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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), *Ancyclostoma 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.

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 *Mycobacterial 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 Leprosum (ENL). Therapeutic interventions linked with immune reconstitution inflammatory syndrome

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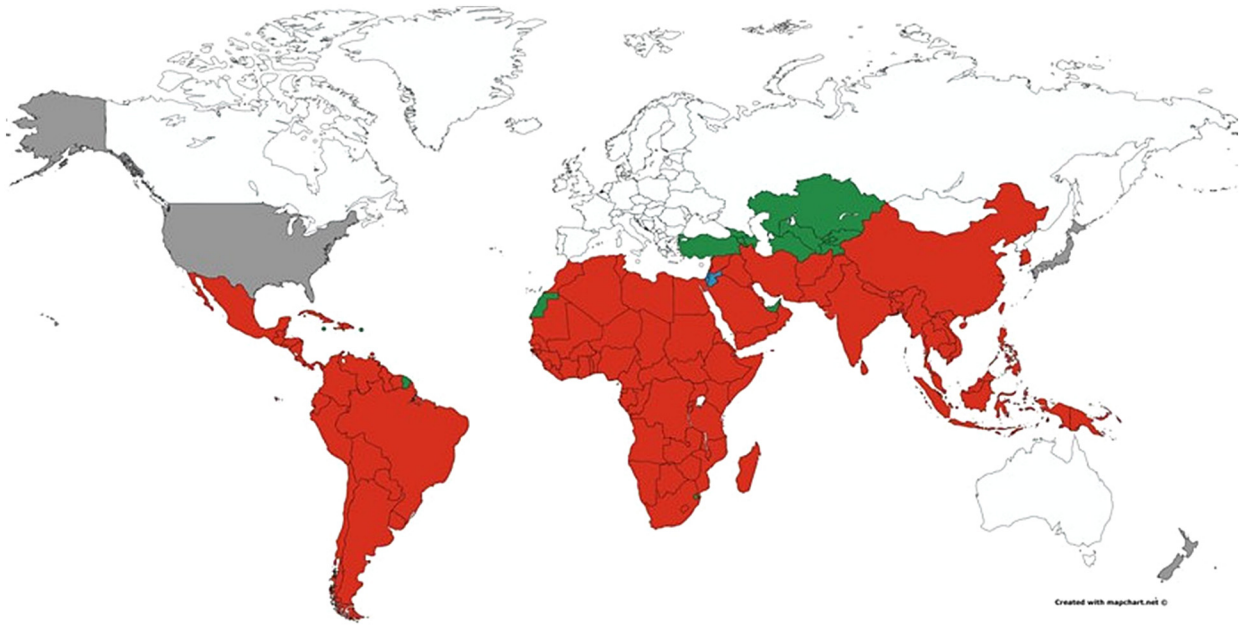

Fig. 1. Global map of reported STH and leprosy. STH and leprosy exist primarily within populations living with significant and sustained sanitation, housing and poverty issues. While the World Health Organization posts regular bulletins, individually updated data is not always submitted; therefore, the 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. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(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 stool was suspended in 200 μl of 2% polyvinylpolypyrolidone in PBS

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
Ascaris lumbricoides	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		
Strongyloides stercoralis	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 CGG CCG TCG CTG C-3′ -black hole quencher 3		
Ancylostoma duodenale	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		
Necator americanus	Na-F	5′-CTG TTT GTC GAA CGG TAC TTG C-3′		
	Na-R	5′-ATA ACA GCG TGC ACA TGT TGC-3’	101 bp	ITS2
	Na-P (MGB probe)	FAM-5′-CTG TAC GCA TTG TAT AC-3′ -nonfluorescent quencher		
Phocine Herpes	PhHV-F	5′-GGG CGA ATC ACA GAT TGA ATC-3’		
	PhHV-R	5′-GCG GTT CCA AAC GTA CCA A-3’	89 bp	gB
	PhHV-P	Cy5–5′-TTT TTA TGT GTC CGC CAC CAT CTG GAT C-3′-black hole quencher 2		

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