Epidemiology of soil-transmitted helminth infections in Timor-Leste

Suzanne Jane Campbell BSc BA MPH

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Statement of Originality

I declare that this thesis is the result of original research, and has not been submitted in any form for credit for any other degree or part thereof.

The research I conducted used baseline data collected for a randomised controlled trial (RCT) that has included a team of research investigators. For the RCT I was centrally involved in questionnaire development and administration via fieldwork, and entry and cleaning of epidemiological data. I have written a database codebook for ongoing use in the RCT. For epidemiological and statistical analyses, I was responsible for variable restructuring and quality assurance, and solely responsible for data analyses and reporting of such. Specifically, I have analysed the prevalence of, and water, sanitation and hygiene (WASH) and demographic risk factors for, soil-transmitted helminths (STH) in communities in Manufahi District, Timor-Leste. I have analysed the association between STH and morbidity outcomes, and the WASH and environmental risk factors for intensity of STH infection in this study population. In this thesis I present results from these analyses to address specific objectives. I also present two published narrative reviews, which have comprised a major component of my research focus, and a published viewpoint for which I contributed 80% of its development. All analyses and reviews are my work, except as indicated by references or acknowledgements in the text.

Signed: _____

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One never decides to do a PhD lightly; it is a major decision. For me it meant leaving an established career, and taking a leap of faith, because of my desire to help others. I could not have anticipated the extent of positive impact that this has had upon my own life.

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Abstract

Soil-transmitted helminths (STH) are significant human parasites, causing long-term morbidity. They are prevalent in impoverished regions lacking adequate water, sanitation and hygiene (WASH).

This PhD has been undertaken during a period of debate around the health benefits of deworming, and global prioritisation of neglected tropical disease (NTD) control and elimination activities. Therefore, this thesis commences by presenting two comprehensive reviews. In one, recent evidence of STH morbidity was analysed, and systematic reviews appraised, to highlight evidence shortfalls for direct morbidity measures indicating possible benefits from chemotherapy. In the second, evidence for chemotherapy, WASH, and current NTD integration were analysed, and the need for "multi-component" integration highlighted, being more holistic integration to achieve more sustainable STH control.

Quantitative epidemiological analyses presented in this thesis used baseline data from a randomised controlled trial (RCT) to analyse STH epidemiology in Manufahi District, Timor-Leste; an impoverished, post-conflict country. This thesis explores: what is the prevalence of STH, and what WASH risk factors contribute towards infections? What are the associations between STH infections and plausible STH-related health outcomes, and finally, what are the associations between aspects of WASH and the village environment with intensity of STH infection, in these communities?

WASH risk factors for STH infections were analysed, stratified by age, using principal component analysis and mixed-effects logistic regression. The main findings were a high prevalence of parasitic infections, however few WASH risk factors significantly associated with STH infection. The impact of STH intensity on community anaemia, and also stunting, wasting and being underweight in children aged one to 18 years, was investigated. An algorithm correlating DNA intensity to eggs per gram of faeces equivalents was used to assign cut-points for PCR-derived *Ascaris* spp. and *Necator americanus* infection intensity. STH were found not to be strong predictors of anaemia, stunting or wasting in the study communities. Finally, given exposure-related risks, and associations between heavy-intensity infection and morbidity, the hypothesis that WASH and environmental risk factors may vary according to infection intensity was tested. Environmental variables, but again few WASH variables, were associated with intensity of STH infection in this analysis. Despite this, WASH is the only identified mechanism that could reduce or prevent transmission in this high-transmission environment. It should be included in integrated control strategies.

All analyses are the first reported examples for Timor-Leste. The thesis findings provide an informed position for establishing national STH control strategies, and a useful baseline for monitoring and evaluating control programmes once implemented. Analyses additionally provide essential baseline information for the RCT in which this research is embedded. Research findings also contribute to international knowledge: few analyses have investigated WASH risk factors stratified by age and STH species separately. These analyses additionally provide the first epidemiological investigation of STH infection

intensity from PCR-diagnosed infection. This requires verification in different epidemiological settings. Finally, the thesis provides the first investigation of adjusted environmental and WASH risk factors in any community setting.

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List of Acronyms and Abbreviations

95% CI	95% confidence interval
AIC	Akaike's information criterion
AIDS	Acquired immune deficiency syndrome
ANU	The Australian National University
AOR	Adjusted odds ratio
ARR	Adjusted relative risk
ASTER	Advanced Spaceborne Thermal Emission and Reflection Radiometer
AUC	Area under the receiver-operating characteristic curve
BCG	Bacille Calmette-Guerin vaccine
BMIZ	Body-mass-index (BMI)-for-age z-score
CLTS	Community-led total sanitation
Ct	Cycle threshold value (used as a measure of DNA concentration in PCR)
DALY	Disability-adjusted life year
DFID	The United Kingdom Department for International Development
DNA	Deoxyribonucleic acid
EPG	Eggs per gram of faeces (a measure of STH in stool)
FRESH	Framework to Focus Resources on Effective School Health
GAHI	Global Atlas of Helminth Infection
GBD	Global Burden of Disease
GDEM	Global digital elevation model

GEMS	Global Enteric Multicentre Study
GPS	Global positioning system
GSEM	Generalised structural equation model
HAZ	Height-for-age z-score
Hb	Haemoglobin
HPA	Hepatopancreatic ascariasis (also called biliary and pancreatic ascariasis; BPA)
HIV	Human immunodeficiency virus
ICDS	Integrated Child Development Services
IDA	Iron deficiency anaemia
IMCI	Integrated Management of Childhood Illness
JMP	Joint Monitoring Programme (JMP) for Water Supply and Sanitation, administered by the WHO and UNICEF
KMO	Kaiser-Meyer-Olkin measure of sampling adequacy
MAL-ED	The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health Study
MDG	Millennium Development Goal
MoH	Ministry of Health (Timor-Leste)
NDVI	Normalised difference vegetation index
NHMRC	National Health and Medical Research Council (Australia)
NGO	Non-government organisation
NTD	Neglected tropical disease
OR	Odds ratio

PCA	Principal component analysis
PCR	Polymerase chain reaction
PSAC	Preschool-aged children
qPCR	Quantitative PCR
RBC	Red blood cell
RCT	Randomised controlled trial
ROC	Receiver-operating characteristic curve
RPC	Recurrent pyogenic cholangitis
RR	Relative risk
SAC	School-aged children
SDG	Sustainable Development Goal
SOP	Standard operating procedure
STH	Soil-transmitted helminth
TDS	*Trichuris* dysentery syndrome
UNICEF	United Nations Children's Fund
UNMIT	United Nations Integrated Mission in Timor-Leste
UQ	The University of Queensland
USAID	United States Agency for International Development
USD	United States dollar
VAD	Vitamin A deficiency
VIF	Variance inflation factor
VIP	Ventilated improved pit latrine
WASH	Water, sanitation and hygiene

WAZ	Weight-for-age z-score
WHO	World Health Organization
WRAML	Wide Range Assessment of Learning and Memory learning test

Chapter 1 Introduction

This introductory chapter highlights the purpose and significance of the PhD research. It provides epidemiological context to the four most common soil-transmitted helminths (STH), *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus* and *Ancylostoma duodenale*, as well as the less commonly reported hookworm *Ancylostoma ceylanicum*, indicating their global importance and the need for effective control programmes. The chapter also introduces the nematode *Strongyloides stercoralis* and the protozoa *Giardia duodenalis*, *Cryptosporidium parvum* and *Entamoeba histolytica*, infections with which are considered in the thesis as secondary research outcomes. These parasites are often concomitant with STH and are thought to be effectively reduced with improved water, sanitation and hygiene (WASH), which in conjunction with chemotherapy (deworming) is central to control of STH infections.

The chapter briefly introduces the current control strategies of chemotherapy and WASH, and introduces how integrating these strategies could achieve a greater, more sustainable reduction in STH burden; these strategies are extensively reviewed in Chapter 3. Following this is a summary of current knowledge of STH in Timor-Leste. The randomised controlled trial (RCT), WASH for Worms, being conducted in Timor-Leste, is introduced as important context within which the PhD research was based. The chapter concludes with the specific aims and objectives of the PhD research, and an outline of the thesis structure.

1.1 Epidemiology and prevalence of soil-transmitted helminths and protozoa

Roundworm (*A. lumbricoides*), whipworm (*T. trichiura*), and hookworms (*N. americanus* and *A. duodenale*) constitute a major global cause of morbidity and mortality, affecting approximately 819 million, 465 million and 439 million people, respectively (1). They are most prevalent in tropical and sub-tropical regions of the developing world (2), particularly Asia, with the highest numbers infected in China and India (1). STH are strongly linked with poverty: transmission involves environmental contamination with eggs, therefore major risk factors include poor personal hygiene and sanitation, which in turn are influenced by differences in socioeconomic status (3). Of the estimated 1.5 billion people infected, the global annual chemotherapy target is 875 million schoolchildren (1,4). The World Health Organization (WHO) has highlighted STH as one of the most significant parasitic infections of humans (5), and there is intense global advocacy for their control.

STH live in the human intestine. STH infections impair the individual's nutrient utilisation, thereby causing anaemia and interfering with growth and development, particularly in children aged less than five years (6-9). This, and other pathways of pathogenesis, may lead to impaired cognitive ability (3,10-12). Heavy hookworm infection can cause iron-deficiency anaemia in young children (6,13,14). Hookworm-induced iron-deficiency anaemia is also recognised as a serious threat to the health of mothers and unborn children (3,15).

STH have a highly aggregated distribution within communities, with a small number of individuals harbouring large numbers of helminths (heavy-intensity infection) and the majority harbouring few or none (3,16-20). Prevalence and infection both follow typical age profiles in endemic areas. *A. lumbricoides* and *T. trichiura* are most prevalent and have highest burden among children, who frequently harbour 60-75% of helminths in a given community (21-23). Children are thus at greater risk of morbidity and can significantly contribute to infecting their environment (24). Hookworm infections tend to reach peak intensity in adolescence and then plateau in adulthood. A particular concern is aggravation of iron-deficiency anaemia in women of reproductive age (3). In China, hookworm intensity has been shown to continue increasing even into older ages (25,26). Whilst the reasons for the different patterns in helminth species are unknown, they may include behavioural and social factors, nutritional status and genetic background (16,19,20,27). Prevalence and intensity of infection among boys has been reported as significantly higher than among girls (16,28,29), but with exceptions (30). In a meta-analysis to determine sex-related prevalence (3), hookworm infections were more prevalent in males than in females, but by contrast, prevalence of *A. lumbricoides* and *T. trichiura* showed no significant sex difference.

A. ceylanicum, a zoonotic pathogen of dogs and cats, has been found to infect humans in parts of Asia. This hookworm, and the nematode *S. stercoralis*, are often not reported by investigators because they require different parasitological techniques for detection compared to the major STH (31). Poor health, abdominal pain, anaemia, lethargy and excessive hunger have been associated with *A. ceylanicum* infections (32,33). Infection

with *A. ceylanicum* is an emerging zoonosis (23) but has generally been overlooked in human parasite surveys because it has been considered a rare or atypical hookworm of humans (32). *S. stercoralis* is distributed worldwide (34), and infects approximately 70 million people in developing countries (35). *S. stercoralis* tends to be most prevalent in tropical regions (36). *S. stercoralis* infection can persist in the host for more than 40 years (37). Clinical manifestations vary from asymptomatic to fatal illness (34).

In addition to helminths, the protozoa *G. duodenalis*, *C. parvum* and *E. histolytica* contribute significantly to diarrhoeal disease in humans. *G. duodenalis* (also called *G. lamblia* or *G. intestinalis*) is the most common intestinal protozoon worldwide (38,39), causing diarrhoea, malnutrition and wasting in approximately 200 million people annually (40). In the two largest multi-site diarrhoeal disease studies that have been conducted (the Global Enteric Multicentre Study, GEMS, and the Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health Study, MAL-ED), *Cryptosporidium* spp. was the second leading cause of infant moderate-to-severe diarrhoea in five of seven sites (GEMS Study), and was one of the four main pathogens exhibiting the highest attributable burden of diarrhoea in the first year of life (MAL-ED Study) (41,42).

Giardia duodenalis and *C. parvum* oocysts are believed to be ubiquitous. They are found in faeces of humans, livestock and wild animals and frequently in surface drinking water sources (43-45). Humans develop giardiasis or cryptosporidiosis after ingestion of faecally-contaminated water or food, contact with faecally-contaminated environmental surfaces,

human-to-human or animal-to-human contact (45). Large outbreaks can occur, particularly associated with warmer temperatures, poor drainage of surface water and poor water supply (43,46). Giardiasis and cryptosporidiosis are also prevalent in child care centres, aged care centres and similar closed environments (38). Studies have found that prevalence of *G. duodenalis* is highest in children (47). Symptoms vary from being asymptomatic to experiencing chronic diarrhoea, malabsorption, weight loss and stunting (48). Giardiasis in early childhood is associated with poor cognitive function and failure to thrive (45). Infected asymptomatic children can readily spread infection (45).

Few human cases of *C. parvum* were reported prior to the acquired immune deficiency syndrome (AIDS) epidemic, however since 1982-3, cryptosporidiosis has been recognised as a severe and life-threatening cause of diarrhoea in patients with AIDS (43). Several studies have shown that cryptosporidiosis in early childhood causes malnutrition and can permanently affect physical and cognitive growth and development (49,50). Cryptosporidiosis is also associated with increased mortality (49). The largest recorded outbreak occurred in 1993 in Milwaukee, United States, with an estimated 403 000 people infected, an attack rate of 52%, mean duration of illness of 12 days and average weight loss of 4.5 kilograms in immunocompetent people (43), being considerably worse in immunocompromised people.

E. histolytica, the causative agent of human amoebiasis, is endemic in most tropical and subtropical countries and is considered responsible for millions of cases of dysentery and liver abscess each year (51,52). *E. histolytica* prevalence ranges from five to 81%, infecting

approximately 480 million people globally (53). It causes approximately 100 000 deaths annually (54), placing it second only to malaria in mortality due to protozoan parasites (52). Low education level, no access to a toilet or tap-water and use of river water are important risk factors for *E. histolytica* infection (55,56). *E. histolytica*-associated dysentery has been associated with malnutrition and lower cognitive test scores in children (57,58). Little is known about *E. histolytica* epidemiology (55). The recent identification of *Entamoeba dispar* as a separate species, which is morphologically identical to *E. histolytica* (52,59), has called into question most of the earlier data on the worldwide prevalence of *E. histolytica* and its importance as a human pathogen (55). *E. dispar* is believed to be ten times more prevalent than *E. histolytica* (60), however only *E. histolytica* causes invasive disease (52). The WHO has recommended that research on amoebiasis epidemiology and treatment be undertaken as a priority (52).

1.2 Pathogenesis and clinical morbidity from soil-transmitted helminths

Most STH species are transmitted by ingestion of helminth eggs after contact with contaminated soil. *A. lumbricoides* and *T. trichiura* are transmitted faeco-orally, whereas transmission of hookworm involves larval penetration of areas of the skin exposed to soil and water (61). As well as cutaneous migration, *A. duodenale* can be orally ingested (3). *A. duodenale* can undergo arrested development in humans (62,63); and can enter human mammary glands during pregnancy (63,64). Neonatal ancylostomiasis has been reported in

China and Africa, resulting in severe disease with profound anaemia (63), and transmission by breast milk may also occur although further studies are required to confirm this (63).

After entering the human host, STH eggs or larvae develop into adult worms directly in the large intestine (*T. trichiura*) or in the small intestine after migration through the vascular system and respiratory tract, where they are subsequently coughed from the lungs and swallowed (*A. lumbricoides* and hookworms) (3,5). In the small intestine hookworms use sharp cutting plates to rupture the intestinal mucosa and feed on blood (3,65). The longevity of STH in the human host results in chronic morbidity: *A. duodenale* can live in the human intestine for an average of one to three years, *N. americanus* for three to four years (66) but up to 18 years (67), and *T. trichiura* for over five years (31). Whilst in the intestine, adult worms reproduce sexually and all species produce large numbers of eggs: *A. lumbricoides* produce up to 200 000 per day, whereas *T. trichiura* and hookworms produce 5 000 to 20 000 per day (5), creating enormous transmission potential. Eggs are excreted, where they, or hatched larvae (in the case of hookworm), can remain viable in the soil for variable periods of time depending on ambient warmth, shade and moisture (2,3).

Blood loss and iron-deficiency anaemia

There is clear evidence that hookworm infection is associated with extensive blood loss (68-71). This begins upon maturity and continues for the life of the hookworm (3); it can thus occur chronically for many years. Hookworms frequently change their location in the gut, causing multiple ulcers (72-74).

Hookworm-associated anaemia occurs when blood loss from hookworm infection exceeds intakes and reserves of host iron and protein (74). Hookworm is considered to be a major cause of iron-deficiency anaemia in young children (3,5,71,72,74,75). Women of childbearing age are also vulnerable because of their high physiologic iron requirements and lower iron stores (3). Anaemia during pregnancy is associated with premature delivery, low birth weight (itself a risk factor for infant mortality), maternal ill-health, impaired lactation, and maternal death (3,76,77). In areas of high transmission with heavy hookworm burdens, blood loss is substantial. *A. duodenale* causes significantly more blood loss than *N. americanus* (daily blood loss of 0.15mL compared to 0.03mL) (69,70), resulting in more severe iron-deficiency anaemia in areas where *A. duodenale* is the predominant species (70). Monospecific *A. ceylanicum* infections have also been associated with anaemia (78).

There is strong evidence of association between intensity of STH infection and morbidity (3), with direct correlations reported between intensity of hookworm infection and reduced haemoglobin and body iron status (71,79-82). In schoolchildren, it has been observed that intensity of hookworm infection explained 35% of the variation in faecal haemoglobin levels, and that infected schoolchildren could lose more than twice their median iron requirements in blood each day (71). In an RCT, children with heavy hookworm infection had more than twofold increased risk of developing moderate-to-severe anaemia (6). Studies have shown even asymptomatic or light-intensity hookworm infections contribute to anaemia in preschool and school-aged children (6,7,11,79,83). Not all studies have found this, however, possibly due to differing levels of hookworm burden (7) or underlying nutritional status. Heavier helminth burdens tend to be harder to cure, particularly in *T.*

trichiura infections (84). There is also a direct relationship between *A. lumbricoides* egg intensity, helminth burden and development of serious pathological conditions (85).

There are further strong links between prevalence and intensity of STH and nutritional factors associated with poverty, such as low dietary iron, and underlying malnutrition. Children with adequate dietary iron intake have been observed to have reduced odds of anaemia irrespective of helminth burdens (86). Anaemia is also closely associated with other diseases, such as malaria, which can make hookworm contribution difficult to assess. Further, in areas of STH and malaria co-endemicity, studies have reported conflicting results of both synergistic and antagonistic effects of malaria-STH coinfection. Few studies have investigated these effects on morbidity outcomes (87-90). Given global prevalence, severity and geographical overlap of malaria and STH, further research on potential *Plasmodium*-STH interactions, and on teasing apart the relative contributions of each disease to anaemia, is required.

T. trichiura infections can cause blood loss in heavy infections, due to dysentery and mucosal damage to the caecum (91). Blood loss caused by *T. trichiura* cannot be re-absorbed since *T. trichiura* resides in the large intestine beyond the region of absorption of iron or protein (91).

Physical development, fitness and worker productivity

A long-term consequence of chronic blood loss is that hookworm infection is linked to stunted growth and wasting in children (92-94), which in turn predisposes to low birth weight in the next generation of infants, which itself predisposes to stunted growth (21). This may then affect productivity either indirectly through early ill-health during childhood with lifetime consequences in terms of failure to achieve growth and cognitive potential (14), or directly as hookworm-associated blood loss and anaemia continues into adulthood. STH infections in adults may reduce the ability to sustain labour (95). Whilst there is negligible direct evidence that STH infections reduce adult productivity, partly due to the lack of well-designed studies (14), direct associations have been reported between reduced haemoglobin concentrations, anaemia, reduced physical ability and ill-health, and lower work tolerance (96,97). Given hookworm-induced haemoglobin reductions and anaemia, it is highly likely that hookworm contributes to lost productivity. This could then translate into economic loss.

Soil-transmitted helminth impact on cognitive development

Associations between STH infection and cognitive development have been reported since the early 1900s (98), but few studies have been well designed (98,99), and evidence is equivocal. Treatment of STH infection has not been clearly demonstrated to improve cognitive performance. Some RCTs have only shown cognitive benefits of anthelmintic treatment on heavily infected children (100,101), some have shown equivocal effects (102) and others have shown no improvements from chemotherapy alone (83,103,104). A

Cochrane systematic review has consistently not found STH treatment to improve cognitive performance (most recently reissued as (105), reviewed in Chapter 2); this has influenced Global Burden of Disease (GBD) estimates for STH with calculations no longer including cognitive impact (106). This, in turn, has major implications for the overall burden of disease estimated to be due to STH and prioritisation of its importance.

