluations, slit skin smear bacterial index (BI) and skin biopsy histopathology. Signed informed ethical consent, participant chart review and data collection with case report forms were performed under approval by the Nepal Health Research Council (NHRC, Approval 101/2011), which conforms to the standards indicated by the Declaration of Helsinki. Patients refusing consent, aged under 18 or above 60 years, with chronic disease, pregnant or lactating were excluded from the study.

Wet mount fecal smear microscopy was performed by a qualified medical technologist. Remaining stool was stored at -80°C until processing. DNA isolation and multiplex qPCR was performed as previously described for detection of *Ascaris lumbricoides* (Al, roundworm), *Strongyloides stercoralis* (Ss, threadworm), and hookworms *Ancylostoma duodenale* (Ad), *Necator americanus* (Na) (Basuni et al., 2011). Primer sequences for multiplex qPCR are listed in Table 1. Briefly, 100 mg

Table 1
Primer sequences for target soil-transmitted helminths (Basuni et al., 2011).

Target organism	Oligo name	Oligonucleotide sequence	Size of the target region	Target gene
<i>Ascaris lumbricoides</i>	Al-F	5'-GTA ATA GCA GTC GGC GGT TTC TT-3'	89 bp	ITS1
	Al-R	5'-GCC CAA CAT GCC ACC TAT TC-3'		
	Al-P	ROX-5'-TTG GCG GAC AAT TGC ATG CGA T-3' -black hole quencher 2		
<i>Strongyloides stercoralis</i>	Ss-F	5'-GAA TTC CAA GTA AAC GTA AGT CAT TAG C-3'	101 bp	18 s
	Ss-R	5'-TGC CTC TGG ATA TTG CTC AGT TC-3'		
	Ss-P	Alexa 680-5'-ACA CAC CCG CCG TCG CTG C-3' -black hole quencher 3		
<i>Ancylostoma duodenale</i>	Ad-F	5'-GAA TGA CAG CAA ACT CGT TGT TG-3'	71 bp	ITS2
	Ad-R	5'-ATA CTA GCC ACT GCC GAA ACG T-3'		
	Ad-P (MGB probe)	JOE-5'-ATC GTT TAC CGA CTT TAG-3' -nonfluorescent quencher		
<i>Necator americanus</i>	Na-F	5'-CTG TTT GTC GAA CCG TAC TTG C-3'	101 bp	ITS2
	Na-R	5'-ATA ACA GCG TGC ACA TGT TGC-3'		
	Na-P (MGB probe)	FAM-5'-CTG TAC GCA TTG TAT AC-3' -nonfluorescent quencher		
<i>Phocine Herpes</i>	PhHV-F	5'-GGG CGA ATC ACA GAT TGA ATC-3'	89 bp	gB
	PhHV-R	5'-GCG GTT CCA AAC GTA CCA A-3'		
	PhHV-P	Cy5-5'-TTT TTA TGT GTC CGC CAC CAT CTG GAT C-3'-black hole quencher 2		

stool was suspended in 200 μ l of 2% polyvinylpyrrolidone in PBS buffer and heated for 10 min at 100 °C. Then, the stool was processed according to the manufacturer's protocol (QIAamp DNA Minikit, Qiagen, Catalog No. 51306). Phocine herpesvirus 1 (PhHV-1) for an internal DNA extraction control was kindly donated by Dr. Martin Schutten (Department of Virology, Erasmus MC, Netherlands) and was added at the AL buffer stage. STH species-specific plasmid-positive controls were donated by Dr. Rahmah Noordin (Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia) which were previously reported with lower detection limits of 10 copies for Al, Ad and Ss and 1000 copies for Na (Basuni et al., 2011). Analyses were performed with Qiagen Multiplex PCR kits in a Rotor Gene Q series machine with Ver.2.1.0 software (Qiagen, Germany). STH burden was assigned based on cycle threshold values as previously described: high (≤ 30), moderate ($>30 \leq 35$), low (>35) (Arndt et al., 2013).

