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Water, sanitation and hygiene related risk factors for soil-transmitted helminth and *Giardia duodenalis* infections in rural communities in Timor-Leste

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ABSTRACT

There is little evidence on prevalence or risk factors for soil transmitted helminth infections in Timor-Leste. This study describes the epidemiology, water, sanitation and hygiene, and socioeconomic risk factors of STH and intestinal protozoa amongst communities in Manufahi District, Timor-Leste. As part of a cluster randomised controlled trial, a baseline cross-sectional survey was conducted across 18 villages, with data from six additional villages. Stool samples were assessed for soil transmitted helminth and protozoal infections using quantitative PCR (qPCR) and questionnaires administered to collect water, sanitation and hygiene and socioeconomic data. Risk factors for infection were assessed using multivariable mixed-effects logistic regression, stratified by age group (preschool, school-aged and adult). Overall, soil transmitted helminth prevalence was 69% (95% Confidence Interval 67–71%), with *Necator americanus* being most common (60%; 95% Confidence Interval 58–62%) followed by *Ascaris* spp. (24%; 95% Confidence Interval 23–26%). *Ascaris-N. americanus* co-infection was common (17%; 95% Confidence Interval 15%-18%). *Giardia duodenalis* was the main protozoan identified (13%; 95% Confidence Interval 11–14%). Baseline water, sanitation and hygiene infrastructure and behaviours were poor. Although risk factors varied by age of participants and parasite species, risk factors for *N. americanus* infection included, generally, age in years, male sex, and socioeconomic quintile. Risk factors for *Ascaris* included age in years for children, and piped water to the yard for adults. In this first known assessment of community-based prevalence and associated risk factors in Timor-Leste, soil transmitted helminth infections were highly prevalent, indicating a need for soil transmitted helminth control. Few associations with water, sanitation and hygiene were evident, despite water, sanitation and hygiene being generally poor. In our water, sanitation and hygiene we will investigate implications of improving WASH on soil transmitted helminth infection in impoverished communities.

1. Introduction

Soil transmitted helminth (STH) and intestinal protozoal infection prevalence remain little studied in several impoverished regions of the world. Very few studies have estimated STH burden in Timor-Leste. This is one of the poorest countries in Asia, ranked 133 of 187 countries on the 2015 Human Development Index (http://hdr.undp.org/en/2015-report). Access to water, sanitation and hygiene (WASH) is necessary for sustainable prevention and control of STH and other enteric infections (Campbell et al., 2014; Strunz et al., 2014). In 2013 the United Nations Children's Emergency Fund (UNICEF) reported that only 18% of rural Timorese communities had improved sanitation (UNICEF, 2013). A recent

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school-based national survey (N = 2198) revealed a 29% STH infection prevalence in school-aged children (SAC), comprising *Ascaris lumbricoides* (21%), hookworm (9.2%), and *Trichuris trichiura* (4.1%) (Martins et al., 2012), however low sensitivity parasitological methods (formalin faecal concentration and microscopic examination) were used, and these figures may be underestimates. No recent studies have quantified STH or intestinal protozoa in communities in Timor-Leste.

STH life cycles involve environmental contamination with faeces containing helminth eggs, therefore major risk factors include poor hygiene, sanitation and access to clean water. These in turn are influenced by poor socioeconomic conditions including inadequate housing, low levels of education, low family income, poor health services, dirty hands and clothing, household crowding, the presence of animals in the house, and poor access to or inadequate sanitation facilities and clean drinking water (Brooker et al., 2004; Traub et al., 2004; Al-Mekhlafi et al., 2007; Knopp et al., 2010; Pham-Duc et al., 2013). Systematic reviews have reported reduced odds of any STH infection amongst people who use treated water, wash hands after defecation, and have access to and use soap (Ziegelbauer et al., 2012; Strunz et al., 2014). These reviews have further reported reduced odds of *A. lumbricoides* or *T. trichiura* infection amongst people who have access to piped water, use available latrines, and wash hands before eating and after defecation, and reduced odds of hookworm infection amongst people with access to sanitation, and those who wear shoes (Ziegelbauer et al., 2012; Strunz et al., 2014). The zoonotic hookworm *Ancylostoma ceylanicum* may be transmitted by dogs, and viable eggs of *A. lumbricoides, Ascaris suum, T. trichiura* and *Trichuris suis* have been recovered from domestic animals, highlighting their potential mechanical role in STH dissemination (Olsen et al., 2001; Traub et al., 2002, 2008; Inpankaew et al., 2014). Other characteristics associated with STH prevalence include age, sex, agricultural occupation, cattle ownership, and consumption of raw vegetables fertilised with human faeces (Traub et al., 2004; Al-Mekhlafi et al., 2007; Knopp et al., 2010; Pham-Duc et al., 2013).