As opposed to direct STH impact on cognitive development, there is considerable evidence for 'downstream' effects on cognitive function, resulting from iron-deficiency anaemia or growth retardation (107), including associations with behavioural anomalies, lower scores on intelligence tests, memory loss, developmental delays, and educational achievement (12,79,91,100,108,109). A systematic review (110) found that iron supplementation modestly improved mental development score in initially anaemic or iron-deficient anaemic subjects and those above seven years of age, but had no effect on mental development in children below 27 months of age (an age group in which STH infections are also rare).

School absenteeism because of ill-health from STH infection is one mechanism whereby cognitive learning in children may be affected. Anaemia arising from STH infection is associated with effects on school attendance among children (14,111). Some studies have found that treating schoolchildren for STH reduced absenteeism by 25%, with largest gains for young children who suffered the most ill-health (111,112). Simply treating disease will not compensate for years of missed learning opportunities (3); good education and psychosocial stimulation are also essential (113).

Additional *Ascaris lumbricoides* morbidity

A. lumbricoides reach up to 40cm in length (90). Due to this large size and their tendency to migrate, *A. lumbricoides* infections can cause life-threatening complications including intestinal obstruction and/or perforation, biliary or pancreatic disease, appendicitis, peritonitis, and liver abscess (114,115). Vomiting of *A. lumbricoides* is frequently reported in endemic regions. *A. lumbricoides* worms have been observed discharging from the umbilicus (116,117), nasal cavities (116), surgical wounds (117), and have been implicated in gastrointestinal bleeding (118). Complications occur in a minority of *A. lumbricoides* infections (115,116,119).

A. lumbricoides intestinal obstruction constitutes approximately 63% of ascariasis-related hospital admissions in endemic areas (120,121), with an estimated case-fatality rate of up to five percent (115). Intestinal obstruction occurs mainly in children aged six months to ten years (117,120), possibly due to the small diameter of their intestinal lumen (120). These children tend to present to hospital being acutely unwell (117), with recurrent colicky abdominal pain, vomiting, constipation, volvulus (twisted bowel) (114,116,120), fever, diarrhoea, dehydration, anaemia (122), peritonitis, acute appendicitis and/or gangrene to the appendix (116), airway obstruction, biliary obstruction, pancreatitis, necrotic ulcers, perforation or gangrene of the intestinal wall (114,116), jejunitis or intussusception (115,117). Any delay in management of intestinal obstruction can cause the bowel to perforate, causing spillage of intestinal contents into the peritoneal cavity (114,115,117,120), which can be fatal (123). Surgery to remove *A. lumbricoides* can be

high risk, especially with acute presentations or underlying malnutrition (116). Surgery if necessary may include intestinal incision and/or resection, anastomosis or appendicectomy (114,116,120). Volumes of worms removed during surgery can be high, with reports of up to four litres of *A. lumbricoides* being removed in a single procedure (114).

Hepatopancreatic ascariasis (HPA) occurs when adult *A. lumbricoides* enter the hepatobiliary or pancreatic duct, or other parts of the biliary canal, producing partial bile duct obstruction (124,125). HPA symptoms can include nausea and/or vomiting (including worm vomiting), fever, rigour, extreme pain in the abdomen and lower chest, biliary sepsis, pancreas and/or gall bladder inflammation, jaundice, strictures, abscess of the liver or pancreas, cholangitis and bleeding into the biliary tree (116,121,124). Patients are often critically unwell, and may suffer septic shock (116). *A. lumbricoides* excrete polypeptides that are chemical irritants, producing allergic manifestations and spasms in the bile duct (124). Worms can move in and out of the bile duct from the duodenum (116), meaning they may not be present in the ducts at time of surgery (116). They can thus remain undetected, leading to difficulties in estimating the magnitude of HPA in endemic areas (116). *A. lumbricoides* that do not migrate back out of the biliary canal usually die, causing severe complications including gallstones (116,124,126).

Trichuris dysentery syndrome

Whilst the majority of *T. trichiura* infections are asymptomatic, *Trichuris* dysentery syndrome (TDS) can occur in heavy infections (1 000 worms or more) (65,127), in which

T. trichiura worms spread throughout the large intestine to the rectum. This causes inflammation of the lower bowel (128), ulcers, mucus-containing stools, chronic dysenteric diarrhoea, stunting, finger clubbing, reduced iron status or iron-deficiency anaemia (91), tenesmus and subsequent rectal prolapse, chronic colitis, generalised abdominal pain, loss of appetite, nausea, vomiting, peripheral blood eosinophilia, and rectal bleeding (65,91). Appendicitis, peritonitis, anorexia and weight loss have also been reported (65,91). It is believed that the colon's absorptive ability becomes impaired, causing the extensive dysenteric diarrhoea (65). TDS is thought to be partly due to host immune response and an elevation of plasma viscosity (129); symptoms resemble inflammatory bowel disease (92) and are sometimes life-threatening (119). *T. trichiura* worms do not have a larval phase of tissue migration, thus lesional pathology is confined to the intestine (65,91,96,129). In observational studies, TDS has been associated with very poor development in young children (119,130), some of which was not reversible with treatment (131).

1.3 Pathogenesis and clinical morbidity from other gastrointestinal parasites

S. stercoralis penetrate the skin and migrate to the small intestine or lungs where they are coughed and swallowed into the gastrointestinal tract (34). *S. stercoralis* is capable of both sexual and asexual reproduction, and autoinfection (13,132). The autoinfective cycle begins when asexually produced larvae invade the intestinal wall or perianal area and enter the bloodstream (34); this cycle can persist in the host indefinitely (133). Infections of *S. stercoralis* include:

- acute (initially local skin rashes and itch, followed by pulmonary symptoms with eosinophilia, before diarrhoea and abdominal pain);
- chronic (often asymptomatic as cell-mediated immune response of the host controls the number of larvae; symptoms can include vomiting, diarrhoea, constipation, recurrent asthma and skin symptoms such as urticaria and larva currens);
- hyperinfection (accelerated autoinfection where signs and symptoms are attributed to increased larval migration); and
- disseminated (where larvae are present beyond the range of respiratory and gastrointestinal symptoms; this does not necessarily imply a greater severity of disease) (34,133). Strongyloidiasis hyperinfection can occur in immunocompromised hosts (34), with a high case-fatality rate (134).

In healthy people *C. parvum* usually causes self-limiting diarrhoea (135) for approximately three to nine days (136). However, in young children and immunocompromised people the diarrhoea can be persistent and severe. It can last for months, causing malabsorption and weight loss, and often leading to death (43,137). Infection begins with the ingestion of oocysts and subsequent infection in the superficial surface of the small intestine, thereby disrupting intestinal function (43). After infection with *C. parvum*, humans develop antibodies (43), although this does not appear to be sufficient to prevent reinfection. For example, in a study in Brazil, secondary household infections occurred in 58% of households with an infected child, despite 95% prevalence of antibody in children more than two years of age (138). Risk factors for *C. parvum* acquisition appear to differ according to immune status (135), with HIV-infected people at greater risk from drinking

tap-water (139,140), and immunocompetent people at risk from foreign travel but not drinking tap-water (141). Host susceptibility (i.e. immune status) may be the primary determinant of developing cryptosporidiosis among AIDS patients (135).

G. duodenalis are ingested as inactive oocysts. After passage through the stomach they become active trophozoites, which feed on duodenal or jejunal mucosa and reproduce, forming cysts again prior to being expelled (40). Despite numerous studies, little is known about the pathogenesis of giardiasis, although the parasite has been reported to damage the intestine, particularly microvilli (142), leading to reduced nutrient absorption (143,144), and affecting vitamin A absorption (145). Once the infection clears, intestinal function returns to normal (145).

E. histolytica is usually present in the large intestine, however amoebae can persist for years as asymptomatic luminal gut infections (56). Occasionally the parasite penetrates the intestinal mucosa and induces colitis or disseminates to other organs, most commonly the liver, where it forms abscesses (52,56). The majority of people infected with *E. histolytica* experience no symptoms, less than 10% have loose stools and a very small percentage suffer from bloody, febrile dysentery or liver abscess (51,52). A study linking hospitalised patients with acute liver abscess to their residential address in Vietnam found that development of acute liver abscess correlated directly with population density (56). There are long latencies between infection with *E. histolytica* and development of acute liver abscess (146-148). A study found that prevalence was higher in females, suggesting that sex-related factors or exposures are important for infection (55). Pregnant and post-partum

women also have an excess risk of severe illness and death (51,56). However, the occurrence of acute liver abscess following *E. histolytica* infection greatly predominates in adult males, with a peak incidence at approximately 40 years of age (51,56).

1.4 Current control strategies

Control strategies can be viewed as having either short-term or long-term objectives (149). Short-term objectives are to substantially reduce STH morbidity and mortality (24). Chemotherapy, the provision of deworming tablets, is the principal way to achieve these short-term objectives (150). Long-term objectives, to reduce the prevalence and intensity of STH transmission, involve the improvement of sanitary facilities, the provision of safe water supplies, the promotion of personal and food hygiene and the safe disposal of waste (24). These objectives are generally more costly, time and labour intensive to achieve and need to be accompanied by social, economic and educational development (149).

Chemotherapy

Chemotherapy has been demonstrated to achieve high levels of morbidity reduction through rapid STH and egg clearance in the human host (3,16,151,152). Benzimidazole drugs (albendazole or mebendazole) are the treatment of choice (153,154). They can be used to treat almost every age group including pregnant women after the first trimester (155) and children from 12 months of age (15). With a strong safety profile, these anthelmintics can be safely and effectively distributed by non-medical personnel (156);

they are provided as single-dose tablets, patients do not need to be weighed (3), and they are cheap. Expiry of patents in the late 1990s has enabled pharmaceutical companies to develop generic drugs for extremely low cost (e.g. $USD0.02 for albendazole) (15); companies have, in turn, donated substantial amounts of anthelmintics in recent years (being, in 2013 alone, close to 1.35 billion treatments (4)), ensuring free provision of anthelmintics (although other issues such as access to these drugs by consumers are still encountered). Regular treatment, despite reinfection, is able to control morbidity in high-transmission areas (154) because, even if prevalence remains high, moderate to heavy infections (responsible for morbidity) decline over time (95).

Five drugs, albendazole, levamisole, mebendazole, pyrantel and ivermectin (against *Strongyloides stercoralis*), are on the WHO model list of essential drugs for control of STH (84,151). Drug efficacy varies, with the cure rate of single-dose albendazole recently reported as 98% for *A. lumbricoides*, 88% for hookworm and 47% for *T. trichiura* (157). Single dose treatments are generally poor against *T. trichiura* infection. Heavy infections of *N. americanus* may also require more than one dose to achieve a cure (158). For single-dose mebendazole, reported cure rate is 95% for *A. lumbricoides*, 36% for *T. trichiura* and 15% for hookworms (159). Albendazole has the added benefit of antiprotozoal activity, although several doses may be required over consecutive days (160,161).

Drug alternatives and combinations have also been investigated, however more research is warranted. Oxantel-pyrantel/mebendazole combination treatment of *T. trichiura* infections has been found to be more effective than treatment with either drug alone (67% cure rate

versus 53% and 38%, respectively) (84). Ivermectin exhibits activity against *A. lumbricoides* infections, and to a lesser extent against *T. trichiura* and hookworms (151). Oxibendazole, which is very similar to albendazole (162) is a drug prospect that requires further investigation. It has exhibited anthelmintic activity in two clinical trials between 1988 and 1990 in China. However, three doses of 15 mg/kg each on three consecutive days were required to obtain high cure rates against *A. lumbricoides*, hookworms and *T. trichiura* (162). Nitazoxanide, which is a nitroimidazole derivative similar to metronidazole, has also been used against *A. duodenale*, *A. lumbricoides* and *T. trichiura* (162). However, twice-daily doses for three or more days are required (163). There do not appear to be any reports about the efficacy of nitazoxanide against *N. americanus* (162). Tribendimine has been reported to have cure rates of 85% to 90% for *A. lumbricoides*, *A. duodenale* and *N. americanus* in open-label trials and RCTs (162). Treating schistosomiasis with praziquantel has been found to yield significant reductions in prevalence of hookworm infection from 75% to 41% among school children (29). Combination therapy with praziquantel and albendazole may be more effective in reducing prevalence and intensity of hookworm infections than administration of albendazole alone (29).

Nitroimidazoles (metronidazole and tinidazole), albendazole, quinacrine hydrochloride, or furazolidone are used for giardiasis, however lack of treatment compliance and side effects can result in treatment failures (38,164). Additionally, drug resistance has been demonstrated against many of these compounds (165), and most of them cause adverse reactions that reduce compliance or have levels of toxicity that restrict their use in women of child-bearing age (166). Furazolidone is a recognised carcinogen and mutagen and is no

longer available for human use in some countries including Australia (167). The antibiotic bacitracin, enhanced by zinc, has been shown to be somewhat effective against giardiasis (166). There are no effective chemotherapeutic agents against *C. parvum* (168,169). Paromomycin has shown modest reductions, but not complete removal, of *C. parvum* oocysts in controlled studies (43). Alternative drugs for giardiasis and cryptosporidiosis are clearly required. Metronidazole is a potent drug for the control of *E. histolytica* (38,52); however it is not considered appropriate for use in asymptomatic patients; therefore chemoprophylaxis against *E. histolytica* is not recommended (52). Invasive disease is treated with a tissue amoebicide (such as metronidazole or a nitroimidazole) followed by a luminal amoebicide (such as diloxanide furoate or paromomycin) (52).

Advantages and disadvantages of chemotherapy for STH are reviewed in Chapter 3, however for context it is important to highlight that the major drawback of chemotherapy is its inability to break STH transmission cycles due to rapid reinfection rates in environments contaminated with infective eggs and larvae (170). Chemotherapy acts only on adult parasites, and there is strong evidence that STH reinfection readily occurs after treatment (3,16,127,171,172) with pre-treatment levels of helminth burden being reached within approximately six months of deworming (171,172). For this reason, and concerns of drug resistance in the context of mass drug distributions, chemotherapy is of questionable long-term benefit.

Reinfections

The rate of reinfection following treatment in those individuals or groups predisposed to heavy infection is more rapid than in the population as a whole (20), but is also linked to the age of the host (173). Additionally, the speed at which reinfection occurs differs between parasite species, being less than one year for *T. trichiura*, one to two years for *A. lumbricoides*, and two to three years or longer for hookworms (6). Therefore, in areas of endemic infection, mass treatment at intervals of approximately two to four months may be necessary for *A. lumbricoides* and *T. trichiura*, and approximately once per year for hookworms, to suppress the average intensity to very low levels (6). Reinfection also occurs with *E. histolytica*. In one study approximately four per cent of the population was reinfected per year (60).

As a result of this high reinfection rate, the WHO recommends that:

- in areas where STH prevalence is 50% or greater, treatment is to be provided twice yearly to preschool and school-aged children, pregnant women and high risk groups of adults;
- in areas where prevalence is 20-50%, annual treatment is to be provided; and
- in areas with prevalence of less than 20%, drugs are to be made freely available at health facilities (174).

The WHO has set a global target of treating 75% of all preschool and school-aged children per annum by 2020 (175). Mass treatment is advocated as the main strategy for control, as

the clinical appearance of STH infection often lacks specific symptoms and may not be recognised by the infected person, even when contributing to significant health damage (95). However, the actual delivery of these drugs to all of the abovementioned groups is problematic, with supply and distribution bottlenecks often preventing anthelmintics from being available to people, particularly those who cannot access treatments in school-based settings. Inequity of access is a recognised issue, and additional health system strengthening initiatives are required to overcome these challenges. Additionally, many endemic countries are not accessing the donated anthelmintics to treat children (176).

Chemotherapy control strategies

In one of the first studies of chemotherapeutic control strategies against STH, Asaolu et al. (24) compared the effectiveness of **mass treatment** (population level application), **targeted treatment** (group treatment according to a group characteristic such as age, sex, social characteristic or infection intensity) and **selected treatment** (individual application where selection is based on intensity of current or past infection). This study illustrated that mass treatment was most effective in significantly reducing *A. lumbricoides* intensity (24). Advantages included elimination of time and cost spent on regular stool examination and the use of relatively unqualified personnel to undertake the programme (24). Further, the safety record and low cost of anthelmintic drugs compared to screening people for positive cases of infection, meant that treatment without prior laboratory diagnosis was considered to be an effective option in places of high prevalence or high transmission (15). Based on studies in Kenya, Stephenson et al. (177) also strongly supported mass treatment in

preference to other chemotherapeutic approaches. Some evidence has indicated that even when mass treatment is provided, intervals as low as two to four months are necessary to reduce *A. lumbricoides* intensity to low levels (6).

However, prior to donated drugs by pharmaceutical companies, concern was expressed that the frequent use of large quantities of anthelmintics in mass chemotherapy would be prohibitively expensive for most developing countries (16). Instead, targeted treatment of school-aged children was advocated, additionally supported because schoolchildren tended to have the highest aggregations of helminths within communities, and were inferred to be the cohort in which chemotherapy might have the greatest impact (3,6). Additional advantages were that, as well as using a smaller quantity of drug (24), most children could be easily reached through attendance at school (177). This key infrastructure factor has enabled several authors to conclude that school-based deworming programs are among the most cost-effective of public health strategies (154,178). Stoltzfus et al. (6,179) and Stephenson et al. (180) have found that treating school-aged children with anthelmintic drugs have resulted in improved nutritional status and growth. Of particular interest is that adults, who receive little or no treatment, also can show a significant reduction in STH following the implementation of school-based deworming programmes. Reductions in infection intensity, often considerable, have been reported for *A. lumbricoides* (24) and *T. trichiura* in young adults (of whom less than four per cent had received treatment) (152). However, hookworm is more common in adults so community-based treatment programmes may be more effective in control of hookworm morbidity than school-based programmes (3). Additionally, the United Nations Children's Fund (UNICEF) has

estimated that 40% of school-aged children in the least developed countries are not enrolled in schools (15), and may therefore miss out on school-based deworming initiatives.

The third strategy, selective treatment, involves selecting and treating those individuals in a community that have heavy worm burdens. This subsequently reduces the morbidity associated with heavy infection and the contamination of the environment with infective eggs and/or larvae (24). Identification of these heavily-infected individuals is not difficult: Haswell-Elkins et al. (181) found that taking a single egg count from individuals' faeces was 65% effective in identifying the heavily infected group. However, Asaolu et al. (24) found that selective treatment had little impact on the prevalence and intensity of *A. lumbricoides* within the community that they studied; nor did it take account of new immigrants into the study area, which means that constant surveillance and stool examination would be required. Additionally, this approach caused considerable resentment when some individuals were selected and others were excluded (24).

Equity of access, concerns of morbidity in non-school cohorts, reinfection rates, plus a shift in focus from morbidity control to the possibility of transmission control (176) has again made community, versus school-based, anthelmintic treatment a current research focus. Mathematical modelling and epidemiological studies are under way to investigate which strategy will have greatest benefit for STH control (176,182).

Water, sanitation and hygiene (WASH)

WASH is the provision of access to a safe water supply, appropriately constructed sanitation infrastructure ensuring safe disposal of human excreta, health education and promotion of hygiene (being personal and household practices aimed at preserving cleanliness and health) (183). WASH is thought to be important in preventing helminth infection and recurrence (3,15). Sanitation is considered to be particularly important: where there is poor sanitation there is little separation of people from human faecal material in the environment; the essential soil-dwelling stage of the STH life cycle provides a direct transmission pathway to the human host. Thus eradication of STH is unlikely to occur in areas where there are no acceptable or hygienic facilities to dispose of human excreta (184,185). Whilst numerous authors have reported on the associations between STH infection and/or protozoa infection, and hygiene and sanitation (reviewed in (186,187)), most studies have been observational in nature. More research needs to be done to confirm and quantify the impact of water, sanitation and hygiene interventions on intestinal worm burdens. There is little experimental evidence for WASH on STH outcomes; whilst this is reviewed in Chapter 3, definitions of each of the WASH components, and a summary of observational and experimental evidence for each, are provided below.

Types of sanitation

Whilst interpretations vary, sanitation can be considered primarily in terms of "hardware" such as toilets, latrines and sewage treatments for disposal of excreta and provision of safe water (95). The accompanying "software" which relate to the use of this infrastructure,

such as personal hygiene, policies and legislation (e.g. regulations on sewerage connections), can come under the umbrella term of "hygiene". Many latrine types have been developed, with pit latrines being the commonest, simplest and cheapest in developing countries (188), although they can suffer excessive disrepair, which can then transmit pathogens in the community. The WHO and UNICEF's Joint Monitoring Programme (JMP) for Water Supply and Sanitation has defined a broad range of latrines according to whether they are 'improved' or 'unimproved', whereby an improved sanitation facility hygienically separates human excreta from human contact and an unimproved one does not (189). Improved sanitation includes piped sewer systems, septic tanks, flush toilets or pour-flush toilets to a pit latrine, ventilated improved pit (VIP) latrines, pit latrines with a slab for squatting on, and composting toilets (189). Unimproved sanitation includes flush toilets or pour-flush toilets that do not go to a pit latrine, pit latrines without a slab, buckets, hanging toilets, or absence of any toilet facilities (189) (Table 1.1).