2.1. Statistical Methods

Data were analyzed with the use of SPSS (version 12, SPSS Inc., Chicago, IL) and STATA 9 (Stata Corporation, TX). The statistical significance of associations between groups were assessed primarily using the chi-squared test. Logistic regression was used to assess helminth positivity as a predictor of reaction outcomes.

3. Results

Among 145 total patients (Fig. 2a), comprehensive diagnosis indicated 21% PB and 79% MB cases across the Ridley-Jopling spectrum: 5 pure neuritic (PN, 3%), 6 TT (4%), 71 BT (49%), 1 BB (1%), 29 BL (20%), 33 LL (23%). Fifty-seven percent (82/145) were in reaction-positive: 49 T1R (34%), 13 ENL (9%), and 20 neuritis (14%). The majority were newly diagnosed (96, 66%) alongside 32 undergoing MDT (25%, range 2 weeks to 12 months), 14 relapse or restart after default (7%) and 3 RFT (2%, range 1–36 months).

Within limited resource contexts, documented medical history for new or referral cases is usually limited or nonexistent. Therefore, participants were asked to self-report history of deworming. Most patients (112/145, 77%) indicated none or unknown within the last year (Fig. 2b). However, 23 remembered deworming within ≤ 6 months, 57% (13) were reaction-positive (11 MB, all BT-LL). Interestingly, out of the 10 patients who reported deworming within ≤ 3 months: 8 patients (80%) were reaction-positive (4 T1R, 3 ENL, 1 neuritis only).

Of 145 total patients, only 6 (4%) cases evidenced parasites by wet mount fecal smear microscopy: 3 (2%) for STH and 3 (2%) for *Giardia* and/or *Entamoeba histolytica* (Fig. 3a). Molecular testing by qPCR, however, identified 79/145 (55%) positive for ≥ 1 STH (STH+): 34% Al+ (49), 18% Ss+ (26), 17% Ad+ (25) and 5% Na+ (7). All microscopy STH+ patients were also STH+ by qPCR. Most STH+ (56/79; 71%) were single species infections with multiple STH infections (polySTH+) evidenced

in 18 (23%), 4 (5%) and 1 (1%) with 2–4 STH species respectively. Across individual STH, Ct values indicated carriage of 18% (19/107) low, 50% (54/107) moderate and 32% (34/107) high infection burdens. Among new leprosy cases (96), 47% (45/96) hosted ≥ 1 STH, with patterns in species and polyparasitism similar to the total patient cohort.

STH co-infection status did not significantly differ among total patients in categories of gender, PB/MB, BI positivity or Ridley-Jopling classification (Fig. 3b). When considering reaction, however, a pattern of inverse presence became apparent (Fig. 3b). STH– cases were significantly more likely to be reaction, with STH+ significantly more likely to be reaction-negative ($p = 0.030$; OR 2.09, CI 1.07–4.08). When divided into reaction types, group numbers reduced and statistical significance was lost. Ongoing MDT (32) patients were mostly reaction-positive (21), 52% (11/21) of which were STH–; while reaction-negative (10) were 83% STH+ (10/12). Out of 17 RFT/Defaulter/RFT patients, 64% (11) were reaction-positive and 94% (16) were STH–.