Numerous other parasites are concomitant with STH and believed to be effectively reduced with improved WASH. Of these, *Giardia duodenalis* is the most common faeco-orally transmitted intestinal protozoon, and is associated with large outbreaks in some countries. Risk factors for giardiasis include recreational freshwater contact, drinking untreated water and eating raw vegetables (Mohammed Mahdy et al., 2008). Additionally, risk factors in developing countries include poverty, inadequate sanitation, high concentrations of domestic animals (Hayes et al., 2003), family members or domestic animals with giardiasis (Traub et al., 2004, 2009), being male, and being aged less than 12–15 years (Mohammed Mahdy et al., 2008).

This cross-sectional analysis is the first known assessment of community-based STH and intestinal protozoal prevalence, and associated risk factors in Timor-Leste. It was conducted as part of a cluster randomised controlled trial (RCT) in Manufahi District, Timor-Leste (Australian and New Zealand Clinical Trials Registry ACTRN12614000680662) (Nery et al., 2015).

2. Materials and methods

2.1. Ethical approval and consent

The study protocol was approved by the University of Queensland (Australia) Human Research Ethics Committee; the Australian National University Human Ethics Committee; the Timorese Ministry of Health Research and Ethics Committee; and the University of Melbourne (Australia) Human Research Ethics Committee. Full details of participant informed consent processes are described elsewhere (Nery et al., 2015); briefly these involved explaining the study purpose and methods, and obtaining signed consent from all adults and parents or guardians of children under 18 years. All individuals resident in participating villages were invited to participate. Children aged less than 12 months were excluded (Nery et al., 2015).

2.2. Study setting, design and collection of data

The RCT commenced in May 2012 and is ongoing, with baseline surveys conducted between May 2012 and October 2013. Baseline data from 18 villages were collected as part of the RCT, with identical surveys used to collect data from six additional villages in Manufahi District. Full details of the study design are provided elsewhere (Nery et al., 2015). Manufahi District is comprised of rural villages not receiving regular systematic deworming programmes at the time of the study.

Single stool samples were collected when field workers were present in villages and fixed in 5% potassium dichromate. Stool samples were examined using multiplex quantitative PCR (qPCR) at QIMR Berghofer Medical Research Institute, Brisbane, Australia, for the presence and intensity of STH and protozoal infection. Full details of this highly sensitive and specific diagnostic method are published separately (Llewellyn et al., 2016).

Three questionnaires encompassing a broad range of potential WASH and socioeconomic risk factors (village level, answered by the village chief; household level, answered by one household member being ideally the female head of household; and individual level answered by all participants with a parent or guardian answering for children under 12 years) were administered by trained field workers. Presence, type and cleanliness of household and village latrines were verified by interviewers; the remaining questions were self-reported. After collection, data were double-entered into a Microsoft Access database and error checks conducted.

2.3. Data analysis

Data were imported to STATA 13.0 (Stata Corporation, College Station, Texas, USA) where each individual observation was linked to its corresponding individual, household and village level information and parasitological outcome. Collinearity was investigated using tetrachoric analysis and the "collin" user written package for STATA.

A household level wealth index was constructed using information on asset ownership (animals, transport and appliances), house floor type, reported income and the presence of electricity (Filmer and Pritchett, 2001). These variables were not individually included in multivariable regression models. Principal components analysis (PCA) was used to develop weights for asset variables. Four principal components (PCs) were retained, each individually contributing 21%, 14%, 11% and 11% of variance explained (cumulatively 57%), with eigenvalues above one. Each included asset variable was weighted according to the proportion of its variance explained by the associated PC (i.e., the normalised squared loading) (Nicoletti et al., 2000). Then each PC was weighted according to its contribution to the proportion of the explained variance in the dataset (i.e., the normalised sum of squared loadings) (Nicoletti et al., 2000), with scorings summed for the four PCs into one resultant socioeconomic score. This score was categorised into quintiles, enabling each household to be classified according to relative poverty.

Prevalence of infection was chosen as the outcome variable in these analyses because intensity of infection classes from qPCR data are still rare. Chi-squared tests were conducted to compare prevalence of infection by age, sex and socioeconomic quintile.

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