Table 1.1: Types of sanitation and how they could affect STH transmission

Type of sanitation	Features	How it could affect STH transmission
Flush toilet	This uses a cistern or holding tank to flush water, and a water seal (which is a U-shaped pipe below the seat or squatting pan) that prevents the passage of flies and odours.	Due to the flushing mechanism these toilets have minimal risk for STH transmission, although if these are squat toilets risks are increased due to potential spillage. If these toilets are not regularly cleaned or maintained, there is a risk of transmission.
Piped sewer system	A system of sewer pipes, also called sewerage, designed to collect human excreta (faeces and urine) and wastewater and remove them from the household environment. Sewerage systems consist of facilities for collection, pumping, treating and disposing of human excreta and wastewater.	This sewerage system is the collection point for the excreta, which may come from one of several toilet types. A piped sewerage system has minimal STH transmission risk if well- maintained, as there is no contact with infected faeces.
Septic tank	An excreta collection device consisting of a water-tight settling tank, which is normally located underground, away from the house or toilet. The treated effluent of a septic tank usually seeps into the ground through a leaching pit. It can also be discharged into a sewerage system.	Septic tanks are considered to have minimal STH transmission risk if well-maintained, as there is no contact with infected faeces.
Flush/pour flush to pit latrine	A pour flush toilet uses a water seal, and uses water poured by hand for flushing (no cistern is used). Excreta are flushed to a hole in the ground or leaching pit (protected, covered).	Due to the flushing mechanism and closed pit, these toilets have minimal risk for STH transmission, although if these are squat toilets risks are increased due to potential spillage. Regular cleaning of spills and maintenance is still required.
Flush/pour flush to elsewhere	A pour flush toilet uses a water seal, and uses water poured by hand for flushing (no cistern is used). Excreta are deposited in or nearby the household environment (not into a pit, septic tank, or sewer). Excreta may be flushed to the street, yard/plot, open sewer, a ditch, a drainage way or other location.	Due to the flushing mechanism, these toilets may have minimal risk for STH transmission, although if these are squat toilets risks are increased due to potential spillage; safety further depends on where excreta are flushed to. If these toilets are not regularly cleaned or maintained, there is a risk of continued transmission.

Type of sanitation	Features	How it could affect STH transmission
Ventilated improved pit latrine (VIP)	A dry pit latrine ventilated by a pipe that extends above the latrine roof. The open end of the vent pipe is covered with gauze mesh or fly-proof netting and the inside of the superstructure is kept dark.	Whilst these toilets are considered improved, their safety in terms of STH transmission depends on whether users are exposed to faeces that can accumulate if people miss the hole when defecating. Whilst having a slab reduces the risk from eggs/larvae growing in surrounding soil, if these toilets are not regularly cleaned or maintained, there is a risk of continued transmission.
Pit latrine with slab	A dry pit latrine that uses a hole in the ground to collect the excreta and a squatting slab or platform that is firmly supported on all sides, easy to clean and raised above the surrounding ground level to prevent surface water from entering the pit. The platform has a squatting hole, or is fitted with a seat.	Whilst these toilets are considered improved, their safety in terms of STH transmission depends on whether users are exposed to faeces that can accumulate if people miss the hole when defecating. Whilst having a slab reduces the risk from eggs/larvae growing in surrounding soil, if these toilets are not regularly cleaned or maintained, there is a risk of continued transmission.
Pit latrine without slab	A hole in the ground for excreta collection that does not have a squatting slab, platform or seat.	These are considered unsafe toilets. Users may be exposed to faeces that can accumulate if people miss the hole when defecating. The lack of slab further adds to the risk as the surrounding soil can enable eggs and larvae to survive in immediate proximity.
Composting toilet	A dry toilet into which carbon-rich material (vegetable wastes, straw, grass, sawdust, ash) are added to the excreta and special conditions maintained to produce inoffensive compost. A composting latrine may or may not have a urine separation device.	Well-constructed composting toilets are generally considered safe according to JMP definitions, however may not be for STH transmission due to the longevity of eggs and larvae in soil, and the fact that it is often soil or compost that is directly added. More evidence is required to determine whether compost toilets prevent STH transmission.
Bucket	Use of a bucket or other container for the retention of faeces (and sometimes urine and anal cleaning material), which are periodically removed for treatment, disposal, or use as fertilizer.	Whilst faeces from a bucket may be disposed of via treatment etc, using a bucket is generally messy and unhygienic. Direct contact with faeces facilitates STH transmission.

Type of sanitation	Features	How it could affect STH transmission
Hanging toilet or hanging latrine	A toilet built over the sea, a river, or other body of water, into which excreta drops directly.	Depositing faeces directly into the water creates an STH transmission risk for all others who use the water supply.
No facilities or bush or field	This includes defecation in the bush or field or ditch; excreta deposited on the ground and covered with a layer of earth; excreta wrapped and thrown into garbage; and defecation into surface water (drainage channel, beach, river, stream or sea).	This "open defecation" is considered to be the riskiest way of continuing STH transmission. The direct depositing of faeces onto the soil, usually with minimal covering, facilitates continuation of the STH life cycle.

(189)

The key feature of improved sanitation is that, for STH, the lack of human contact with human faeces means that there is no direct path for continuation of the STH life cycle. This, whilst inherently biologically plausible, has been under-researched in terms of specific STH associations, and those studies that have been done are less than definitive in their conclusions. Two systematic reviews reported latrine use to be associated with reduced odds of STH infection (186,187), but could not differentiate by latrine type. Reductions in STH prevalence have not been seen across all studies, including an RCT (190). Other factors, such as level of community use, and inadequately reduced open defecation behaviour, may have contributed to the lack of prevalence reduction (191). There is observational evidence that open defecation is associated with higher odds of hookworm infections (192). There has been little research specifically comparing improved and unimproved sanitation. Further, an improved latrine may be a transmission point for disease if it is not well maintained or correctly used (172,184,193). Similarly, sharing latrines across households potentially increases STH transmission risk. Further research is necessary to determine whether shared sanitation is either safe or appropriate (194), and to determine an evidence base for improved versus unimproved designs.

Water supply

According to JMP definitions, improved types of water supply include piped water into the dwelling, piped water to the yard, public taps or standpipes, tubewells or boreholes, protected dug wells, protected springs, bottled water and rainwater (189). Unimproved types of water supply include unprotected springs, unprotected dug wells, carts with small

tanks or drums, tanker-trucks, surface water and bottled water (189). Bottled water is either an improved or an unimproved water supply, according to the source of the water. Improving water supply involves not only improving its quality, but primarily improving its quantity (195), as it allows for better personal and domestic hygiene (196) (Table 1.2).

Table 1.2: Types of water supply and how they could affect STH transmission

Type of water supply	Features	How it could affect STH transmission
Piped water into dwelling	A water service pipe connected with in-house plumbing to one or more taps (e.g. in the kitchen and bathroom).	Piped water into the dwelling is regarded as safe for transmission, but also depends on the original source.
Piped water to yard/plot	A piped water connection to a tap placed in the yard or plot outside the house.	Piped water to an outside tap is generally regarded as safe in terms of the water itself (depending on the source), however the surrounding areas, which often get muddy, may present a transmission risk, particularly if shoes are not worn.
Public tap/standpipe	A public water collection point. Public standpipes can have one or more taps and are usually made of bricks, masonry or concrete.	Piped water to an outside tap is generally regarded as safe in terms of the water itself (depending on the source_, however the surrounding areas, which often get muddy, may present a transmission risk, particularly if shoes are not worn. The more public nature of this, compared to piped water supplies used by individual households, can be regarded as increasing transmission risk.
Tubewell or borehole	A deep hole that has been driven, bored or drilled, to reach groundwater. They are built with casing, or pipes, to protect the water source from contamination. The water is delivered through a pump. Boreholes/tubewells are usually protected by a platform around the well, which prevents contamination at the well head.	Prevents infected faeces from entering the water supply. Boreholes/tubewells need to be properly built and well-maintained to prevent damage.
Protected dug well	A dug well that is protected from runoff water by a well lining or casing that is raised above ground level and a platform that diverts spilled water away from the well. A protected dug well is covered, so that bird droppings and animals cannot fall in.	Prevents infected faeces from entering the water supply. The cover needs to be properly built and well-maintained to prevent damage.

Type of water supply	Features	How it could affect STH transmission
Unprotected dug well	A dug well that is not protected, either from runoff water, or bird droppings and animals.	Does not prevent infected faeces from entering the water supply. These tend to be frequently used water collection/water use points in many communities.
Protected spring	A spring that is protected from runoff, bird droppings and animals by a "spring box", built around the spring so that water flows directly out of the box into a pipe or cistern, without being exposed to outside pollution.	Prevents infected faeces from entering the water supply. The spring box needs to be properly built and well-maintained to prevent damage.
Unprotected spring	A spring that is not protected from runoff, bird droppings, or animals. Unprotected springs do not have a "spring box".	An unprotected spring does not prevent infected faeces from entering the water supply. These tend to be frequently used water collection/water use points in many communities. Runoff may flow into other surface water.
Rainwater	Rain that is collected or harvested from roof or ground catchment surfaces and stored in a container, tank or cistern.	Rainwater may be generally safe in terms of STH transmission (but not other pathogens), however is often stored in large containers which are less safe. People extracting water from these containers, for example with short-handled ladles which allow hand contact with the water, may facilitate transmission if hands are soiled.
Cart with small tank/drum	Water sold by a provider who transports it into a community.	Depending on the source, and handling of, the water in the drum, this may have minimal STH transmission risk.
Tanker truck	Water brought by truck into a community and sold directly from the water truck.	Depending on the source, and handling of, the water in the truck, this may have minimal STH transmission risk.
Surface water	Water located above ground including rivers, dams, lakes, ponds, streams, canals, and irrigation channels.	Surface water, often being a lake or other still water body, is often located downhill from a community, and may receive run-off from springs or surrounds when it rains. Faeces/eggs/larvae could be washed in, potentially making it unsafe; more evidence is needed to confirm this.
Bottled water	Bottled water may have an unbroken seal and come direct from a supplier. However it is common practice in many developing countries to refill bottles from a local water	Bottled water with an unbroken seal carries no transmission risk. Refilled bottles must be regarded as unsafe. Additionally, bottled water is rarely in sufficient quantity to

	source and resell these.	be available for purposes other than drinking, therefore it often does not lend itself to practicing safe hygiene, such as washing hands.

(189)

There is extremely limited information as to associations with improved or unimproved water supply and STH outcomes. It is known that *N. americanus* larvae can survive and remain infective for several days in water, although with reduced longevity (197). Although biologically plausible, there is little direct evidence that STH infections can arise from ingesting contaminated drinking water. The link between water supply and STH may be more likely to come from what the water is used for, particularly the quality, and quantity, available for people to practice hygienic behaviours. Hygiene is discussed further below.

A review of water and sanitation studies found a 29% decrease in *A. lumbricoides* prevalence following implementation of improved water and/or sanitation facilities (196). Studies have focused on diarrhoea outcomes, with a meta-analysis showing water quality improvements to be associated with a 17% reduction in diarrhoea risk (198), and a systematic review (199) calculating a reduced risk of 39% for household point-of-use treatment interventions from studies identified as being of sound quality. This review found no statistically significant reduced risk of diarrhoea from water supply interventions such as piped water or household connection but studies were very limited (199). Distance to water source has been linked to volume utilised for household activities such as drinking, cooking and hygiene (200,201); with greater distances implicated in diarrhoea, poorer child nutritional status, and higher under-five child mortality (200,202,203). Observational analysis confirms mothers of young children are more likely to wash their hands if they have a piped water supply (204), which is likely because increased water quantity makes hygiene possible. Inadequate water supplies curtail efforts to improve sanitation and hygiene behaviours (203).

Water and sanitation infrastructure is underpinned by, and itself underpins, hygiene and health education. This is because simply providing the infrastructure will not necessarily lead to its use. Behavioural change is required. If available facilities are not used correctly environmental contamination will still occur and overall health may not improve (193,184). Public latrines are insufficient for the excreta disposal needs of a community, as they are not usually accessible at night or by the elderly, those with disabilities or young children (195). Even where latrines may be available to all households, some of the community may not use them (205) for reasons that may include latrine dysfunction, defecating outdoors being perceived to be more hygienic and concern that children may have accidents if using an open latrine unattended (206). In a study of slum-dwellers in Botswana, Zambia and Ghana, Feachem et al. (207) found that provision and use of piped water and different levels of sanitation facilities did not protect families from infection when the overall level of faecal contamination in the environment remained high.

Broader than STH, improvements in the overall health status of study populations have been reported after improving water supply and sanitation facilities (208,209). Shuval et al. (210) proposed the threshold-saturation theory, in which the overall outcome of investments in community water supply and sanitation facilities depends largely on the coverage and general socioeconomic conditions of communities. In an observational study comparing two rural villages with different sanitation characteristics in Nigeria, Asaolu et al. (28) found that, in spite of better facilities in one town, both communities were endemic for *A. lumbricoides* with higher mean intensity of infection in the village with better facilities, where the individual with the highest worm burden was found. Thus, if only a

few individuals in a community are provided with better facilities while the general conditions of the majority remain poor, the impact of the investment on human health is minimal (28). This is supported by other researchers who have identified that sanitation does not become effective until it covers a high percentage of the population (172,196), with coverage of properly built, used and maintained sanitation required to be higher than 90 per cent to have any effects on worm transmission (193). This is because of the infectivity of STH eggs and larvae in the soil; if insufficient proportions of people in a community have access to sanitation, those who have latrines will still be at risk of infection (205).

Therefore, investment in community-wide interventions is deemed most beneficial for STH reduction. Asaolu et al. (28) also recommend that improvements to water supply and sanitation should be accompanied by a programme of health education to promote the value and appropriate use of the facilities. Preventable measures and simple community based measures such as increasing public awareness about the impact of open-air defecation, safe disposal of waste water and safe handling of drinking water could yield effective short-term results (211).

Hygiene

The most important component of personal hygiene is handwashing with soap at appropriate times, potentially including before meal preparation, after contact with animals, after urinating, defecating, wiping body fluid or assisting children with toileting, and after

contact with garbage or other 'dirty' items. In a systematic review (187) and two RCTs (212,213), reduced odds of STH infection, particularly *A. lumbricoides*, have been reported for handwashing before eating and after defecating, and soap use or availability. However, there is little evidence that clearly indicates on which occasions handwashing is of greatest importance (187).

Health education can be tailored to audiences, and utilise a range of different media. However, health education programs are unlikely to guarantee uptake of interventions; numerous local issues including risk perception, intervention acceptability, socio-cultural factors and practical issues also influence behaviour (214). Therefore, as with many other health interventions, strong community engagement strategies are required. Community Led Total Sanitation (CLTS) and sanitation marketing have been advocated as innovative methods, focusing on demand creation and changes to social norms that drive behaviour change, to mobilise communities to eliminate open defecation (215). Although CLTS has been implemented in more than 20 countries (215), data from monitoring and evaluation of the approach is required to assess its effectiveness (216). The extent to which communities actually change behaviour and reduce open defecation is unclear (216,217). Insufficient evidence has been demonstrated on the effects of CLTS on parasitological or health parameters (217), and more evidence on this and other forms of community mobilisation is needed.

WASH and economic development

It is apparent that improving WASH leads to improvements in general health. An efficient sanitation infrastructure removes the underlying cause of most poverty-related communicable diseases (which themselves contribute to poverty in turn) and thus supports the economic development of a community (95). The living standard in developed countries may be attributed to uninterrupted safe water supply and widespread provision of facilities for sanitary disposal of human excrement (184,193). It is therefore concluded that whilst WASH is essential for long-term reductions in infections from STH and protozoa, the long time required to improve sanitation and change behaviours necessitates rapid complementary measures such as chemotherapy to control STH while progress is made on addressing WASH deficits (218).

1.5 Integrated soil-transmitted helminth control measures

Integrated STH control is extensively reviewed in Chapter 3. The integration of chemotherapy with WASH has been advocated for sustainable STH control for many years (20,151,219); the cumulative effect of the additional WASH measures being to further reduce transmission and the potential for reinfection than is possible through chemotherapy alone. Integration of multiple interventions may reduce the need to have 10-12 years of repeat doses of benzimidazole, thereby reducing the theoretical possibility of (as yet undetected) individual harm associated with long-term usage, or development of parasite drug resistance (220-221).

In many countries, the approach towards STH control has been to integrate STH interventions into wider health priorities. Many countries use an integrated approach to provide mass drug administration against several endemic neglected tropical diseases (NTDs) (222,223). In Uganda, interventions include provision of health education and administration of both praziquantel (for schistosomiasis) and albendazole simultaneously, provision of albendazole with immunisations, and the introduction of Child Health Days (224,225). Many preschool children can be reached by adding STH chemotherapy to vitamin A distribution or immunisation campaigns (95). STH control measures can also be added to public health initiatives such as Integrated Management of Childhood Illness (IMCI), Integrated Child Development Services (ICDS) programs, school health programmes, Roll Back Malaria programmes, micronutrient initiatives and reproductive health initiatives (95,226).

Cooperation between different sectors is of paramount importance. In 2000, a framework to Focus Resources on Effective School Health (FRESH) was launched to provide a consensus approach of agreed good practice for the effective implementation of health and nutrition services within school health programmes (2). This has encouraged more countries to implement school health reforms (2), and increasingly, these programs are including chemotherapy for STH.

Whilst integrating chemotherapy and WASH could be of considerable benefit, the evidence base is weak. Only one school-based RCT has evaluated the impact of integrated chemotherapy and WASH interventions on STH (227). This provides justification for

undertaking the community-based "WASH for Worms" trial, an RCT that aims to investigate the impact of integrated chemotherapy and WASH on STH at the community-level for the first time. It is hoped that this study will provide the evidence base for scaling up integrated chemotherapy and WASH programmes in Timor-Leste and other developing countries.

1.6 Soil-transmitted helminths in Timor-Leste

Timor-Leste is a recently independent country. Previously a colony of Portugal, it achieved independence of nine days standing in 1975 prior to occupation by neighbouring Indonesia. Following a vote for independence in 1999, re-declaration of independence was finally made in 2002 after three years of violence in which thousands of people were killed or injured and more than 80% of the country's infrastructure destroyed (228). Since this time there have been short periods of civil unrest resulting in the need for a peacekeeping role from the United Nations Integrated Mission in Timor-Leste (UNMIT) (229), but there is a strong underlying culture of resilience. UNMIT ceased at the end of 2012 (229). A period of relative peace and rebuilding has ensued: during this PhD Candidature alone, Timor-Leste has progressed from 147th to 133rd on the Human Development Index (230,231). There is still widespread poverty, which has led to a national poverty definition of less than $USD0.55 per day (as opposed to the more regularly encountered $USD1 per day); even at this definition more than 40% of the country live below the poverty line (232).

Very few studies have been conducted to estimate STH burden in Timor-Leste. STH have been reported in a Portuguese survey in the 1950s (233). There has been a published intestinal parasite survey in the 1970s (234), which found STH to be endemic, and a PhD thesis from 2010 detailing a prevalence survey of three villages. This latter study found 33%, 1.3% and 2.4% of hookworm, *A. lumbricoides* and *T. trichiura* prevalence respectively (235). A recent school-based national survey revealed STH prevalence of 29% in school-aged children, comprising *A. lumbricoides* (21%), hookworm (9.2%) and *T. trichiura* (4.1%) (236), however insensitive diagnostic methods were used. No other reported studies have quantified the STH or intestinal protozoal burden in Timor-Leste. With only 18% of rural Timorese communities having improved sanitation (216), community STH prevalence is suspected to be high.

1.7 The WASH for Worms randomised controlled trial

In mid-2011, the University of Queensland (UQ) was successful in receiving a National Health and Medical Research Council (NHMRC) Partnership Grant with WaterAid Australia to conduct an RCT in villages in Timor-Leste. This trial, known as "WASH for Worms", will assess the impact of a community-based WASH programme on infection with intestinal parasites following mass albendazole chemotherapy. The trial commenced in early 2012 in Manufahi District, Timor-Leste and is ongoing. In February 2014, the trial was transferred to the Australian National University (ANU), with key trial personnel.

The aims of the trial are:

1. To determine the effectiveness of a community-based WASH programme integrated with mass albendazole chemotherapy in reducing the incidence (hookworm, roundworm, and whipworm) or prevalence (threadworm and gastrointestinal protozoa) of parasitic infection, in rural villages in Timor-Leste.

2. To determine the reduction in intensity of infection and in parasitic disease-related morbidity in children, including anaemia, stunting and wasting, achieved by implementation of the integrated WASH and mass albendazole programme.