Among new leprosy cases ($n = 96$) (Fig. 4a), inverse STH/reaction trends were significant with the majority of reaction-negative (56/96; 58%) also STH+ ($p = 0.023$; OR 2.59, CI 1.13–5.92). Conversely, the majority of reaction-positive (62/96; 65%) were STH– (comparison within reaction cases: $p = 0.037$; OR 2.06, CI 1.04–4.10). Among new LL patients (21), 8 of 9 reaction-positive were STH–/LL (89%) (5 neuritis, 1 T1R, 2 ENL); conversely, 8 of 9 (89%) STH+/LL were reaction-negative (Fig. 4b). STH– cases were primarily reaction-positive: 65% of total (43/66) and 61% (31/51) of new leprosy cases. When considering individual STH species (Fig. 5), the majority positive was reaction-negative: 59% Al+ (16/27), 60% Ss+ (9/14), 62% Ad+ (8/13) and 67% Na+ (4/6) of new leprosy cases. Of polySTH+ (12), 75% (9) presented reaction-negative. Most STH infection burdens were moderate (Ct values of 30–35). Most Al+ (78%, 21/27) and Ss+ (54%, 8/15) were mono-STH infections, while most Ad+ (85%, 11/13) and Na+ (67%, 4/6) were polySTH+ (i.e., had ≥ 1 of the other STH tested). If using an alternate scale with a narrower definition of high burden (Ct values of <25) broadening moderate burden range (Ct values 25–35) significant differences evidenced. Moderate Al+ cases were significantly reaction-negative (65%, 13/20) in comparison to STH– cases ($p = 0.043$). The majority (82%, 9/11) of moderate Ad+ co-infections significantly presented reaction-negative ($p = 0.014$) but were also mostly (7/9) polySTH+.

Corticosteroid treatment for reactions is known to increase susceptibility to Ss infection (De Souza et al., 2014). Among the 145 total patients, 8 had received corticosteroids prior to sampling: 4 T1R and 4 ENL cases of which 4 were STH+. Only one new case was under corticosteroid treatment at sampling (T1R, low burden Al+).

4. Discussion

Our study results demonstrate that STH co-infection is inversely associated with leprosy reactions within a co-endemic setting; therefore,

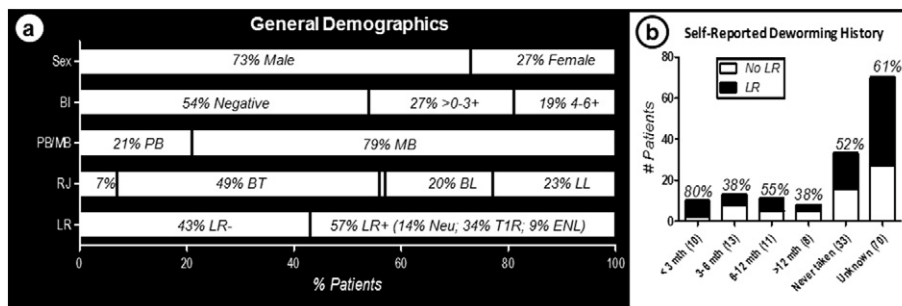


Fig. 2. Demographics for 145 Nepalese leprosy patients screened for soil-transmitted helminths (STH). a) Leprosy demographics within the sampled cohort were similar to those normally reported overall for patients attending Anandaban services (data not shown) including sex, bacterial index (BI), WHO leprosy classification of paucibacillary (PB) or multibacillary (MB), Ridley-Jopling classification (7% were perineural or tuberculoid (TT); BT = borderline tuberculoid; BL = borderline lepromatous; LL = lepromatous) or leprosy reaction (LR: Neu = neuritis, T1R = Type 1 reaction, ENL = erythema nodosum leprosum). b) Most participants (77%) either could not recall or denied having taken deworming treatment within the previous year. However, of those who did self-report deworming within the previous 3–6 months, 80–57% developed LR (respectively).

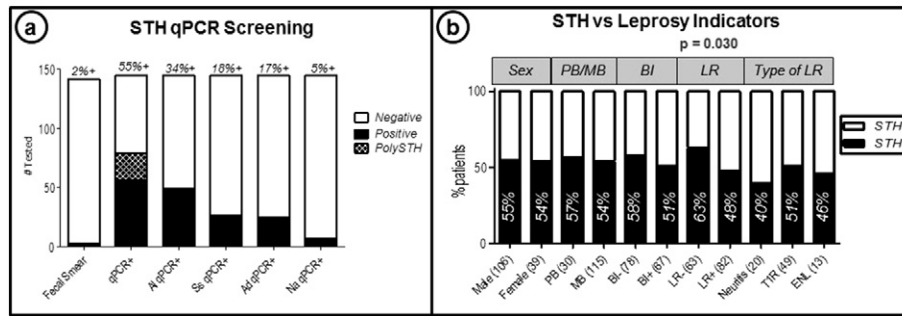


Fig. 3. Direct microscopy and qPCR result for 145 Nepalese patients and STH prevalence by clinical parameters. a) Fecal samples from 145 patients were screened by simple smear microscopy (first bar) or by quantitative PCR (qPCR) for soil-transmitted helminths (STH) depicted as combined (qPCR+ column) or individually for *Ascaris lumbricoides* (Al), *Strongyloides stercoralis* (Ss), *Ancylostoma duodenale* (Ad) and *Necator americanus* (Na). Samples negative for any STH are clear in color whereas those positive for one or multiple STH are shaded black or hatched, respectively. b) When compared against common leprosy indicators, STH presence was significantly associated with presence of LR (Neuritis, Type 1 reaction (T1R) and Erythema Nodosum Leprosum) but not associated with gender, WHO classification (paucibacillary (PB) or multibacillary (MB)), or slit skin smear bacterial index (BI).

implicating reaction development as a clinical outcome associated with absence or disturbance of a once chronic and pathologically complicit STH co-infection. STH infection disturbances can occur in response to contextual transitions or changes implemented for individuals or populations, including: immigration, health care access, housing and sanitation improvements, Mass Drug Administration (MDA) or other unprogrammed deworming events. Effective against Al and hookworms, single dose albendazole is one of the most commonly prescribed dewormers. However, prolonged or other treatments are required for other STH (i.e., 2 × daily/7 days for Ss); therefore, non-specific deworming with single dose albendazole may interrupt but not eliminate other chronic STH infection (Hu et al., 2013). Since 2003, a 15 year anti-filaria MDA program began in Nepal, employing a single dose of diethylcarbamazine partnered with albendazole in 5 year annual treatment cycles staggered across grouped districts (Go, 2010). Also beginning in 2003, annual leprosy reaction patient visits within our programs tripled and have maintained increased levels ever since (data not shown). While it may be anecdotal, the correlation corresponds with possible deworming-related IRIS events within a co-endemic population.

In limited resource contexts, it can be clinically impossible to validate deworming history or accurately differentiate recent, absent, interrupted, chronic or combined STH infections. Within our study, most Nepalese (77%, 112/145) self-reported unknown or no exposure within ≤12 months prior to sampling. Of those who did recall deworming within ≤3–6 months, the majority (80–57% respectively)

had presented reaction-positive: neuritis, T1R and ENL. Most, but not all, were patients who generally carry more *M. leprae* burden: MB, BI+, BT-LL. Both T1R and ENL development have been reported outcomes from pharmacological IRIS (Ramien et al., 2011; Sopirala et al., 2011). Although deworming can remove the governing force behind immune dysregulation, multiple cellular mediators (i.e., macrophages, dendritic cells) commissioned under the previous administration may actively persist or be retained (T regulatory cells) for variable lengths of time until repopulation occurs, potentially resulting in defense management conflicts (Salgame et al., 2013; Steinfelder et al., 2016; Taylor et al., 2012).

Our findings mark pioneering use of STH multiplex qPCR among leprosy patients and in Nepal. Similar to other reports (Easton et al., 2016; Llewellyn et al., 2016), qPCR unveiled similar proportions (i.e., Al > Ad and Na (hookworms) > Ss) yet higher levels of STH infection (55% positive) as compared to local microscopy (2% positive) or previous studies (Pullan et al., 2014; Kunwar et al., 2016).