3. To understand the planning and implementation of the sanitation component, as well as the barriers and enablers associated with the acceptability and uptake of the WASH programme (237).

Study setting

With suspected high STH burden, Timor-Leste is an appropriate setting for this study. The Timor-Leste Ministry of Health (MoH) has responsibility for hygiene and sanitation, including district water and sanitation offices, but has had little or no resources to fund water and sanitation work in rural areas. The partner organisation for WASH for Worms, WaterAid, commenced a programme to improve hygiene and sanitation in Manufahi in May 2012. It is the only active, international non-government organisation (NGO) providing such programmes in the district. In 2005, the MoH initiated a deworming programme, with support from the WHO, however this ceased in 2008 due to insufficient funding (237). The MoH recently developed a national integrated plan for the control of

NTDs, which will include STH deworming; limited resources meant deworming re-started in late 2015 in a limited number of districts (238).

The trial is conducted in Manufahi District, selected by WaterAid on the basis of poor existing WASH infrastructure (237). Manufahi District is an agricultural region, and is comprised of rural villages not receiving regular systematic deworming programmes at the time of the study (Figure 1.1).

Figure 1.1: District map of Timor-Leste indicating the study setting, Manufahi District (239)

Study design

WASH for Worms is a two-arm cluster RCT, with cluster units being small rural villages in Manufahi district. WaterAid works with government ministries and local district and subdistrict officials to agree on a priority list of communities to be supported by WaterAid according to annual scheduling. A sampling frame was established and inclusion and exclusion criteria applied to select communities (237). Ethical approval has been obtained from the UQ Human Research Ethics Committee; the ANU Human Ethics Committee; the Timorese Ministry of Health Research and Ethics Committee; and the University of Melbourne Human Research Ethics Committee. This study is funded by a Partnership for Better Health – Project grant from the NHMRC (APP1013713). This study is registered with the Australian New Zealand Clinical Trials Registry (Registration number ACTRN12614000680662) (237).

The study protocol has been published (237), and the study design is provided at Figure 1.2. Baseline surveying of 24 villages was undertaken, however, six villages were subsequently withdrawn from the RCT due to not meeting study criteria (237) (Figure 1.3). The analyses in this thesis are not affected, as all data were collected according to the protocol. The remaining 18 intervention (n=1 318) and control (n=1 200) communities receive mass chemotherapy with albendazole on a six-monthly basis, as recommended by the WHO. Ten intervention communities also receive the community-based WASH programme. This involves (i) constructing safe, adequate and convenient water supplies; (ii) constructing sanitation, particularly household latrines together with a CLTS-inspired

programme for triggering latrine construction and use; and (iii) promoting hygiene, especially handwashing (237). WaterAid Australia is implementing the WASH programme in partnership with local, community-based NGOs. As the follow-up period is two years after programme implementation, this trial is ongoing. At baseline, 2 921 people from 24 villages were enrolled. Baseline data are used in all PhD analyses.

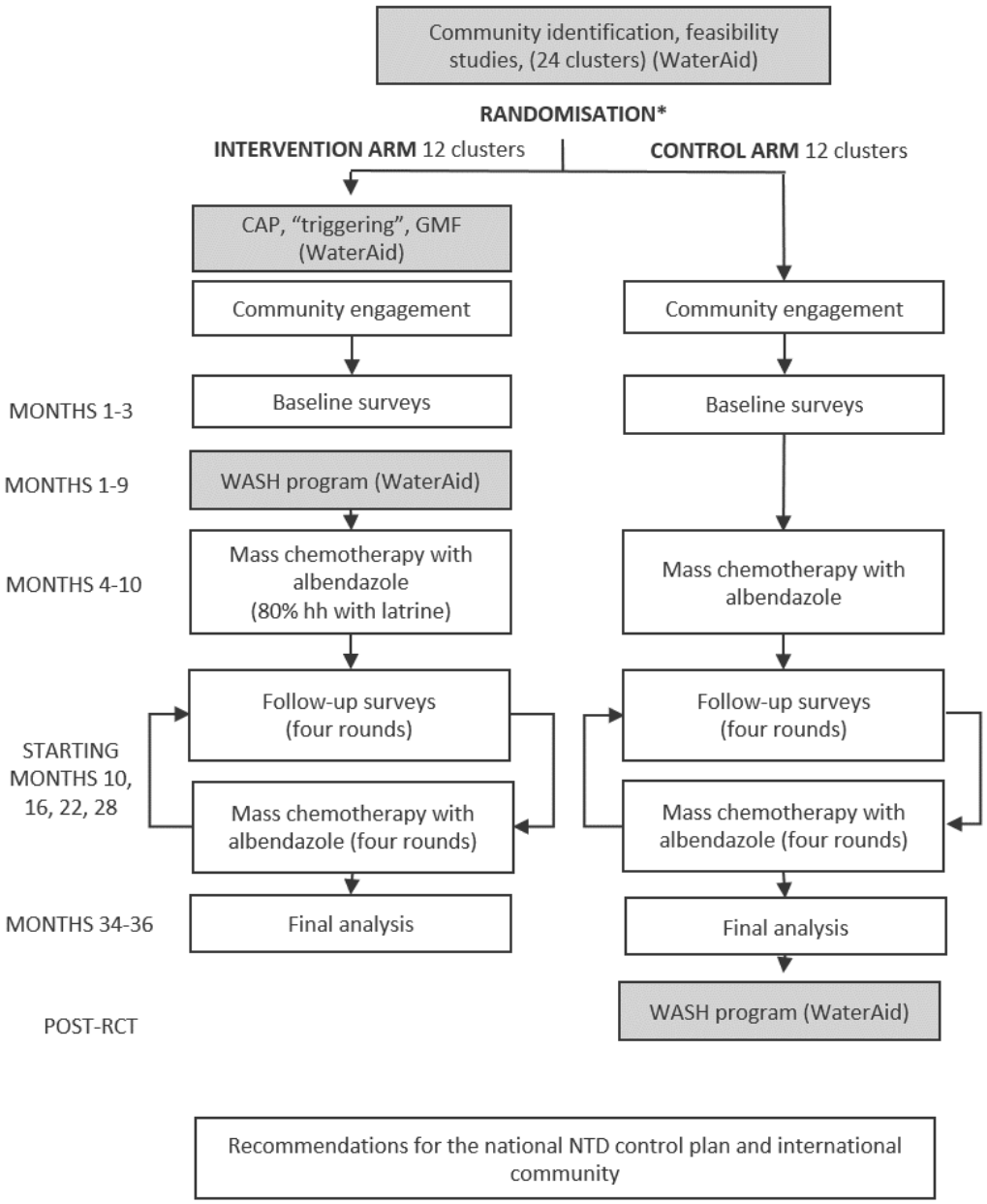

Figure 1.2: Flow diagram of the WASH for Worms cluster randomised controlled trial (237)

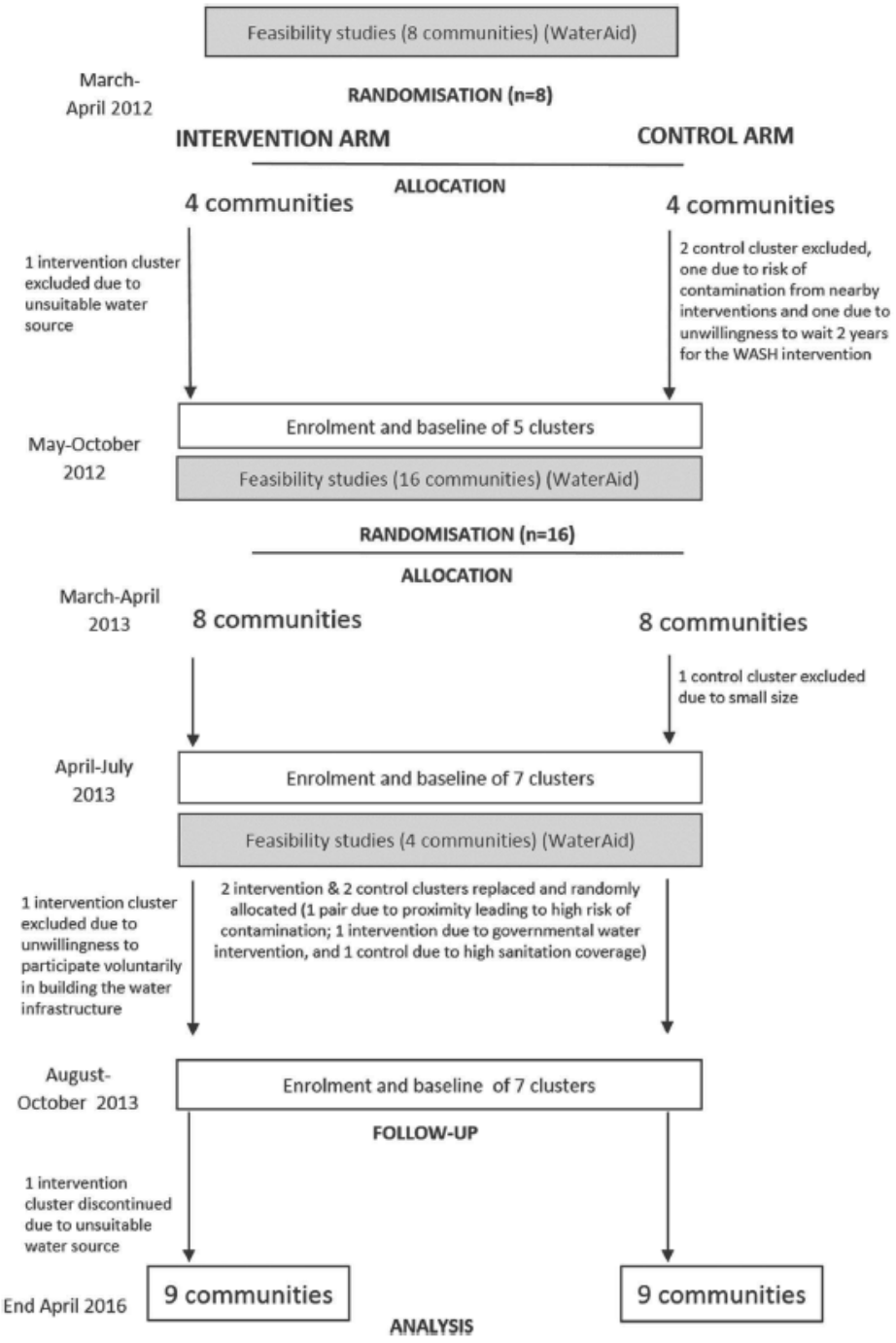

Figure 1.3: Flow diagram of the trial enrolment process, demonstrating randomisation, allocation, and cluster replacement

(237)

Real-time polymerase-chain reaction for STH diagnosis

Diagnosis of STH infections can be undertaken by a range of microscopy-based techniques which, although well accepted, may not be as sensitive as more recently available molecular techniques (240). Multiplex polymerase-chain reaction (PCR) may be more sensitive at parasite detection as well as more accurate at determining infection intensity than microscopy, along with superior ability to detect polyparasitism (240). In the WASH for Worms trial, previously-developed multiplex PCR assays (241) were modified by parasitologists to determine the prevalence and intensity of intestinal parasite infections (240). The qPCR assay has had previously reported 100% specificity and sensitivities of 100% and 99.5% for the detection of *N. americanus* and *A. duodenale*, in controlled experiments (using well-defined DNA and stool samples as controls, and faecal samples known to contain L3 larvae of at least one of the species after coproculture). The excretion and distribution of parasitic DNA in faeces is expected to be less variable than the number of eggs (241). With regards measurement error, there is the potential that use of qPCR to report on prevalence may mean that specific covariate relationships with heavy relationships get obscured by light infections, therefore making it less useful for investigating the most important risk factors for transmission. Kato Katz will pick up heavier intensity infections, and might better indicate the intensity of transmission. Alternatively, there may be less measurement error with qPCR and therefore associations may be more likely to be found. Comparisons were undertaken by parasitologists of the STH results from the PCR to STH results from a sodium nitrate flotation technique on stool specimens preserved in 10% formalin. The primary reason for the flotation technique being

chosen as the comparator, rather than Kato-Katz, is because the sensitivity of detection has been shown to be lower than a single faecal float using sodium nitrate (242). PCR-derived data were used in all PhD analyses. For all analysis chapters, *Ascaris* spp. is referred to instead of *Ascaris lumbricoides* because the PCR assay was not species-specific for *Ascaris*.

1.8 Significance of this PhD

This PhD presents original research to analyse STH epidemiology in a little-studied part of the world. Each of the quantitative analyses provide the first reported examples for Timor-Leste of community STH prevalence, risk factors, and associations with morbidity. As such, this thesis provides important information for developing STH control strategies in Timor-Leste, and a useful baseline for monitoring and evaluating control programmes once implemented. Research findings also contribute to international knowledge: few analyses have investigated WASH risk factors stratified by age and STH species separately. These analyses have additionally provided the first epidemiological investigation of STH infection intensity from PCR-diagnosed infection. This requires verification in different epidemiological settings. The thesis has provided the first investigation of adjusted environmental and WASH risk factors for intensity of STH infection in any community setting. Finally, the thesis provides two narrative reviews investigating the evidence and important research agendas for STH morbidity, and STH control strategies, globally.

1.9 The PhD aims and objectives

The research presented in this thesis aims to provide information on the baseline burden of STH in Manufahi District, Timor-Leste. This is a direct contribution to the RCT aims 1 and 2. Investigations of STH prevalence, intensity of infection, risk factors for infection, and associations with morbidity outcomes will be presented. The goal of this research is to contribute to the evidence base for WASH- and environmental-related risk factors on STH infections, contribute to the evidence base for STH prevalence and intensity in Timor-Leste, and provide essential quantitative information on the epidemiology of STH at baseline for the RCT, so as to have accurate information upon which effectiveness of interventions can be measured at trial completion.

In addition to quantitative epidemiological analyses, the PhD research aims to provide both an updated picture of known contributions to STH morbidity, and a critical evaluation of STH control strategies. This is because the PhD project has been undertaken during a period of rapidly-evolving global prioritisation of (i) clarifying STH contribution to morbidity; and (ii) advocacy of NTD control and elimination programmes encompassing STH.

To meet the stated aims, the PhD project has the following specific objectives:

1. To review and describe current global evidence of STH morbidity (Chapter 2);
2. To review and critically appraise strategies for STH control, in the context of broader NTD control and elimination priorities (Chapter 3);

3. To determine the baseline prevalence and risk factors for STH and protozoa in Manufahi District, Timor-Leste (Chapter 4);

4. To determine the baseline haemoglobin concentration of the study population and anthropometric measurements of children aged 1-18 years (height-for-age, weight-for-age, and height-for-weight z-scores) and the associations between STH and anaemia, stunting, wasting and being underweight (Chapter 5);

5. To determine the baseline intensity of STH infection, and WASH and environmental risk factors for intense infections in this community (Chapter 6); and

6. To make recommendations on implementing STH control programmes in Timor-Leste and on progressing the broader international STH research agenda (Chapter 7).

1.10 Research and thesis structure

This thesis consists of seven chapters; an introduction, and five chapters comprising six journal publications (either published, in press, or under review), and a general discussion and conclusions. The first three publications include two narrative reviews and a viewpoint. To undertake the narrative reviews, extensive literature retrieval and investigation was undertaken. Over 2000 articles were obtained and reviewed, with condensing of these as knowledge gaps were identified, key arguments formed and manuscripts drafted. These are followed by three drafted publications that present original epidemiological research. All of the chapters commence with an introduction of the context of the paper to explain its fit to the overall thesis structure, and additionally include an overview of statistical methods to

provide a rationale for the techniques used to address the research objectives. References for the introduction and discussion (Chapters 1 and 7) appear in the Bibliography of Works at the end of the thesis, but references for Chapters 2–6 (drafted publications) appear at the end of each chapter.

Chapter 1 describes the global significance of STH and gastrointestinal protozoa, and current strategies for control. It summarises known evidence of STH in Timor-Leste and an overview of the WASH for Worms RCT in which the PhD research is set. It also presents the thesis justification, aims and objectives.

Chapter 2 presents a literature review, structured as a journal paper, investigating the current evidence for STH on morbidity and mortality outcomes, and critically appraising a recent Cochrane systematic review, to investigate possible reasons for equivocal evidence of deworming on morbidity outcomes. This narrative review highlights areas where further research into STH morbidity is crucially required. 118 references from published international literature were reviewed, with prioritisation of publications from 2000 onwards. This manuscript was published in May 2016 in *PLoS Neglected Tropical Diseases*.

Chapter 3 presents a second literature review, presented as a publication, critically appraising the current context for STH control strategies. This review provides an overview of the evidence base for chemotherapy and WASH on STH outcomes, and investigates in detail the current focus on integration of drugs for NTDs. It concludes by describing

"multi-component" integration, whereby chemotherapy is augmented with other intervention strategies, for more sustainable STH control, and a description of the research required to provide evidence for this, and current global opportunities in which such research could be conducted. 84 references from published international literature were referenced in this review, with prioritisation of publications within the last five years. This manuscript was published online in January 2016 in *Trends in Parasitology*.

Chapter 3 additionally presents a viewpoint publication, which critically appraises the importance of WASH for STH and schistosomiasis control, and raises concerns about programmatic advice on STH and schistosomiasis control programme implementation guidelines that have been developed by the WHO. This publication was intentionally written as a discussion-provoking viewpoint to encourage development of national STH and schistosomiasis guidelines that comprehensively manage risks and guidance for helminth control programmes. This manuscript was published in *PLoS Neglected Tropical Diseases* in April 2014.

Chapter 4 uses baseline data from the RCT to present an epidemiological investigation of STH prevalence in Manufahi District, and the first detailed investigation of WASH risk factors for STH in Timor-Leste. This analysis uses contemporary epidemiological methods to investigate risk factors and construct a wealth score to allow adjustment for relative poverty within this community. This chapter is in press at the *International Journal for Parasitology*.

Chapter 5 uses baseline data from the RCT to present the first analysis of STH impact on morbidity in Manufahi District, Timor-Leste. This contributes to the overall knowledge of STH impact on morbidity, particularly through the use of PCR-derived DNA cycle threshold (Ct) values as the measure of intensity of STH infection. This has required, for the first time in epidemiological analyses, assignment of cut-points for Ct-values to categorise these into indicative measures of heavy and moderate-light intensity infections. Investigations of intensity of infection on mean haemoglobin concentrations (as an indicator of anaemia burden), child stunting, child wasting and child underweight are presented. This chapter is under review with *Parasites and Vectors*.

Chapter 6 utilises the cut-points for DNA Ct-values developed for Chapter 5, in an investigation of intensity of STH infection and associated risk factors in the trial population at baseline. This is the first investigation of intensity of STH infection in Timor-Leste, and of specific underpinning WASH and environmental risk factors. To my knowledge this is the first epidemiological analysis that adjusts for both WASH and environmental risk factors for STH in a community setting using intensity of infection as outcome; this provides important information on modifiable and non-modifiable STH infection risk. This chapter is under review with *PLoS Neglected Tropical Diseases*.

The discussion and conclusion (Chapter 7) summarises the key research findings, and highlights the main implications, limitations and future directions of the research. It provides recommendations for the implementation of STH control programmes in Timor-Leste and in other endemic countries.

In addition to the chapters are four appendices. The PhD project has involved working with the research team conducting the RCT since its inception. In addition to conducting epidemiological analyses of STH burden, the PhD Candidate has been heavily involved in the establishment, questionnaire development and field validation, data management and cleaning of trial data, prior to conducting epidemiological analyses. Appendix 1 covers these trial-based activities, and provides detail on data preparation for epidemiological analyses. Appendices 2-4 are the individual, household and village questionnaires that were developed.

Chapter 2 The burden of soil-transmitted helminths

2.1 Chapter context

Narrative reviews synthesise information from published literature to critically appraise a topic and highlight research priorities. They differ from systematic reviews as they do not involve a systematic selection of literature according to specified inclusion and exclusion criteria. Narrative reviews can instead focus on studies based on author selection. Whilst this can be seen as a methodological weakness due to possible selection bias, it can also be seen as a strength, as not applying selection criteria can allow the author to consider studies of different designs, that may have had diverging results or conclusions, that still inform the knowledge picture. Narrative reviews can thus allow an investigation into the total pool of knowledge on a subject, as opposed to a systematic review which is a powerful means of capturing the strength of evidence on a topic (for example by considering RCTs only).