The fact that 55% of all and 47% of new leprosy cases attending a national leprosy referral hospital were STH+, even within an era of MDA, is significant and indicative of the pervasive co-infection background in which *M. leprae* continues to transmit and persist as a disease. Secondly, STH occurrence was similar among all cases in regard to common leprosy indicators except reactions, dynamic situations previously associated with IRIS (Deps & Lockwood, 2008). Inverse STH/reaction relationships were repeatedly demonstrated across total patient, new case, individual STH species and polySTH categories. Moderate STH burden was most

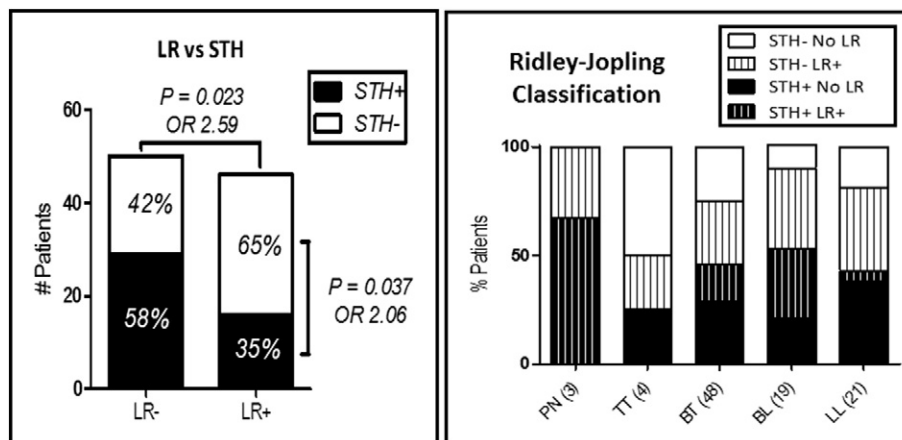


Fig. 4. Soil-transmitted helminths (STH) co-infection and leprosy reaction associations in new leprosy cases. Of the 145 total patients sampled, 96 were new leprosy cases. Significant inverse relationships between presence and STH and reaction became more evident. a) The significant majority of those without reaction (LR-) were co-infected with ≥ 1 STH (STH+); whereas, the significant majority of those with reaction (LR+) were not co-infected with STH (STH-). b) STH presence was not significantly associated with Ridley-Jopling classification: pure neuritic (PN), tuberculoid (TT), borderline tuberculoid (BT), borderline lepromatous (BL) and lepromatous (LL) leprosy. Nevertheless, 89% of STH-/LL patients reported LR+, while 75% new STH+/LL reported LR-.

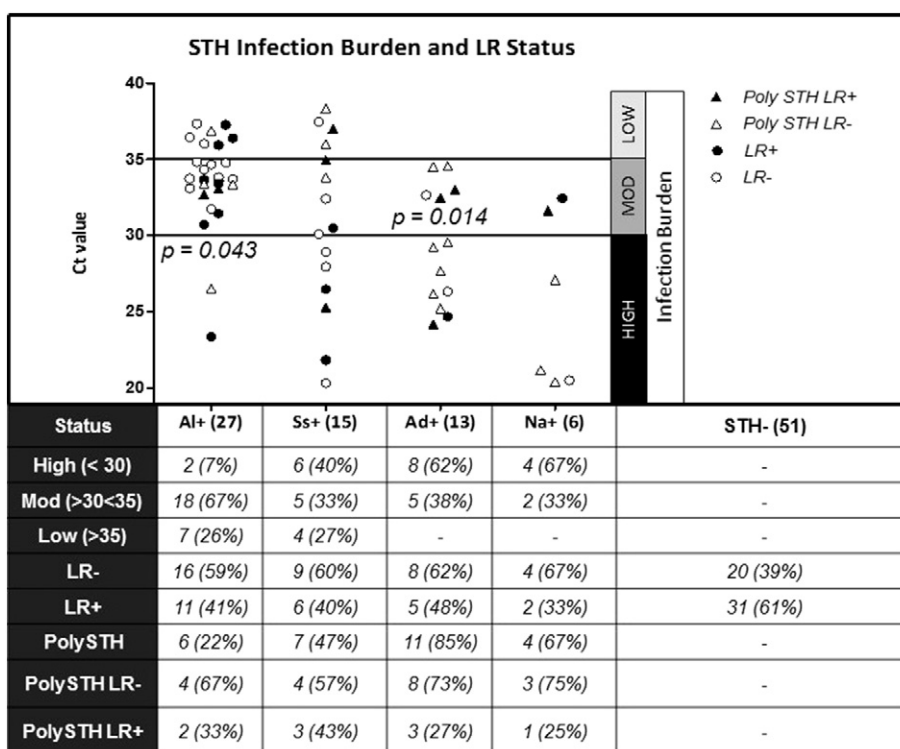


Fig. 5. Ct values corresponding to respective soil transmitted helminths. Cycle threshold values from multi-plex quantitative PCR (qPCR) were used to group STH infection burdens into high (Ct < 30), moderate (Mod, Ct > 30 < 35), and low (Ct > 35) (Arndt et al., 2013). STH mono-infections are represented by circles, whereas those within ≥ 1 STH infections (polySTH) are represented by triangles. Open characters represent LR-, while closed characters represent LR+. The majority of STH+ were mono-infections of moderate to high burden. Regardless of STH infection burden or whether polySTH, the majority of each STH species positive cases trended LR-.

common and, in groups with sufficient numbers, significantly associated with reaction-negative. Tools to comprehensively evaluate STH burden remain limited and contextually challenging; therefore, most STH immune dysregulation has been reported in association with presence regardless of burden (Salgame et al., 2013; van den Biggelaar et al., 2004; Elias et al., 2008; Ivan et al., 2015).

While trends were often evident in the majority, association was not absolute: some were reaction-positive/STH+ or reaction-negative/STH-. A 10 year Brazilian retrospective chart study of fecal microscopy reported chronic STH co-infection more often in MB and LL cases: findings which were not replicated in our study (Diniz et al., 2010). This dissimilarity highlights the transitory qualities involved in STH co-infection immune dysregulation and reconstitution. There were no indications of deworming history or context reported in the Brazilian cohort. Fecal evidence for STH detection, either by microscopy or qPCR, can rapidly disappear after deworming (Verweij, 2014) whereas, evidence of leprosy pathology or *M. leprae* residues can persist for years after effective MDT (Scollard et al., 2006a). Consequently, significant correlation between leprosy and chronic STH for historical extrapolations can be skewed by deworming activities within a population. Additionally, those who receive therapeutic deworming without concomitant infrastructure changes such as sanitation improvements are at high risk for re-infection within co-endemic settings. A recent small study reported microscopy STH+ association with ENL (Oktaria et al., 2016). Confoundingly for comparisons, however, the 8 with either active or historical ENL were grouped (two very different immunological states), all of which had received extended corticosteroid treatment prior to sampling. Nevertheless, in regard to reactions: 75% (8/12) of our new STH-/LL patients reported reaction-positive, while 89% (1/9) new STH+/LL reported reaction-negative. Therefore, 76% (16/21) of LL (most severe leprosy growth) new cases strongly displayed an inverse STH/reaction status.

Our results provoke a clinical dilemma: can deworming a leprosy patient from a co-endemic setting precipitate reaction and disability

development? As there are no standardized recommendations, leprosy care providers necessarily but variably practice deworming within co-endemic settings. Notably, aside from diagnosis coincidence, leprosy reactions most often develop during the first 6–12 months of MDT: a season corresponding with likely improved health care access and deworming-associated immune reconstitution durations (Scollard et al., 2006a; Ranque et al., 2007).