The purpose of this chapter is to review shortfalls in recent evidence for STH morbidity, to identify where more research is needed to strengthen morbidity evidence. As such, it focuses on the pool of knowledge of both observational and experimental evidence of STH in humans. This chapter presents the most recent comprehensive review of STH morbidity, and the first review to investigate why systematic reviews have differed in their findings on evidence of morbidity from STH. This is highly topical; currently there is a controversial debate on replication analyses (243,244) of a policy-influencing deworming trial (110), and

a 2015 re-issue of a Cochrane systematic review on the impact of anthelmintic treatment on morbidity (105). The debate has centred around whether evidence demonstrates measurable benefit of anthelmintic treatments on morbidity from STH. One of the key points of the debate has been whether it is appropriate for systematic reviews to include experimental evidence only. There are important considerations to be taken into account for observational and experimental studies that report on STH-attributable morbidity, and, for context, the methodological flaws of these are highlighted in Table 2.1.

Table 2.1: Types of epidemiological study and their methodological weaknesses for assessing STH-attributable morbidity

Observational studies: **Cross-sectional, cohort, case-control**	**Experimental studies:** **Randomised controlled trials (RCTs)**	**All studies**
These study types do not permit distinction between cause and effect. They are investigator-observed, there is no intervention and therefore, for STH, no direct 'evidence' of deworming *per se*.	RCTs may be unethical or impossible to conduct in some settings e.g. withholding a treatment. Further, it is unethical to include people who are acutely unwell, but these may be those most likely to benefit from the intervention.	More agreement is needed on measurement tools to determine what constitutes an appreciable effect. E.g. an agreed definition on changes to height or weight in children (and length of time for this to be achieved and measured). This needs to be balanced by STH reinfection rates.
Observational studies may not adequately control for confounding. They cannot control for unmeasured variables, e.g. poverty, or nutrition, each of which could confound STH associations with morbidity.	Blinding in RCTs may not be possible for all morbidity investigations. This may be blinding of investigators, or of participants. Responses may be affected if they are aware of their allocation.	Participant bias may occur if people know they are being studied. Results may be affected by participant recall bias (or responder bias) for data collected via questionnaire or interview, which is the main way that data on risk factors or possible confounders is collected.
Cohort and cross-sectional studies will be limited if the primary outcome (e.g. a particular STH) is rare. Power may not be achieved.	Contamination between trial arms in field epidemiology settings needs to be carefully controlled and requires vigilance if people in control arm try to access the intervention (if intervention is not blinded). Further, other interventions may be introduced, affecting some participants e.g. an unrelated organisation establishment of an electricity grid in some communities.	Morbidity outcomes for STH are likely underpowered to detect effects if power calculations have been based on prevalence or intensity of infection, as opposed to the morbidity outcome itself.
Low response rates and loss to follow up can also affect power and results. This could affect internal and external validity. Significant differences between responders and non-responders may be missed.	Low response rates and loss to follow up can affect power and results. This could affect internal and external validity. Significant differences between responders and non-responders may be missed.	Diagnostics may be imperfect for accurate measurement of disease state. Data analysis methods also need to be carefully undertaken with consideration of the research question, primary and secondary outcomes, structured appropriately and reported in sufficient detail for independent verification.

Observational studies: **Cross-sectional, cohort, case-control**	**Experimental studies:** **Randomised controlled trials (RCTs)**	**All studies**
Cross-sectional study measurements can only be interpreted as being correct at the time of measurement if disease or morbidity states fluctuate.	The correct treatment groups for detecting the effect should be selected. E.g. treatment in school-aged children may miss the main child growth trajectory that could be affected by STH (e.g. for morbidity measures of stunting and wasting). Similarly, for deworming, the tablet type and brand need to be the same, as different efficacies have been reported.	Power may be affected, sometimes badly, in determining associations between explanatory variables and outcomes in situations where either present in low numbers. The same can occur in situations where there is a very large proportion of an explanatory variable, as the ubiquity of the factor makes differential associations hard to detect with any statistical significance.
Case-control studies can only assess one outcome variable (the one that cases entered the study with). They can be affected by matching problems. Often cases can't be random sampled. Sampling bias, observation and recall bias can be problematic.	Length to follow up the effect of the intervention needs to be carefully considered. E.g. long enough to detect effect, short enough to control for reinfection. There needs to be more consensus on this.	Missing data or poorly captured data will have a major bearing on analyses that can be done, and on results.
	RCTs should have baseline assessment done to determine whether there are systematic differences between interventions and controls. This may include STH heterogeneity across different sites (which will lead to differential treatment effects), and also morbidity heterogeneity (which is assumed to be the same in study arms, but needs to be specifically checked).	

This review therefore extensively analyses the evidence in published studies to investigate existing evidence gaps in morbidity from STH, and highlights where research is required to address them. Whilst this is a narrative review, a systematic search strategy has been applied to identify and select studies. The 2015 Cochrane systematic review and other systematic reviews have been analysed in detail. Challenges to the systematic review methodology have been made, which should further inform the pursuit of an evidence-based research agenda. Control of NTDs, including of STH, is currently a major international funding priority for the Bill and Melinda Gates Foundation, the United Kingdom Department for International Development (DFID), USAID, and other agencies. In view of this, the research and implementation communities are at a crucial stage for determining correct approaches to sustainable control and even elimination. This manuscript is intended to challenge the NTD community to direct research efforts in key evidence-strengthening areas as productive investment that could eventually lead to proven benefits of deworming.

In addition to inproved knowledge of current STH evidence gaps, this review provides background on the insidious impact of STH within the human host. It therefore becomes an important precursor to remaining chapters of the thesis. This review addresses objective 1 of the thesis. It was published in *PLoS Neglected Tropical Diseases* in May 2016. The article utilises 118 references from international published literature. Priority is given to recent primary evidence, focusing particularly on publications from 2000 onwards.

For this paper, the PhD Candidate was responsible for 90% of the conception, 100% of the literature collection, and 90% of the analysis, interpretation, drafting and writing of the paper. Clements A was responsible for 10% of the conception and analysis. Clements A, Nery S, Doi S, Gray D, Soares Magalhães R, McCarthy J, Traub R and Andrews R were collectively responsible for 10% of the interpretation and writing of the paper.

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REVIEW

Complexities and Perplexities: A Critical Appraisal of the Evidence for Soil-Transmitted Helminth Infection-Related Morbidity

Suzy J. Campbell[1]*, Susana V. Nery[1], Suhail A. Doi[1], Darren J. Gray[1,2], Ricardo J. Soares Magalhães[3,4], James S. McCarthy[5,6], Rebecca J. Traub[7], Ross M. Andrews[8], Archie C. A. Clements[1]

1 Research School of Population Health, College of Medicine, Biology, and Environment, The Australian National University, Canberra, Australian Captial Territory, Australia, **2** Molecular Parasitology Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia, **3** Children's Health and Environment Program, Queensland Children's Medical Research Institute, The University of Queensland, Brisbane, Queensland, Australia, **4** School of Veterinary Science, The University of Queensland, Gatton, Queensland, Australia, **5** School of Population Health, University of Queensland, Brisbane, Queensland, Australia, **6** Clinical Tropical Medicine Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia, **7** Faculty of Veterinary and Agricultural Science, The University of Melbourne, Melbourne, Victoria, Australia, **8** Menzies School of Health Research, Charles Darwin University, Darwin, Northern Territory, Australia

* suzanne.campbell@lstmed.ac.uk

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Abstract

Background: Soil-transmitted helminths (STH) have acute and chronic manifestations, and can result in lifetime morbidity. Disease burden is difficult to quantify, yet quantitative evidence is required to justify large-scale deworming programmes. A recent Cochrane systematic review, which influences Global Burden of Disease (GBD) estimates for STH, has again called into question the evidence for deworming benefit on morbidity due to STH. In this narrative review, we investigate in detail what the shortfalls in evidence are. Methodology/Principal Findings: We systematically reviewed recent literature that used direct measures to investigate morbidity from STH and we critically appraised systematic reviews, particularly the most recent Cochrane systematic review investigating deworming impact on morbidity. We included six systematic reviews and meta-analyses, 36 literature reviews, 44 experimental or observational studies, and five case series. We highlight where evidence is insufficient and where research needs to be directed to strengthen morbidity evidence, ideally to prove benefits of deworming. Conclusions/Significance: Overall, the Cochrane systematic review and recent studies indicate major shortfalls in evidence for direct morbidity. However, it is questionable whether the systematic review methodology should be applied to STH due to heterogeneity of the prevalence of different species in each setting. Urgent investment in studies powered to detect direct morbidity effects due to STH is required.

Introduction

Soil-transmitted helminth (STH) infections are among the most prevalent of the neglected tropical diseases (NTDs), characterised by chronic and subtle impacts on human health and development. They rarely cause direct mortality; instead they are major contributors to morbidity. Morbidity effects of STH infection are difficult to quantify given the long duration of infection, often over many years, presence of other concurrent diseases, and factors such as poverty and malnutrition, to which they are strongly linked, and which can confound measures of STH-associated morbidity. Accurate quantification of STH-associated morbidity and disease burden is critical to rationalise large-scale deworming programmes.

Debate exists around the health benefits and cost-effectiveness of STH intervention strategies, rising to prominence with Cochrane systematic reviews reporting equivocal evidence of health benefits [1–4] and a statistical re-analysis of a major deworming trial from 1998–1999 that found differing results to original author conclusions [5,6]. In response, concerns have been raised about methodological bias in systematic reviews [7], the importance of not confining anthelmintic treatment to infected children [7,8], the economic importance of deworming [9], and the conclusions drawn in the replication analyses [9,10]. The Cochrane systematic review, the replication analyses, and the resultant discussions are positive insofar as they will further progress an evidence-enhancing research agenda, but there is a strong underlying imperative not to adversely influence international policy for mass deworming.

Global burden of disease (GBD) studies quantifying STH burden, with the most recent estimates published in 2012 [11], have also been subject to debate, in part due to the exclusion of certain morbidities because available evidence is deemed insufficient to justify inclusion. The findings from Cochrane systematic reviews influence what morbidities are included in GBD estimates, which, also, can be used to influence policy; therefore, it is important to understand what the underlying shortfalls are.

It is timely to revisit the pool of knowledge for STH impact on health, with a particular focus on how recent evidence contributes to knowledge gaps, and to critically appraise the approaches being used in systematic reviews, particularly the Cochrane systematic review, to disentangle why results are consistently inconclusive.

In this narrative review, we critically appraise narrative and systematic reviews and consider the recent observational and experimental literature, covering both STH morbidity and treatment associations with health outcomes. We explore the reasons for the reported lack of effect in the most recent Cochrane systematic review and investigate why conclusions regarding STH impact on haemoglobin do not concur with other systematic reviews [12,13]. This paper is not a meta-analytic paper (which would replicate previous reviews). By considering both well-known and more recent evidence, we provide an updated perspective of where evidence is insufficient to enable conclusions on STH morbidity to be drawn and highlight where research needs to be directed in future.

Methods

We searched scientific literature in the MEDLINE database (January 2000 to January 2016) for evidence of any morbidity or mortality outcomes associated with *Ascaris lumbricoides*, *Trichuris trichiura*, and the hookworms *Necator americanus*, *Ancylostoma duodenale*, and *Ancylostoma ceylanicum*. The nematode *Strongyloides stercoralis* was not included in this review. Specifically, the following combination of text and Medical Subject Headings (MeSH) terms was used: ("*Necator americanus*" or "*Ancylostoma duodenale*" or "*Ascaris lumbricoides*" or "*Trichuris trichiura*" or "*Ancylostoma ceylanicum*" or hookworm or "soil-transmitted helminth") and (morbidity or mortality or anaemia or stunting or retardation or wasting or

malnutrition or cognitive or cognition or impair). The search was limited by English language. This search aimed to (1) identify narrative and systematic reviews of STH morbidity and (2) identify recent research papers on STH-associated morbidity. Article abstracts were reviewed and literature was retrieved if there was specific reference to a morbidity or mortality outcome from STH or if they could not be excluded (i.e., if the abstract did not clearly indicate morbidity outcomes). Reference lists of identified articles were cross-checked. We further cross-checked peer-reviewed literature with information from United Nations, World Health Organization (WHO), and several other non-government organisation websites.

Once identified, narrative and systematic reviews were analysed to determine current knowledge and evidence gaps (Tables 1 and 2). Critical appraisal checklists were used to analyse systematic reviews [14]. For the systematic reviews, we focussed on the research question being investigated (null hypothesis), search and selection criteria, trials selected, inclusion or exclusion of factors such as concurrent diseases or interventions, definitions given by authors to "quasi" randomised controlled trials (RCTs), evidence rankings from authors, definition of trial participants, baseline measures, classes of infection intensity, intervention and outcome measures, consideration of absolute versus relative outcome measures, length of follow-up, pooling of results and sub-group analyses undertaken, and the conclusions drawn within manuscripts. We did not analyse bias or adjusting of intra-class correlations in cluster RCTs, but we did check that the systematic reviews had investigated these. We did not investigate the specific meta-regression methods of the models. Given the requirement for regular updates of Cochrane systematic reviews, we only included the most recent of these. We report our findings in relation to the most recent Cochrane systematic review.

We further investigated recent evidence to determine whether knowledge gaps identified in earlier reviews were being addressed. Informed by the evidence summaries (Tables 1 and 2), we repeated the literature search (Fig 1, Table 3), applying the following criteria:

(i). If studies related to hookworm impact on haemoglobin or anaemia, we included only experimental evidence since the 2010 systematic review was done;

(ii). If studies related to impact on haemoglobin or anaemia of other STH, or any STH impact on physical (e.g., stunting or wasting) or cognitive measures of morbidity, we included studies from 2006 (the date of the last major STH review) and considered all observational and experimental evidence, with the exception of case series and case reports; and

(iii). In the absence of any identified epidemiological investigations into *A*. *lumbricoides* or *T*. *trichiura* acute complications, we included five case series, but not individual case reports.

We looked at coinfections among STH species. For STH and non-STH parasite interactions, STH and vaccine interactions, and associations with allergies, atopy, and asthma, we note that effects on morbidity are likely to be synergistic and that research is under way to investigate these effects. However, discussion of these is beyond the scope of the current review.

After applying the above search criteria, studies were further excluded from Table 3 if they had been analysed in one of the systematic reviews, if they did not report results for STH species separate from other parasites, or results on haemoglobin, anaemia, or a physical or cognitive measure (with the exception of the case series for *A*. *lumbricoides* and *T*. *trichiura*). For this reason, studies that reported prevalence or intensity of infection only (indirect morbidity measures), or changes in these following interventions, were not reported. Furthermore, this means that for some studies, not all study outcomes were included in our evidence tables. Critical appraisal checklists were again followed to assess these studies [14].

Table 1. Summary of existing evidence from narrative reviews of soil-transmitted helminth morbidity published since 2000.

Topic area: STH entry and establishment*	
Citation	**Reported evidence**
O'Lorcain and Holland, 2000 [15]; Crompton, 2001 [16]; Bethony et al., 2006 [17]; Albonico et al., 2008 [18]; Brooker, 2010 [19]; Periago and Bethony, 2012 [20]; Craig and Scott, 2014 [21]; Ojha et al., 2014 [22]	Direct oral infection of *A. duodenale* can cause Wakana disease, characterized by nausea, fever, vomiting, pharyngeal irritation, cough, shortness of breath, and hoarseness. Penetration of hookworm larvae into the skin causes intense itching and often a cutaneous rash. *A. lumbricoides* and hookworms migrate through the vascular system and respiratory tract before being coughed from the lungs and swallowed, with accompanying clinical symptoms including (for hookworms) mild cough resembling Löffler's syndrome, sore throat and fever, and (for *A. lumbricoides*) *Ascaris* pneumonia and hypersensitivity, occasionally manifesting as asthma. Larval migration to the gastrointestinal tract causes symptoms including rising peripheral eosinophilia, moderate to severe epigastric pain, intense nausea, exertional shortness of breath, pain in the lower extremities, palpitations, abdominal tenderness, joint and sternal pain, headache, fatigue, impotence, flatulence, weight loss, and/or moderately severe diarrhoea. Infections cause clearly discernible intestinal damage. Deworming children has been shown to clearly reduce intensity of helminth burden and associated symptoms.
Topic area: Blood loss and anaemia	
Citation	**Reported evidence**
Crompton, 2000 [23]; Guyatt, 2000 [24]; O'Lorcain and Holland, 2000 [15]; Drake and Bundy, 2001 [25]; Stephenson, 2001 [26]; WHO, 2002 [27]; De Silva, 2003 [28]; Brooker et al., 2004 [29]; Hotez et al., 2004 [30]; Savioli et al., 2004 [31]; Bethony et al., 2006 [17]; Hotez et al., 2006 [32]; Larocque and Gyorkos, 2006 [33]; Albonico et al., 2008 [18]; Brooker, 2010 [19]; Elliott et al., 2011 [34]; Tchuem Tchuenté, 2011 [35]; Hall et al., 2012 [36]; Periago and Bethony, 2012 [20]; Ojha et al., 2014 [22]	Hookworms cause intestinal blood loss leading to iron deficiency (including iron deficiency anaemia [IDA]) and protein malnutrition. Effects worsen with increased intensity of infection. Anaemia, in turn, is strongly linked to poor underlying iron status, malaria, poverty, and other factors. *T. trichiura* is associated with blood loss, chronic inflammation, iron deficiency, and protein loss in children. Blood loss from *T. trichiura* infection is likely to contribute to anaemia, particularly if hookworms are also harboured or the individual has low dietary iron intake. No evidence that *A. lumbricoides* infection leads to iron malabsorption and IDA in children. IDA during pregnancy is linked to severe maternal anaemia. Both IDA and severe anaemia in pregnancy contribute to poor maternal health, maternal mortality, premature delivery, low infant birthweight, and impaired lactation. *A. duodenale* infection during pregnancy may be lactogenically transmitted to neonates. Deworming children clearly shows improvements in iron status and reduced chance of developing IDA and severe anaemia. Deworming will reduce helminth infections, but nutritional interventions are required to restore health. Some (but not all) studies show that deworming pregnant women, with or without iron folate supplementation, can improve maternal haemoglobin (Hb) levels, reduce severe anaemia and stillbirths, and improve infant birthweight and survival. Albendazole treatment during pregnancy may increase risk of eczema in the population.
Topic area: Physical development, fitness, worker productivity	
Citation	**Reported evidence**
Crompton, 2000 [23]; Guyatt, 2000 [24]; O'Lorcain and Holland, 2000 [15]; Stephenson et al., 2000 [37]; Stephenson, 2001 [26]; WHO, 2002 [27]; De Silva, 2003 [28], Brooker et al., 2004 [29]; Hotez et al., 2004 [30]; Savioli et al., 2004 [31]; Bethony et al., 2006 [17]; Hotez et al., 2006 [32]; Hall, 2007 [38]; Albonico et al., 2008 [18]; Brooker, 2010 [19]; Tchuem Tchuenté, 2011 [35]	Chronic hookworm, *A. lumbricoides*, and *T. trichiura* infections associated with reduced weight, height, skinfold thickness/arm circumference, and appetite in children, with effects more pronounced in heavy infections. Even light infections are considered likely to contribute to growth deficits if underlying nutrition is poor. Severe stunting can occur in *Trichuris* Dysentery Syndrome (TDS). *A. lumbricoides* associated with lower vitamin A absorption and lactose intolerance in children. Other physical effects of hookworm include dermatitis, anasarca, oedema of face and limbs, potbelly, waxy skin, and worm passing. Most of these effects may be attributable to iron deficiency. Many (but not all) RCTs found improvements in height, weight, arm circumference, appetite, and physical activity (step tests) after deworming schoolchildren and younger children. STH is associated with poor economic productivity, but direct evidence is lacking. Difficult to quantify, as iron deficiency and IDA are characterized by weakness and fatigue in adults and some studies have linked IDA (measured Hb level) to productivity. An economic analysis estimated that curing hookworm infection in United States of America led to 25% increased likelihood of children attending school than untreated children, translating into 45% higher earnings by 1940.