If sufficiently immunosuppressive, could STH re-infection clear or reduce the severity of leprosy reactions? Trials have employed therapeutic STH infection for inflammatory conditions such as allergy, multiple sclerosis, type 1 diabetes and Crohn's disease, however, typically not within STH endemic contexts (Wammes et al., 2014). Various helminth excretory secretory (ES) proteins or exosomes containing diverse cargo including microRNAs are also under investigation for effects including: functional mimicry of TGF- β , suppressive T cell signaling, inhibition of integrin binding that blocks neutrophils, dendritic cell switch to Th2 pathways, macrophage activation inhibition, inhibition of antigen presentation by B cells, production of IL-10, reduction of IL-12, and reduction of IL1R1/ST2 transcripts (Coakley et al., 2016). Remarkably, some of our STH-/reaction-positive patients later became STH+ within a few months; however, reaction did not necessarily subside (data not shown). It may be that once a reaction is triggered by immune reconstitution, the complicated cascade of networked events cannot be easily reversed. Since corticosteroids increase risk for potentially fatal disseminated Ss hyperinfection, it is not clinically recommendable for reaction patients in STH-endemic settings to be permitted to retain or become re-infected with STH. The ideal scenario would be for STH and leprosy co-infections to be cured without instigation of immunopathological complications; however, the practical logistics to achieve this are currently unclear.

STH-mediated immune dysregulation, deworming and immune reconstitution in regard to leprosy may bring insight in areas of host susceptibility (or augmentation thereof), transmission (extended subclinical status), vaccines and immunodiagnostics. Disease

diagnostics often target immune biomarkers that mounting evidence now indicates map directly into STH-influenced pathways, which can result in false negative, skewed or muted test results (Salgame et al., 2013; Coakley et al., 2016; Thomas et al., 2010). If durations for arousing detectable immune reconstitution in *M. leprae* infections prove similar to tuberculosis (2–3 months), contacts or suspect cases could be dewormed at an initial screening, provided WASH (Water, Sanitation and Hygiene) training and then asked to return for immunodiagnostics or immunotherapy at 3 months coincidental with existing post-exposure chemoprophylaxis treatment schedules for contacts (Elias et al., 2008; Richardus & Oskam, 2015). Admittedly, it could be theorized that some subclinical cases would develop clinical symptoms including leprosy reaction during immune reconstitution. Nevertheless, this would happen 1) earlier in disease than otherwise, 2) with scheduled follow-up and supervision by a health staff. Both aspects could promote earlier detection and reduced risk of disability development, a major objective of global leprosy programs.

There were limitations in this study, several of which will be addressed in ongoing further investigations. Increasingly available qPCR primers and rapid tests for additional STH species can improve comprehensive endemic screening. Although cause and effect between deworming and leprosy reaction has not yet been demonstrated, a larger cohort with standardized deworming, WASH training, longitudinal sampling and immune biomarker monitoring will permit mapping of expected changes during immune reconstitution specific to *M. leprae*, as well as potentially identify associated risk factors.

This study provides opening evidence that STH are not only significantly relevant to leprosy patients but also reaction occurrence. Recognition of STH as a complicit accomplice to leprosy outcomes has proverbially “opened a can of worms.” Yet, by unearthing this factor, programs could also gain impetus and insight from the current thrust behind neglected tropical disease (NTD) research and elimination initiatives. Where chronic STH are engaged for elimination, *M. leprae*-affected skin and nerves may befall aroused immunological crossfire potentially leading to self-healing or pathology. After STH elimination, future populations may better host natural immunity necessary to resist *M. leprae* infection – possibly on par with areas where leprosy has been successfully eradicated. Perhaps by the time STH leprosy co-infection relationships are better defined alongside other NTD complicities, answers to these questions and better options for mitigating reaction and disability development risks will be on the horizon.

Contributor's Statement

DAH, CBK, PP, DRSJBR, RT, AG performed literature searches. Study Design was developed among DAH, CBK, KDN, MS, IBN and LBA. Onsite management and data collection was performed in Nepal by DAH, KDN, CBK, PP, DRSJBR, RT, MS and IBN. Data Analysis and interpretation as well as writing, figure development and review involved all co-authors.

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There are no conflicts of interest relevant to this article for any of the authors.

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