(*Continued*)

Table 1. (*Continued*)

Topic area: Cognitive development	
Citation	**Reported evidence**
Crompton, 2000 [23]; Guyatt, 2000 [24]; O'Lorcain and Holland, 2000 [15]; Stephenson et al., 2000 [37]; Crompton, 2001 [16]; Drake and Bundy, 2001 [25]; Stephenson, 2001 [26]; WHO, 2002 [27]; De Silva, 2003 [28]; Brooker et al., 2004 [29]; Hotez et al., 2004 [30]; Savioli et al., 2004 [31]; Bethony et al., 2006 [17]; Hall, 2007 [38]; Albonico et al., 2008 [18]; Brooker, 2010 [19]; Tchuem Tchuenté, 2011 [35]	Strong hookworm associations with intellectual growth retardation, cognitive performance in school, reduced school attendance, and educational deficits; however, evidence is not yet considered causal. Most of these effects may be attributable to iron deficiency. There are agreed strong associations between iron deficiency and cognitive performance. TDS also associated with a marked decrease in cognitive score tests. Regular deworming of schoolchildren has shown improvements in some measures of cognitive performance, educational achievement, and school attendance.
Topic area: Multiparasitism and other disease interactions**	
Citation	**Reported evidence**
Drake and Bundy, 2001 [25]; Brooker et al., 2004 [29]; Bethony et al., 2006 [17]; Borkow and Bentwich, 2006 [39]; Mwangi et al., 2006 [40]; Albonico et al., 2008 [18]; Geiger, 2008 [41]; Hotez et al., 2008 [42]; Pullan and Brooker, 2008 [43]; Steinmann et al., 2010 [44]; Elliott et al., 2011 [34]; Gerns et al., 2012 [45]; Webb et al., 2012 [46]	Helminth species, including *Schistosoma mansoni*, are often comorbid in humans. Individuals harbouring multiple helminth species often harbour higher-intensity infections from each species than those with single-species infections; this may lead to additive impacts on morbidity as infection intensity is closely linked to severity of disease. STH are often co-endemic with malaria and Human Immunodeficiency Virus (HIV) and may increase host susceptibility to these and possibly other infectious diseases such as tuberculosis. STH and malaria in particular are known aetiological contributors to anaemia; the extent to which they may interact and potentially worsen anaemia is unknown. *A. lumbricoides* may reduce post-vaccination immune responses to tetanus vaccine and STH infection has been suspected to lower the efficacy of Bacille Calmette—Guerin (BCG) vaccine. Some studies have found no effect of anthelmintics on response to immunisation.
Topic area: Additional *A. lumbricoides* morbidity	
Citation	**Reported evidence**
O'Lorcain and Holland, 2000 [15]; Crompton, 2001 [16]; Savioli et al., 2004 [31]; Albonico et al., 2008 [18]; Brooker, 2010 [19]; Hesse et al., 2012 [47]; Ojha et al., 2014 [22]	Intestinal obstruction due to *A. lumbricoides* is regularly encountered in children from developing countries. Children tend to harbour more *A. lumbricoides* than adults. Complications of the biliary system occur in adults. Both of these conditions can be fatal. Numerous ectopic sites for *A. lumbricoides* migration have been encountered, often with risks, e.g., airway obstruction. Mortality rates from ascariasis are difficult to estimate. Deworming has been clearly shown to reduce the frequency of complications. However, intestinal blockage arising from anthelmintic purging of *A. lumbricoides* has been reported.
Topic area: Additional *T. trichiura* morbidity	
Citation	**Reported evidence**
Stephenson et al., 2000 [48]; Drake and Bundy, 2001 [25]; Savioli et al., 2004 [31]; Albonico et al., 2008 [18]; Brooker, 2010 [19]; Wang et al., 2013 [49]; Ojha et al., 2014 [22]	TDS associated with heavy *T. trichiura* infection mainly occurs in children and leads to severe growth stunting and cognitive deficits that might not be reversible. Other TDS symptoms include chronic dysentery, rectal prolapse, anaemia, and clubbing of fingers. TDS can be fatal. Lighter *T. trichiura* infections are also problematic, especially in children.
Topic area: *A. ceylanicum*	
Citation	**Reported evidence**
Traub, 2013 [50]	*A. ceylanicum* may have important morbidity, but sparse evidence is available. In experimental infections, *A. ceylanicum* can cause "ground itch" and moderate to severe abdominal pain, diarrhoea, and occult blood in the faeces accompanied by peripheral eosinophilia. Patent *A. ceylanicum* can produce chronic infections that may occur in high enough burdens to produce anaemia.

* This evidence is grouped by topic area as many narrative reviews referred to the same primary evidence.

** Not being considered further in this review.

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Table 2. Evidence from systematic reviews of soil-transmitted helminth morbidity published since 2000 (selected according to specified criteria, arranged by date of most to least recent).

Citation	Included studies and focus areas	Reported results	Comments
Taylor-Robinson et al., 2015 [4] Previous versions: Dickson et al., 2000 [1]; Taylor-Robinson et al., 2007 [2], 2012 [3].	RCTs and quasi-RCTs, comparing deworming in any of the four STH species with placebo/no treatment in children aged 16 years or less, reporting on weight, haemoglobin (Hb), intellectual development, school attendance, school performance, and mortality.	Treating infected children with a single dose of deworming drugs may increase weight gain over 1–6 months. There is insufficient evidence to know whether treatment of known infected children has effects on Hb, school attendance, cognitive functioning, or physical well-being. Treating all children living in endemic areas with a single dose of deworming drugs probably has little or no effect on average weight gain, average Hb, or average cognition. Regularly treating all children in endemic areas with deworming drugs, given every 3–6 months, may have little or no effect on average weight gain, average height, average Hb, formal tests of cognition, exam performance, or mortality. Very limited evidence to assess an effect on school attendance. Insufficient evidence to do subgroup analysis by age.	See main text for detailed comments. Study selection criteria included all STH species and all anthelmintics included in the WHO Model List of Essential Medicines. They only included studies with other interventions (e.g., micronutrients) in which these interventions were given to the intervention and control arms. They only included studies of children. They considered 37 (of 45 total) trials for which no baseline screening of STH prevalence was done, although studies were from endemic areas. Observational evidence not included on the basis of reducing residual confounding. Some of the conclusions differ from those of Smith and Brooker, 2010 and Gulani et al., 2007.
Yap et al., 2014 [51]	RCTs and prospective cohort studies assessing nutrition influence, with and without anthelminthic drugs, on STH infection and reinfection.	Positive effects of nutritional supplementation or the host's natural nutritional status on (re-)infection with STH were reported in 14 studies, while negative effects were documented in six studies. Multi-micronutrients did not significantly impact on STH re-infection rates. Current evidence for effect of nutrition on (re-) infection with STH is weak and of low quality.	Studies were investigated by different STH.
Smith and Brooker, 2010 [12]	Observational and experimental evidence investigating hookworm impact on anaemia (non-pregnant populations). Compared Hb concentration between uninfected and hookworm-infected individuals (of different intensities). Meta-analysis of RCTs to investigate deworming impact on Hb and anaemia.	Observational studies: moderate- and heavy-intensity hookworm infections associated with lower Hb in school-aged children. All infection intensities associated with lower Hb in adults. Intervention studies: albendazole corresponded to increased mean Hb; mebendazole had no impact. Greatest mean Hb increase when albendazole co-administered with praziquantel. No measured benefit of benzimidazoles with iron supplementation. For anaemia, benzimidazoles alone had small impact on moderate anaemia. Larger impact on anaemia from benzimidazoles with praziquantel.	Study selection criteria included hookworms only, benzimidazole treatments (alone, and with praziquantel or iron supplementation) from 1980 onwards, and required baseline hookworm assessment for inclusion. Considered effects of different treatment types: albendazole alone, mebendazole alone, albendazole plus praziquantel, albendazole plus iron supplementation. Could not differentiate by hookworm species. Some of the conclusions differ from those of Taylor-Robinson et al., 2015.
Haider et al., 2009 [52]	Three RCTs investigating effect of administration of anthelmintics during the second or third trimester on maternal and child health outcomes.	Single dose of anthelmintic in second trimester of pregnancy had no associated impact on maternal anaemia in the third trimester. Single dose of anthelmintic plus iron supplementation in the second and third trimester of pregnancy had no associated impact on maternal anaemia in the third trimester compared to iron supplementation alone. No impact was found for low birthweight, perinatal mortality, or preterm birth. Impact on infant survival at six months of age not evaluated. Current evidence insufficient to recommend use of anthelmintic for pregnant women after the first trimester of pregnancy.	Assessed the same two RCTs as Brooker et al., 2008, with inclusion of a third RCT. Whilst Haider et al., 2009 undertook meta-analysis, they have the same conclusions as Brooker et al., 2008; that evidence is currently insufficient. More RCTs of pregnant women should be undertaken to strengthen evidence.

(*Continued*)

Table 2. (*Continued*)

Citation	Included studies and focus areas	Reported results	Comments
Brooker et al., 2008 [53]	Observational and experimental evidence investigating hookworm impact on Hb concentration in pregnant women. Compared Hb concentration between uninfected and lightly-infected women, and between lightly-infected and heavily-infected women.	Observational studies: increasing hookworm infection intensity was statistically associated with lower Hb levels in pregnant women in poor countries. Intervention studies: two RCTs identified; other evidence also included. Could not quantify benefit of anthelmintics in RCTs due to different outcome measures. RCTs showed deworming benefit on maternal or child health. Concluded that there are insufficient data to quantify the benefits of deworming.	More RCTs of pregnant women should be undertaken to strengthen evidence.
Gulani et al., 2007 [13]	RCTs and quasi-RCTs assessing routine deworming impact on Hb, in any population.	Ten of the studies used albendazole as the anthelmintic drug, three used mebendazole, and one used bephenium. Routine administration of anthelmintics results in a marginal increase in Hb.	This review did not distinguish between different STH species or account for intensity of infection, which may have underestimated true treatment effect [12,54]. Some of the conclusions differ from those of Taylor-Robinson et al., 2015.

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Findings from Observational and Experimental Evidence

Tables 1 and 2 provide a summary of evidence for STH morbidity derived from previous narrative and systematic reviews. These have been used to determine current knowledge and evidence gaps. Table 3 summarises the information for recent observational and experimental evidence, including 44 studies by key topic area. Whilst some general statistical and epidemiological comments have been provided on study design, processes, analyses, and limitations, we have not applied formal scoring criteria to these studies.

STH entry and establishment within the human host

No recent epidemiological studies addressing STH entry and establishment in the human host were identified. Sequelae (Table 1) associated with STH entry into the human host tend to be regarded as transient events, often reported as features of the STH life cycle rather than in terms of quantifiable morbidity on the host. Narrative reviews have reported a broad range of symptoms following larval migration to, and establishment within, the gastrointestinal tract (Table 1). The only epidemiological studies likely to be ethically acceptable to quantify these symptoms would be cohort studies being conducted on people moving to endemic areas for

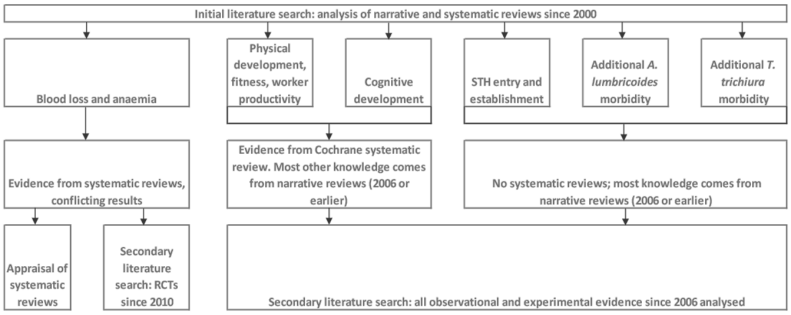

Fig 1. Literature selection flow diagram.

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Table 3. Observational and experimental contributions to soil-transmitted helminth morbidity; evidence since 2006.

Citation and country	Sample size and population	Study design and description	Results	Comments
Haemoglobin and anaemia*				
Gyorkos et al., 2011, 2012 [55,56], Peru	935 pregnant women (474 intervention, 461 control)	Double-blind RCT; mebendazole plus iron supplements (intervention), placebo plus iron supplements (control). Assessed impact of mebendazole on maternal STH intensity and prevalence. Recruited second trimester; followed up at delivery. Randomisation reported in [57]: computer-generated allocation. STH diagnosed by Kato-Katz.	Recruited 1,042 women; 935 (89.7%) had complete information for analyses. Baseline (second trimester) prevalence of hookworm 46.3%, *A. lumbricoides* 63.9% and *T. trichiura* 82.0%. Women in highest two *T. trichiura* intensity quintiles had significantly lower mean Hb concentrations than lowest quintile. Higher baseline intensities of hookworm and *T. trichiura* infections associated with higher risk of anaemia in third trimester. Overall, women with moderate/heavy *T. trichiura* infection were at higher risk of anaemia; highest risk among those with moderate/heavy hookworm co-infection. Mebendazole significantly reduced prevalence and intensity of all STH infections, but not anaemia risk.	Data originated from an RCT investigating the effect of mebendazole on birth weight [33]; these data assess different trial outcomes.
Ndyomugyenyi et al., 2008 [58], Uganda	832 pregnant women with confirmed STH infection at baseline (198 ivermectin, 194 albendazole, 199 ivermectin plus albendazole, 241 uninfected women [reference])	Open-label RCT; investigating efficacy and recorded adverse events among STH-infected women of ivermectin only, albendazole only, and ivermectin plus albendazole against STH infections on maternal health and neonatal outcomes. Reference group of uninfected women (non-randomised). STH diagnosed by Kato-Katz. Recruited second trimester; followed up at delivery.	832 women provided baseline stool; 636 (76.4%) followed up. Baseline STH prevalence 71% (hookworm 66.6%, *T. trichiura* 4.6%, *A. lumbricoides* <0.1%). Other parasite prevalence: 35.2% *Plasmodium falciparum*; 3.7% *Schistosoma mansoni*. Maternal anaemia prevalence reduced in all groups with no significant between-group difference at 36 weeks. Significant difference in mean Hb levels between first antenatal care visit and at 36 weeks of gestation for most (but not all) treatment groups and also reference group. Significant difference in mean Hb levels at 36 weeks between some (but not all) treatment groups and reference group. No significant difference in infant anaemia between treatment and reference groups. Mean infant Hb at birth not significantly different among groups, although significant difference was noted between one treatment group and reference group. No significant difference for mean birth weight, frequency of low birth weight, premature birth, stillbirth, or neonatal mortality.	Infection status determined pre-randomisation, but randomisation method not detailed. Women with severe anaemia excluded, which could have resulted in lack of difference in anaemia status in trial arms at 36 weeks. Study not powered to detect rare effects (adverse pregnancy outcomes) [58].
Quihui-Cota et al., 2010 [59], Mexico	73 School-aged children (SAC) aged 6–10 years, of confirmed *T. trichiura* infection or no parasite infection (excluded children with other parasite infections or co-infections)	Cross-sectional (longitudinal follow-up of 5 weeks); investigated association between trichuriasis and iron status	*T. trichiura* prevalence 45.2%. Height for age significantly higher in the *T. trichiura*-uninfected than *T. trichiura*-infected children. Significantly higher baseline concentrations of Hb, haematocrit, blood cell count (RBC), and serum iron in *T. trichiura*-uninfected compared with *T. trichiura*-infected children. Trichuriasis and Hb associated in adjusted analyses. Hb, RBC, and serum ferritin concentrations significantly increased in infected children 5 weeks after treatment.	Not large numbers. Regression analysis adjusted for sex and age.

(*Continued*)

Table 3. (*Continued*)

Citation and country	Sample size and population	Study design and description	Results	Comments
Physical development, fitness, worker productivity—Preschool-aged children (PSAC)				
Awasthi et al., 2008 [60], India	3,935 PSAC (aged 1–5 at baseline) (1852 intervention, 1860 controls for analyses)	Open-label cluster RCT of 50 urban slums; 25 received albendazole plus usual care (intervention), 25 received usual care only (control). Computer-generated random allocation. Investigated impact of five rounds of anthelmintic treatment administered every 6 months over 2 years on PSAC height and weight.	Baseline recruitment 3,935 children; 3,712 (94.3%) followed up. Community-based programme; no baseline STH assessment done. At final follow up, albendazole-treated arm had similar height gain but a 35% greater weight gain, equivalent to an extra 1 kg over 2 years.	2-year follow-up carefully considered in power calculations for ability to detect treatment effect in a generally malnourished population. No measure of egg counts from STH (but treatment effects are attributable to deworming intervention in this trial design).
Joseph et al., 2014, 2015 [61,62], Peru	1,760 PSAC (12 and 13 month olds) in a deworming trial (NB: baseline analyses are for subgroup of 880 children with stool analysed by Kato Katz)	Double-blind RCT, investigating deworming impact on growth in one-year-old children. Children enrolled at 12 month clinic visit, followed up at 18 and 24 month visits. Computer-generated random allocation to: (i) deworming at 12 months and placebo at 18 months; (ii) placebo at 12 months and deworming at 18 months; (iii) deworming at both 12 and 18 months; (iv) placebo at both 12 and 18 months (control group). Cross-sectional baseline survey (nested) conducted to assess association between malnutrition (i.e., stunting and underweight) and STH infection. Primary RCT outcome: weight gain at 24 month visit.	Baseline recruitment 1,760 children; follow-up of 1,563 children (88.8%). STH prevalence 14.5% (*A. lumbricoides* 11.5%, *T. trichiura* 4.5%, hookworm 0.6%). Risk factors for stunting included infection with at least one STH species and a lower development score. No statistically significant association found between any STH infection and being underweight. At RCT follow-up, there was no statistically significant difference in weight gain in any of the deworming intervention groups compared to the control group.	Baseline survey reported in [62]. Baseline analyses adjusted for sex, age, birth weight, any hospitalizations since birth, development score, and socioeconomic status. Models run as any versus no STH infection, not by species.
LaBeaud et al., 2014 [63], Kenya	545 PSAC aged 0–3 years	Prospective maternal-child cohort followed up every 6 months for 3 years. Assessed the prevalence of parasitic infections and their association with growth in very early childhood.	STH prevalence 19% (undifferentiated) by three years of age; point prevalence of hookworm and *A. lumbricoides* not known. Malaria 12%, filariasis 4.8%, schistosomiasis 2.9% by age three years. Hookworm infection by 24 months associated with below average growth in length and head circumference at 24 months and relative deficit in overall weight gain over first 36 months of life. Hookworm infection by 30 months associated with a decreased z-score for head circumference at 30 months, infection by 36 months of age associated with a relative decrease in weight gained at 36 months. *A. lumbricoides* infection by either 12 or 18 months associated with a decrease in weight z-scores at each age milestone, respectively. *A. lumbricoides* infection by 24 months associated with decreased length achieved at 24 months. For 180 infants followed up at every visit through 24 months, hookworm infection by 24 months associated with decrease in weight and head circumference z-score at 24 months. *A. lumbricoides* infection by 24 months associated with decrease in linear growth (length z-score) at 24 months.	Analyses adjusted infant's sex, birth weight, birth length, birth head circumference, and maternal education.

(*Continued*)

Table 3. (*Continued*)

Citation and country	Sample size and population	Study design and description	Results	Comments
Suchdev et al., 2013 [64], Kenya	205 PSAC, mean age 3.2 ± 0.08; 487 SAC, mean age 11.4 ± 0.13	Cross-sectional; to evaluate nutritional impact of STH infection	STH prevalence 39.7% (*A. lumbricoides* 23.0%, *T. trichiura* 26.3%, hookworm <0.1%). Any STH infection, and *A. lumbricoides* infection significantly associated with vitamin A deficiency (VAD). Children with moderate-intensity *A. lumbricoides* infection more likely to have VAD than children without *A. lumbricoides* infection. Associations remained when models adjusted for reported history of vitamin A supplementation. High-intensity *A. lumbricoides* infection positively associated with anaemia. No nutritional associations significant in SAC.	Analyses adjusted for age, sex, and socioeconomic status
Casapía et al 2007 [65], Peru	252 PSAC aged <5 years	Cross-sectional; to determine prevalence of malnutrition and its risk factors	STH prevalence: *T. trichiura* 38.9%, *A. lumbricoides* 32.1%, hookworm 3.2%. Moderate- to high-intensity *T. trichiura* infection and any hookworm infection were risk factors for moderate to severe wasting. Moderate- to high-intensity *T. trichiura* infection was a risk factor for moderate to severe underweight. STH were not risk factors for moderate to severe stunting.	Analyses adjusted for child gender and maternal age
Gutierrez-Jimenez et al., 2013 [66], Mexico	250 PSAC aged <5 years from three impoverished municipalities	Cross-sectional	STH prevalence 38.8% (*A. lumbricoides* 33.6%, *T. trichiura* 1.2%, hookworm not reported). Malnutrition associated with presence of intestinal parasites in children from one village.	Unadjusted analyses; results for malnutrition not tabulated.
Gyorkos et al., 2011 [67], Peru	349 PSAC; 164 aged 7–9 months, 185 aged 12–14 months	Cross-sectional; to (1) examine prevalence patterns of helminth infection in early childhood; (2) assess association between helminth infection and socio-demographic characteristics; and (3) examine effect of intensity of helminth infection on stunting and anaemia	STH infections first appeared in children at eight months of age. In the 7–9 month age group STH prevalence was 4.3% (*A. lumbricoides* 1.8%, *T. trichiura* 1.2%, hookworm 1.2%). For the 12–14 month age group, STH prevalence was 29.2% (*T. trichiura* 21.2%, *A. lumbricoides* 12.4%, hookworm 0.5%). Among infected children, moderate-to-heavy infection of any STH was associated with stunting.	Very low numbers—seven moderate- to heavy-infected children. Analyses adjusted for age, sex, mother's education, and intensity of STH infection
Physical development, fitness, worker productivity—mixed PSAC and SAC				
Hürlimann et al., 2014 [68], Côte d'Ivoire	852 individuals (anthropometry for children aged <18 years only)	Cross-sectional survey (two communities); investigated patterns of polyparasitism, including associations and interactions between infection, clinical indicators, and self-reported morbidity.	Prevalence of hookworm 32.4% and 26.8% in the two communities, respectively; *A. lumbricoides* and *T. trichiura* infections rare. Most STH infections were light intensity. Polyparasitism common. In analysis of hookworm mono-infection, infected children had significantly lower odds of anaemia.	Opposite direction of effect to anticipated. All models were adjusted by age group, sex, and socioeconomic status.
Oninla et al., 2010 [69], Nigeria	749 children aged 3–19 years	Cross-sectional; assessed STH impact on nutritional status	Baseline STH prevalence 30.0%. Hookworm infection a significant risk factor for underweight, wasting and stunting. *T. trichiura* a significant risk factor for stunting.	Reported as multivariable model results, but not tabulated and adjusted variables not noted.

(*Continued*)

Table 3. (*Continued*)

Citation and country	Sample size and population	Study design and description	Results	Comments
Staudacher et al., 2014 [70], Rwanda	622 children aged 4–18 years across two sites (301 rural, 321 urban)	Pre and post (15 week follow-up), assessed prevalence, associated factors, and manifestation of STH infection, cure, and reinfection rates. Intervention: single dose mebendazole.	Baseline STH prevalence 25.4% (38.2% urban, 13.4% rural); *A. lumbricoides* 24.3%. STH infection associated with reduced height-for-age, stunting, any clinical finding, anaemia and low Hb levels in urban but not rural children (who exhibited worse clinical parameters). Clinically assessed malnutrition associated with STH infection only in rural children.	Analyses adjusted for factors found to be univariately significant.
Cabada et al., 2015 [71], Peru	240 children aged 3–12 years	Cross-sectional; to evaluate prevalence of soil-transmitted helminth infections, anaemia, and malnutrition	Parasite prevalence: *A. lumbricoides* 14.2%, *Fasciola hepatica* 9.6%, *Hymenolepsis nana* 9.3%, *T. trichiura* 1.3%, hookworm 1.7%, *Strongyloides stercoralis* 0.8%, *Giardia intestinalis* 27.5%. Stunting and wasting not associated with helminth infections.	Low STH prevalence. Analyses adjusted for socioeconomic and demographic variables, and variables $P \leq 0.1$ in bivariate analysis. Regression results not tabulated.
Sekiyama et al., 2014 [72], Indonesia	418 children aged 0 to 12 years at recruitment	Prospective cohort (3 year follow-up); to investigate growth trajectories relative to nutrition, disease, and hormonal status	Baseline STH prevalence: *A. lumbricoides* 30.6%, *T. trichiura* 23.4%. STH infection a significant predictor of low height-for-age z-scores in childhood.	Not differentiated by species. Analyses adjusted for sex, age group, and anaemia.
Sayasone et al., 2015 [73], Lao PDR	1313 children, aged 6 months to 12 years	Cross-sectional; to assess and quantify relationship between single and multiple species helminth infection	Parasite prevalence: hookworm 51.0%, *Opisthorchis viverrini* 43.3%, *A. lumbricoides* 16.8%, *T. trichiura* 10.7%, *S. mekongi* 5.6%, *Taenia* spp. 1.2%. Hookworm infection associated with anaemia. Positive association between hookworm infection and pale sub-conjunctiva.	Analyses adjusted for sex, age group, socioeconomic status, nutritional status, and personal hygiene.
Physical development, fitness, worker productivity—SAC				
Müller et al., 2011 [74], Côte d'Ivoire	156 SAC aged 7–15 years	Cross-sectional; investigating relationship between schistosomiasis, STH, and physical performance of children	Prevalence: *S. haematobium* 85.3%, *Plasmodium* spp. 71.2%, *S. mansoni* 53.8%, hookworm 13.5%, *A. lumbricoides* 1.3%. Maximum volume of oxygen (VO2 max) influenced by sex and age, but not by hookworm infection or intensity.	Low numbers: 21 hookworm-infected children overall. Analysis adjusted for temperature, relative air humidity, sex, and age.
Degarege and Erko, 2013 [75], Ethiopia	403 SAC aged 5–15 years	Cross-sectional; investigating association between helminth infection and nutritional status of schoolchildren	Prevalence: hookworm 46.9%, *S. mansoni* 24.6%, *A. lumbricoides* 4.2%, *T. trichiura* 1.7%. Hookworm infection associated with low body mass.	Adjusted analysis for age and sex.
Wolde et al., 2015 [76], Ethiopia	450 SAC aged 7–14 years	Cross-sectional; investigating determinants of underweight, stunting, and wasting	Prevalence of helminths (undefined species) 64.3%; *A. lumbricoides* 57.6%; hookworm and *T. trichiura* prevalence not reported. Ascariasis not associated with being underweight or stunted; reported odds of being stunted increased four times for children who had *T. trichiura* infection than children who do not have *T. trichiura* infection (but see comment).	Very low numbers—hookworm and *T. trichiura* numbers in regression models may be too low to assess anthropometry. Analyses adjusted for sex, age, family size, maternal education, monthly income, household food insecurity, and infection with ascariasis, trichuriasis, or hookworm.
Oliveira et al., 2015 [77], Angola	328 children aged 5–12 years	Cross-sectional; investigating association between helminth infection, anaemia, and stunting	Prevalence of intestinal parasites was 44.2%, comprising *A. lumbricoides* (22.0%), *G. lamblia* (20.1%), *H. nana* (8.8%); hookworms 0.2%. No significant association between *A. lumbricoides* infection and anaemia or stunting.	Analyses adjusted for variables $P \leq 0.05$ in bivariate analysis.

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Table 3. (*Continued*)

Citation and country	Sample size and population	Study design and description	Results	Comments
Casapía et al., 2006 [78], Peru	1,074 SAC aged 8–16 years	Cross-sectional; to determine risk factors for stunting only, and stunting and underweight	STH prevalence 86% (*T. trichiura* 77.9%, *A. lumbricoides* 60.4%, hookworm 21.3%). Prevalence of either *H. nana* or *Enterobius vermicularis* was 10.9%. Hookworm a significant risk factor for stunting and underweight. Moderate and heavy *T. trichiura*-*A. lumbricoides* co-infection a significant risk factor for stunting only.	Analyses adjusted for sex and variables of P≤0.1 in univariable analysis.
Verhagen et al., 2013 [79], Venezuela	390 SAC aged 4–16 years from three rural areas	Cross-sectional; to investigate prevalence and associations between intestinal helminth and protozoan infections, malnutrition, and anaemia	Parasite (multiple species) prevalence 68%. Hb levels significantly lower in children with hookworm infection but not *A. lumbricoides* infection. Children infected with hookworm had significantly lower weight-for-height/BMI-for-age compared to uninfected children. Children with *A. lumbricoides* infection showed reduced weight-for-height/BMI-for-age compared to uninfected children, but only significant for moderate-intensity *A. lumbricoides* infection.	Analyses adjusted for age and sex
De Gier et al., 2015 [80], Cuba and Cambodia	1,389 SAC; mean age 8.14 ± 2.07 (Cuba), 2,471 SAC mean age 9.68 ± 2.27 (Cambodia)	Cross-sectional height; to analyse STH and/or zinc associations with height for age z-scores and whether STH and zinc were associated.	STH prevalence 8.4% (Cuba) and 16.8% (Cambodia). In Cuban children, STH infection had strong negative association with height for age.	No species investigations. Analyses adjusted for sex and age.
Yap et al., 2012 [81], China	69 SAC aged 8–15 years	Cross-sectional; to assess feasibility of measuring children's physical fitness and to relate it to STH infections.	STH prevalence: *T. trichiura* 81%, *A. lumbricoides* 44%, hookworm 6%. V02 max estimate used within one min during exhaustive exercise of *T. trichiura*-infected children lower than that of non-infected children. *T. trichiura*-infected children completed fewer 20m shuttle run tests until exhaustion. No significant association between stunting and any STH infection.	Analyses adjusted for age, sex, stunting, and infection status.
Ahmed et al., 2012 [82], Malaysia	289 SAC aged 6–13 years	Pre and post (3 month follow-up after albendazole treatment); to assess risk factors for anaemia and malnutrition, and nutritional impacts of STH infections.	Baseline STH prevalence *T. trichiura* 84.6%, *A. lumbricoides* 47.6%, hookworm 3.9%. Moderate-to-heavy ascariasis was risk factor for anaemia. Stunting associated with moderate-to-heavy ascariasis and trichuriasis. Post-treatment assessment showed no difference in the mean increments in growth indices between negatively-to-lightly infected and moderately-to-heavily infected groups.	Analyses adjusted for age, gender, mother's educational level, and household monthly income. *A. lumbricoides* not adjusted for *T. trichiura* as *T. trichiura* was not significant in univariable model. Follow up may not have been long enough to detect changes in anthropometric measurements.
Papier et al., 2014 [83], Philippines	667 SAC aged 10–14 years	Cross-sectional; to investigate whether poor nutrient intake may increase susceptibility to parasitic diseases	Parasite prevalence: *S. japonicum* 20.1%, *A. lumbricoides* 54.4%, *T. trichiura* 71.4%, hookworm 25.3%. Hookworm infection a risk factor for stunting.	Analyses adjusted for variables of P≤0.1 in univariable analysis.

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Table 3. (*Continued*)

Citation and country	Sample size and population	Study design and description	Results	Comments
Physical development, fitness, worker productivity—pregnant women, neonates				
Ndibazza et al., 2010 [84], Uganda	2,507 pregnant women: (i) 629 albendazole + placebo, (ii) 628 praziquantel + placebo, (iii) 628 albendazole + praziquantel, (iv) 630 placebo + placebo.	Double-blind RCT; 2x2 factorial design: (i) albendazole and placebo, (ii) praziquantel and placebo, (iii) albendazole and praziquantel, (iv) placebo and placebo. Aimed to investigate benefits of deworming during pregnancy on maternal and child health outcomes. Recruited women at antenatal visit (treatment after first trimester); follow-up at delivery and 14 weeks thereafter. Software-generated randomisation. STH diagnosed by Kato-Katz.	2,507 women at baseline; 2,051 (82%) provided post-delivery stool; 1,918 (81%) provided post-delivery blood sample; 1,964 infants (82%) had birthweight recorded; 2,365 infants (99%) were assessed for congenital anomalies at birth. Baseline parasite prevalence 68% (hookworm 45%, *S. mansoni* 18%). At delivery no overall effect of albendazole on maternal anaemia, but suggestion of benefit of albendazole among women with moderate to heavy hookworm. No effect of albendazole on mean birth weight or on proportion of low birth weight. Anthelmintic use during pregnancy showed no effect on perinatal mortality or congenital anomalies.	Sample size calculations based on different trial outcomes. Study powered to detect 0.3g/L difference in maternal Hb and 70g difference in infant birthweight for either intervention [84]. Adequate power to detect whole group effects but not perinatal mortality effects in subgroup analyses [84]. Excluded women with clinical requirements to treat low Hb; may have excluded those most likely to benefit from interventions [84]. Loss to follow-up did not vary between trial arms. Analyses adjusted for albendazole or praziquantel (according to treatment being investigated in the model).
van Eijk et al., 2009 [85], Kenya	390 pregnant women	Cross-sectional; to investigate STH risk factors and effects among pregnant women	STH prevalence: 76.2% (*A. lumbricoides* 52.3%, hookworm 39.5%, *T. trichiura* 29.0%). STH infections not associated with clinical symptoms or low body mass index. Hookworm infection associated with a lower mid upper arm circumference.	Models adjusted for malaria, marital status, treatment of water and a report of soil eating, and other STH.
Fairley et al., 2013 [86], Kenya	696 pregnant women	Cross-sectional; to investigate associations of maternal helminth infection and malaria-helminth co-infection on birth outcomes	Prevalence in mothers: *P. falciparum* 42.7%, *S. haematobium* 30.6%, filariasis 36.2%, hookworm 31.5%, *T. trichiura* 5.9%. Hookworm or trichuriasis infection not associated with neonatal low birth weight or a low weight z-score.	Model adjusted for maternal age, socioeconomic status, education level, marital status, gravida, and area of residence.
Aderoba et al., 2015 [87], Nigeria	178 women with singleton pregnancy	Cross-sectional; investigating STH prevalence during pregnancy and associated adverse maternal and infant outcomes	STH prevalence 17.4% (8.4% *A. lumbricoides*, 4.5% *T. trichiura*, 14.0% hookworm). Mean Hb concentration lower among STH infected women than those without. Mild anaemia more common among STH infected women. Mean birth weight lower for infants of STH-infected women; prevalence of low birth weight higher in this group.	Very low numbers (31 women infected); no species investigations. Analysis adjusted for maternal weight, parity, social class, HIV status, and pregnancy duration at booking.
Boel et al., 2010 [88], Thai-Burmese border	339 pregnant women (in 1996), 490 (in 2007)	2 cross-sectional studies (1996 and 2007); to assess relationship between STH infection and the progress and outcome of pregnancy	Overall STH prevalence 70% (hookworm 43%, *A. lumbricoides* 34%, *T. trichiura* 31%). Hookworm infection associated with an increased risk of anaemia. Hookworm associated with low birth weight.	Adjusted models used variables significantly associated in univariate analysis, including malaria, primigravid/multigravid status, and participation in the 1996 survey.

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Table 3. (*Continued*)

Citation and country	Sample size and population	Study design and description	Results	Comments
Physical development, fitness, worker productivity—all ages				
Baird et al., 2011 [89], Kenya	Original cohort were approx. 30,000 SAC from 75 schools [90]	Longitudinal economic analysis, examined impact of deworming programme on adult living standards by following participants from deworming program that began in 1998.	Followed up 83% of deworming programme participants over a decade. Self-reported health, years enrolled in school, and test scores improved significantly, and hours worked increased by 12% in the treatment group. Treated individuals reported eating an average of 0.1 additional meals per day. Within the subsample working for wages, earnings were >20% higher for the treated group. Most of earnings gains are explained by sectoral shifts, including doubling of manufacturing employment. Small business performance improved among the self-employed. A lower bound on the annualized social internal rate of return to deworming is large, at 83%.	Unclear if this has been independently peer reviewed.
Degarege et al., 2013 [91], Ethiopia	480 febrile outpatients aged 1–80 years; mean age (SD) 23.1 (14.2) years	Cross-sectional; to investigate associations between helminth infections and ABO blood group, anaemia, and undernutrition	Parasite prevalence 50.2% (*A. lumbricoides* 32.7%, *T. trichiura* 12.7%, *S. mansoni* 11.9%, hookworm 11.0%). Individuals infected with only *T. trichiura* had significantly lower mean haemoglobin level compared to uninfected individuals. Odds of developing anaemia higher in individuals infected only with hookworm compared to uninfected individuals. Increased intensity of *A. lumbricoides* infection significantly associated with decrease in haemoglobin level and with increase in anaemia prevalence. Odds of being underweight significantly higher in *A. lumbricoides*-infected individuals aged $\leq$5 and *T. trichiura*-infected individuals aged $\geq$20 years, compared to uninfected individuals.	Analyses adjusted for age, sex, and nutritional status.
Cognitive development[#]				
Ebenezer et al., 2013 [92], Sri Lanka	1,190 SAC (615 intervention, 575 control)	Unblinded cluster RCT (grade 4 in schools); 49 schools received deworming and weekly iron supplementation for 6 months (intervention), 49 schools received placebos for both anthelmintic and iron (control); follow-up 6 months. Aimed to assess impact of school-based deworming and iron supplementation on individual cognitive abilities. Software-generated randomization. STH diagnosed by Kato-Katz.	Baseline enrolment of 1,621 children; 1,190 children (73.5%) included in analyses. Baseline STH prevalence 25.7% (*A. lumbricoides* 21.0%, *T. trichiura* 6.1%, hookworm 5.3%). No impact of deworming and iron supplementation found on Hb levels, anaemia, cognitive test, or educational test scores.	Analyses adjusted for age, sex, baseline nutritional status, individual socioeconomic indicators (parental education), school-level indicators (whether or not school had an ongoing midday meal programme), interaction between treatment and low nutritional status, and high-intensity worm burden. Hb level and anaemia prevalence differed between treatment and control groups (adjusted in models). Loss to follow-up of two control schools, but similar loss to follow-up overall across treatment and control arms [92].

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Table 3. (*Continued*)

Citation and country	Sample size and population	Study design and description	Results	Comments
Thériault et al., 2014 [93], Peru	1,088 SAC, mean age 10.3 ± 1.1 years (517 intervention, 571 control for analyses)	Cluster RCT (nine intervention schools, nine control schools); both trial arms received deworming treatment; intervention arm received 4 months of health hygiene education aimed at increasing knowledge of STH prevention; follow-up 4 months. Aimed to measure impact of health hygiene education on absenteeism. Software-generated randomization. STH diagnosed by Kato-Katz.	1,486 students at baseline; 1,088 (73.2%) analysed. Baseline STH prevalence 72.1% in intervention schools, 78.1% in control schools. At completion, overall absenteeism rates at intervention and control schools not significantly different. Post-trial non-randomized analyses showed students with moderate-to-heavy *A. lumbricoides* infections and light hookworm infections 4 months after deworming had, respectively, missed 2.4% and 4.6% more schooldays during follow-up period than uninfected counterparts.	Data from [94]. Analyses adjusted for socioeconomic status and age. 4 months may not have been long enough to detect changes in absenteeism from health education intervention. Infection status between control and intervention groups differed; may have affected estimates. Study powered to detect a different outcome, therefore analyses may be underpowered [93]. Attendance measured by teacher logs rather than direct observation (systematic bias affecting both trial arms) [93].
Ahmed et al., 2012 [95], Malaysia	289 SAC aged 6–13 years (same cohort as reported in [82])	Pre and post (3 month follow-up after albendazole treatment); to determine possible relationship between intestinal helminthiasis and school absenteeism	Baseline STH prevalence: *T. trichiura* 84.6%, *A. lumbricoides* 47.6%, hookworm 3.9%. Infection of moderate-to-heavy worm burdens and anaemia identified as significant risk factors of high absenteeism among the subjects. Following treatment of infected children, school absenteeism reduced significantly by about 16% among the pupils.	Multivariable models included ascariasis, trichuriasis, anaemia status, stunting, underweight, mother's employment status, mother's education, and father's education. Multivariable results a bit unclear, particularly how models were built and whether consideration was given to anaemia, stunting, and underweight as potential effect modifiers. Given this, and short follow-up, very interesting association between STH infection and absenteeism.
Ezeamama et al., 2012 [96], Philippines	253 *S. japonicum*-infected SAC aged 7–19 years	Prospective cohort study followed for 18 months; to determine whether treatment of intestinal parasitic infections improves cognitive function. Separately assessed changes in cognitive test scores for: (i) treatment-related *S. japonicum* intensity decline, (ii) spontaneous reduction of single STH species, and (iii) ≥2 STH infections among *S. japonicum*-infected children.	At baseline, 97% concurrent infection with *S. japonicum* and ≥1 STH species. Baseline prevalence of *A. lumbricoides* 79.9%, *T. trichiura* 95.6%, hookworm 50.6%. At follow-up, a decline versus no change/increase of any individual STH species and joint decline of ≥2 STH species associated with higher scores in Wide Range Assessment of Learning and Memory (WRAML) learning test. Hookworm and *T. trichiura* declines independently associated with improvements in WRAML memory scores as was the joint decline in ≥2 STH species. Baseline coinfection by ≥2 STH species associated with low Philippine nonverbal intelligence test (PNIT) scores.	Adjusted analyses by age, sex, and socioeconomic status. Considered potential effect modification from helminth infection intensity, underweight, and anaemia.
Liu et al., 2015 [97], China	2179 SAC aged 9–11 years	Cross-sectional; to examine relationship between STH infections and developmental outcomes	STH prevalence: 42% (*A. lumbricoides* 31%, *T. trichiura* 22%, hookworm 1%). Infection with ≥1 STH associated with worse cognitive ability, worse nutritional status, and worse school performance than no infection. Children with *T. trichiura* infection, either single infection or co-infected with *A. lumbricoides*, experienced worse cognitive, nutritional, and schooling outcomes than uninfected peers or children infected with only *A. lumbricoides*.	Analyses adjusted for gender, age, boarding status, ethnicity, ever eats uncooked meat / vegetables, ever drinks unboiled water, and socioeconomic characteristics
Additional *A. lumbricoides* morbidity				

(*Continued*)

Table 3. (*Continued*)

Citation and country	Sample size and population	Study design and description	Results	Comments
Alam et al., 2010 [98], Bangladesh	138 consecutive cases of biliary and pancreatic ascariasis (BPA) in adults, mean age 36.8 ± 16.1 years	Case series	Comparison of clinical BPA morbidity from dead and living *A. lumbricoides* worms. 98 patients had living worms, 40 had dead worms. Males were more prone to develop dead worm BPA. Presentations for biliary colic (131; 94.9%), acute cholangitis (30; 21.7%), obstructive jaundice (19; 13.8%), choledocholithiasis (20; 14.5%), acute pancreatitis (10; 7.2%), acute cholecystitis (6; 4.3%), liver abscess (2; 1.4%), hepatolithiasis (3; 2.2%), stricture of common bile duct (2; 1.4%), pancreatic abscess (1; 0.7%), and cirrhosis of liver (1; 0.7%). Surgical intervention required in five patients. Recurrences of stone and cholangitis occurred only in those with dead worms. Biliary ascariasis with dead worms deemed more dangerous than that with living worms.	
Baba et al., 2009 [99], India	207 patients admitted with diagnosis of intestinal obstruction aged 3–14 years	Case series	131 patients diagnosed as having obstruction due to ascariasis. Most patients 3–5 years of age. 64 patients needed operative intervention of either enterotomy, milking of worms or resection anastomosis. Appendicular perforation was seen in four and worm in gall bladder in one patient. Surgical complications were wound infection in 17, burst abdomen in four and faecal fistula in three patients.	
Mukhopadhyay, 2009 [100], India	42 cases of hepatobiliary ascariasis, adults aged between 20–50 years	Case series	Most common presentation was upper abdominal pain in 95.2% of the patients (40 patients). Complications included obstructive jaundice in 28.6% (12 patients), cholangitis in 16.7% (seven patients), acute pancreatitis in 2.4% (one patient), and hepatic abscess in 2.4% (one patient). History of worm emesis present in 38.1% (16 patients). History of previous cholecystectomy present in 16.7% (seven patients) and endoscopic sphincterotomy in 4.8% (two patients). Conservative management successful in 83.3% (35 patients). During follow-up, worm reinvasion of biliary system occurred in 7.1% (three patients).	
Additional *T. trichiura* morbidity				
Khuroo et al., 2010 [101], India	Ten cases of TDS in adults	Case series	No patients had growth retardation, malnutrition, or immunodeficiency. Abdominal symptoms in one patient; nine had no abdominal symptoms. Large numbers of actively motile *T. trichiura* in right colon (seven patients), ileum (one), left colon (one), and whole colonic mucosa (one). Mucosal changes included petechial lesions, blotchy mucosal haemorrhages, and active mucosal oozing.	TDS has not previously been described in adults.

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Table 3. (*Continued*)

Citation and country	Sample size and population	Study design and description	Results	Comments
Kaminsky et al., 2015 [102], Honduras	Children aged 12 years or younger	Hospital-based study; severe trichuriasis cases identified by routine stool examination from hospitalised patients.	11,528 faecal samples examined between March 2010 and September 2012; of these, 122 (1.0%) *T. trichiura* infections were diagnosed. Morbidity included dysentery of several months' duration, severe anaemia, and stunting. Heavy *T. trichiura* infections included egg counts from 232 to 3,520 eggs, Hb 3.4 to 10.8 g/dL, eosinophilia, severe malnutrition, and growth stunting. Orally administered drugs prescribed at different dosages and duration; no cure or egg excretion control was exercised before patient release. A range of 340 to about 10,000 worms were recovered after treatment from eight patients.	Cases selected on basis of high *T. trichiura* egg counts. Descriptive analysis; some morbidity data (e.g., persistence of dysentery) self-reported. There is a need to conduct detailed community studies in trichuriasis morbidity, effective treatment assessment, and clinical response in severely malnourished parasitised children [102].

* RCTs are focus given prior systematic reviews, however one cross-sectional study for *T. trichiura* is included.

Two replication analyses [5,6] are not included in this table as they were analysed in [4].

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the first time, or on very young children being exposed to STH for the first time. Quantifying infection-related or gastrointestinal-related morbidity from STH does not seem to be a current research focus.

Blood loss, haemoglobin, and anaemia

The evidence for negative impact of STH infections is strongest for morbidity due to blood loss and anaemia. There is clear evidence that hookworms in particular cause blood loss from feeding on mucosal tissue in the small intestine [23], thereby causing iron-deficiency anaemia. However, anaemia is of multifactorial origin [103], and it is difficult to disentangle the effect from all other potential confounders. Furthermore, there are likely to be different impacts of anaemia in different age groups. With the exception of two systematic reviews investigating impact in pregnant women [52,53] and one that investigated observational evidence separately for school-aged children and adults [12], systematic reviews have not investigated age-dependent effects. Whilst this is likely due to lack of evidence, this is particularly important for anaemia indicators.

Evidence from systematic reviews and recent experimental studies is mixed; not all studies have found an impact of hookworm (or STH more generally) on either haemoglobin levels or anaemia. Three systematic reviews (one in non-pregnant populations, one in pregnant populations, and one across populations), confirm previous observational direct correlations between intensity of hookworm infection and reduced haemoglobin, or improvements in haemoglobin levels following deworming [12,13,53]. Two other systematic reviews (one in children aged 0 to 16 years, one in pregnant women) [4,52] found no evidence for reduced anaemia following deworming. Two RCTs found changes in STH intensity or haemoglobin levels, but not in levels of anaemia, following deworming [55,58]. This is possibly due to differing levels of hookworm burden [104] and hookworm species, different anthelmintics used, underlying nutritional status, or other confounding factors, as well as specific differences in systematic reviews as discussed below.

It is only since 2002 that deworming has been advocated during pregnancy (after the first trimester), with the result that experimental data on maternal and child outcomes are relatively recent and more evidence is needed. Whilst data are somewhat dated, iron-deficiency anaemia has been suggested to be responsible for 20% of maternal deaths worldwide [23], with estimates that hookworm causes at least 30% of moderate or severe cases of anaemia among this population group [105]. The WHO estimates that more than half of pregnant women in developing countries have morbidity related to iron-deficiency anaemia [27]. Given this high prevalence, quantitative investigations into the role of hookworm in anaemia-related maternal mortality are required. No estimates of hookworm-associated mortality are currently included in GBD calculations.

Studies vary with regards the involvement of *T. trichiura* in blood loss and anaemia. *T. trichiura*-associated blood loss has been previously inferred to only become significant in heavy infections [48]; newer evidence [59] is in agreement with this. Interestingly, in one recent study undertaken in a high *T. trichiura* and *A. lumbricoides* (but low hookworm) endemic environment, moderate to heavy *A. lumbricoides* infection was a risk factor for anaemia in school-aged children [82]. Further experimental evidence investigating the impact of STH on haemoglobin and anaemia, particularly in preschool-aged children and pregnant women, is required.

Physical development, fitness, and worker productivity

Whilst this is the focus area for which we found the greatest quantity of recent studies, results for impact of STH on measures of height, weight, and head circumference are mainly

observational and vary widely. Some studies found impacts of *T. trichiura*, *A. lumbricoides*, and/or hookworm on height but not weight, weight but not height, both height and weight, height and head circumference, and some studies found no impacts at all (Table 3). Underlying prevalence and infection intensity varied considerably by geographic location and, although most studies adjusted for some potential confounders, it is difficult to control for the role of malnutrition within populations, which could have had a major influence on results. Generally, more studies reported associations with stunting and wasting than studies that did not. The Cochrane systematic review found that deworming may increase weight gain [4], but was inconclusive on other physical health measures.

There are inherent difficulties in detecting growth changes in school-aged children [106], with, amongst other things, an appropriate length of follow-up time required to adequately assess these. However RCTs need to balance sufficient follow-up to detect effects with the potential for STH re-infection. Given rapid growth and potentially greater STH morbidity (evidenced by intensity of infection data) in preschool-aged children and the fact that, in some areas, preschool-aged children are now included in deworming programmes, greater emphasis on investigating morbidity in this cohort is required. It could be that this age group is where the strongest evidence of effect may lie.

It is biologically plausible that young girls who grow poorly become stunted women with a greater chance of giving birth to low birth weight infants who are likely to be stunted in adulthood [37]. Whilst longitudinal investigations into the impact of chronic STH infection in girls, following their general health status as they become mothers, have not been conducted, studies investigating pregnancy outcomes are being conducted. In terms of albendazole impact on pregnant women and neonates, an RCT not included in the systematic reviews provides further evidence of the lack of clear benefit of albendazole on maternal or neonatal health [84]. Some observational studies (but again, not all) [88] reported associations between hookworm infection and low birth weight.

We found one recent attempt in the published literature to investigate effects of STH on longer-term schooling and worker productivity; this analysis found improved economic outcomes for a cohort of dewormed individuals followed over approximately 10 years [89], similar to a previous economic analysis [107]. There is negligible other direct evidence that STH infections reduce adult productivity. However, the health consequences, such as anaemia, are known to affect productivity, and hookworm could be a major contributor to this. More well-designed longitudinal analyses are needed.

STH impact on cognitive development, school performance, and absenteeism

The impact of STH on cognitive development is the area that has come under greatest scrutiny over the years. It is extremely complex to measure accurately. Cognitive psychology is a dynamic field, encompassing different theories of the interplay of a broad range of psychological and environmental factors. Impaired cognition is rarely from a single cause, with an array of cognitive tests needed to assess impacts such as STH on a range of cognitive functions [106]. It is perhaps not surprising that despite much research undertaken to investigate whether STH contribute to cognitive impairment in children, few conclusions have been able to be drawn. The Cochrane systematic review investigated three RCTs undertaken in children of known high-intensity infection status, specifically designed to measure cognitive outcomes, but did not meta-analyse these due to different outcome definitions and therefore drew no new conclusions. Recent experimental and observational studies further illustrate this. Firm evidence continues to be elusive. Recent evidence investigating school absenteeism is sparse.

Additional clinical morbidity from STH species

Severe clinical complications such as trichuris dysentery syndrome (TDS), or intestinal obstruction and hepatopancreatitis as a result of *A*. *lumbricoides* infections, are relatively rare and represent only a small portion of the disease burden [108], although they are sufficiently serious to warrant attention. Apart from very few case series, there is an almost complete lack of epidemiological investigation into quantifying these acute complications over recent years. This is important to highlight, as ascariasis is the most common of the STH infections and causes the majority of STH mortality [109]. In the absence of recent information, it is unclear how mortality estimates have been derived [110]. It is additionally unclear whether any recent estimates exist for one of the most serious presentations of ascariasis, hepatopancreatic ascariasis (HPA), or a disease known as recurrent pyogenic cholangitis (RPC) (caused by stone formation, usually around dead *A*. *lumbricoides* in the bile duct). This has been epidemiologically linked to recurrent biliary invasion by *A*. *lumbricoides* in endemic areas [111]. Similarly, there appear to be no detailed quantitative investigations of trichuriasis, particularly TDS, in recent years. TDS can cause major, acute disease that is sometimes life-threatening [28]. There are no recent empirical data on either global incidence of TDS or any attributable mortality.

Current STH prevalence and burden estimates do not include *Ancylostoma ceylanicum*, which recent data show to be the second most prevalent hookworm species after *N*. *americanus*, in some Asian countries [50]. Whilst evidence is scant, *A*. *ceylanicum* may have more severe morbidity than *A*. *duodenale* [112,113]. Studies of *A*. *ceylanicum*-associated morbidity are somewhat dated and highlight the evidence gap arising from a failure to investigate morbidity associations. Diagnosis of *A*. *ceylanicum* requires coprodiagnostic molecular biology techniques not readily available in developing countries; however, in the current era of large-scale deworming programmes, there is growing impetus for utilisation of contemporary diagnostic methods, hence renewed focus on this hookworm species is needed.

It is clear that many morbidities associated with STH cannot be investigated in an experimental design and, further, that many associations (such as maternal mortality from hookworm) are not feasible to investigate at all. In assessing evidence for deworming on STH-associated morbidities, the Cochrane Collaboration has exclusively considered RCT and quasi-RCT evidence in its systematic reviews of deworming. As has been raised elsewhere [7,8], this major limitation results in lack of consideration of a vast quantity of broader evidence of association. The Cochrane systematic review is assessed in detail below.

A Critical Appraisal of a Cochrane Systematic Review

Cochrane systematic reviews are regarded as the benchmark for high-quality evidence, utilising rigorous methodologies undertaken in accordance with a set protocol. In the most recent Cochrane review [4] on the health benefits of deworming, the authors considered length of trial follow-up and different assessment points. They undertook analyses by classes of STH infection intensity. They further investigated dose number as either single dose or multiple dose treatments. They were not able to undertake analyses by age group due to insufficient data. The authors considered only absolute measures of heights and weights. As is appropriate, trials were not pooled where the outcome definitions varied, such as cognitive tests.

Under the rules of the Cochrane Collaboration, the systematic review protocol must be produced prior to undertaking the review [114]. It is clear from the report that a protocol was developed. However, the protocol is not publicly available on the Cochrane website and we could not verify whether key points raised below were written in the protocol, or whether changes were made during the review process, possibly due to lack of data. The research objective of the Cochrane systematic review was "to summarise the effects of giving deworming

drugs to children to treat soil-transmitted intestinal worms, in weight, haemoglobin, and cognition; and the evidence of impact on physical well-being, school attendance, school performance, and mortality" [4]. We believe this is a situation in which the null hypothesis and the intended subgroup analyses need to be clearly stated and be verifiable with the protocol. One interpretation of the null hypothesis from the stated objective is that "deworming does not improve the listed health outcomes." This is supported by the description of the participants as "infected children identified by screening in community trials. All children must have lived in endemic areas" [4]. This does not clearly imply consideration of unscreened children. Yet, for data synthesis, the participants are separately analysed according to these two groups: infected children and all children living in an endemic area. Thirty-seven of the 45 included RCTs were based on mass drug distribution of an unscreened population [4]. Therefore, the alternative interpretation of the null hypothesis also follows: "mass drug distribution as delivered to all children in endemic areas does not improve health outcomes." The objective, participants, and intended subgroup analyses need to be more clearly explained, as the two hypotheses require a major conceptual shift in interpretation and raise different questions in terms of included studies and how outcomes are determined. The issue of considering unscreened children has already been strongly criticised, primarily because international policy promotes treating all children in endemic communities, and a systematic review is not required to establish that treating uninfected children will have no health benefit on these children [7,8].

With the analysis of unscreened children, trials have been pooled irrespective of STH species, treatment types, and drug distribution strategies. Conceptually, pooling trials in systematic reviews is appropriate; however, for STH there is a very high level of heterogeneity, and this approach is prone to methodological flaws. By considering RCTs conducted in different locations in which screening has not been done, there is no baseline assessment of STH prevalence and/or intensity. The underlying assumption is that baseline prevalence is the same between intervention and control groups, which enables post-intervention assessment attributable to the intervention (provided randomisation adequately enabled control for confounding and that there was no systematic differential bias between groups). Whilst justifiable for RCTs, this causes a difficulty for systematic review methods, as there is marked STH heterogeneity in different endemic areas and, if baseline testing is not done, there must be another estimate to determine the STH of greatest prevalence in the population (e.g., from other epidemiological surveys) to ensure that heterogeneity is addressed when pooling RCTs. In the systematic review, no baseline of STH prevalences are reported. Whilst evidence is limited, there is sufficient prior knowledge of differential impacts of STH on morbidity outcomes, e.g., the role of hookworms, but not *A. lumbricoides*, in blood loss, to indicate that pooling of STH is not an accurate way of assessing morbidity. Similarly, it is also not accurate to pool different anthelmintic treatments of known and well reported [115,116] very different efficacies according to STH. Lastly, drug distribution strategies, particularly targeted delivery to school-aged children versus mass drug administration to all community members [117], is currently a major area of research investigation; differential impacts between school and non-school child cohorts are likely according to delivery strategies of targeted school programmes versus broader community treatments [118], or different frequency treatments as are recommended according to endemicity [117]. Such pooling of studies would cause dilution of effects due to different STH responsiveness to treatments with known differential efficacies, potentially delivered according to different strategies. Thus, the Cochrane authors appear to have not pooled like with like. It comes as no surprise that few conclusions can be drawn from the Cochrane systematic review.

The rationale for pooling studies may have been data-driven or aimed at replicating the deworming programme context. A more robust approach would be to apply the method of Smith and Brooker [12], who used known baseline prevalence of hookworm infection, analysed

by hookworms only, and by anthelmintic drug classes separately. It is very probable indeed that there is insufficient evidence to undertake meta-analyses for many morbidity outcomes by different STH and different anthelmintics. However, this is not a valid rationale for pooling them. If such meta-analyses cannot currently be done, there is insufficient evidence to say that deworming does not contribute to health outcomes. As has been indicated by others [8], this is not the same as a lack of effect.

The lack of evidence is correctly and clearly pointed out by the Cochrane authors with regards treatment of children with known STH infection. Participant and trial numbers for many outcomes were very low and many of the results were not sufficient to meta-analyse. Lack of sufficient data was also the reason why subgroup analysis by age was not conducted. This is, however, an extremely important evidence gap given differing age prevalence profiles of STH and the likely greater morbidity in preschool-aged children (with the consequence that these effects, too, could be diluted across age groups). The authors conclude that most evidence is of very low, low, or moderate quality. The most useful and important conclusion of the systematic review is that there is a major shortfall in evidence for most morbidities to feed into meta-analyses in the first place. Until such time as evidence is generated, meta-analyses will not be able to appropriately assess the health benefits of STH interventions.

If the protocol was publicly available, we could specifically ascertain whether the included trials met the protocol, whether excluded trials did not meet the protocol, and whether other trials should have been identified under the terms of the published search strategy. Other authors have noted omission of RCTs that ideally should have been considered [8]. Further evidence that has not been provided in the most recent Cochrane systematic review include GBD estimates since 2003 and a 2010 systematic review that found statistically significant effects for some anthelmintics on haemoglobin [12]. Finally, the authors have raised the issue of young children choking on deworming tablets in their discussion, referring to an unreferenced WHO newsletter. Their viewpoint is not derived from their systematic analyses.

Conclusion

In this manuscript, we have found that there is a paucity of recently collected data to inform our knowledge of STH morbidity. In particular, relatively little quantifiable evidence of STH morbidity has been forthcoming in recent years. Of the evidence that has been provided, few firm conclusions can be drawn. Perhaps this, too, is a reflection of the insidiousness of STH. Alternatively, this could be partly a result of trials that are powered to measure different primary outcomes; secondary morbidity outcomes may therefore be inadequately powered for effects to be detectable. Furthermore, this may be because intervention trials are impossible to conduct over sufficient time periods to assess deworming impacts on morbidity in the manner that such programmes are delivered in real-world settings (i.e., repeated rounds administered throughout childhood). There is also a possibility that our review has applied selection criteria that could have excluded key evidence. We considered that applying these criteria was the most accurate way to differentiate between direct and indirect STH morbidity measures. The main discrepancy that the Cochrane systematic review highlights is insufficient and heterogeneous underlying evidence. This is reinforced by our own findings.

We do have an evidence problem regarding STH morbidity and health effects of deworming. The use of prevalence and, to a lesser extent, intensity of infection as indicators for intervention planning, monitoring, and evaluation may have reduced the impetus to investigate more direct morbidity measures. As a consequence, we might not currently be able to prove the benefits of deworming. Furthermore, evidence of morbidity may become increasingly hard to detect over time if prevalence and intensity continue to reduce in populations. This is

obviously a good outcome, but a poor basis upon which to make any assessments. Our main conclusion is that further investments in appropriately designed studies that are powered to measure changes in direct STH morbidity indicators are urgently required.

Key Learning Points

- There is a paucity of quantifiable evidence of STH morbidity in recent years when assessed by direct morbidity measures such as changes in height, weight, haemoglobin, and cognition.
- The most recent Cochrane systematic review has assessed possible benefits of deworming on morbidity outcomes by pooling RCTs of deworming regardless of individual infection status, STH species, type of anthelmintic drug, and distribution strategy. In our opinion, this is methodologically inaccurate given current knowledge of STH heterogeneity. There may be insufficient evidence to prove benefits of deworming, but this is not the same as the authors' conclusion of lack of an effect.
- Careful consideration needs to be given to use of systematic reviews of RCTs for measuring improvements in morbidity from deworming. Furthermore, there needs to be clarification of the role of observational evidence for assessing STH-associated morbidity given that not all morbidity investigations are feasible within an RCT design.
- Studies designed to detect direct morbidity from STH are urgently required to strengthen evidence for deworming.

Top Five Papers

1. Taylor-Robinson DC, Maayan N, Soares-Weiser K, Donegan S, Garner P. Deworming drugs for soil-transmitted intestinal worms in children: effects on nutritional indicators, haemoglobin, and school performance. Cochrane Database Syst Rev. 2015;7: CD000371.
2. Smith JL, Brooker S. Impact of hookworm infection and deworming on anaemia in non-pregnant populations: a systematic review. Trop Med Int Health. 2010;15(7): 776–95.
3. Gulani A, Nagpal J, Osmond C, Sachdev HP. Effect of administration of intestinal anthelmintic drugs on haemoglobin: systematic review of randomised controlled trials. BMJ. 2007;334(7603): 1095.
4. Brooker S, Hotez PJ, Bundy DA. Hookworm-related anaemia among pregnant women: a systematic review. PLoS Negl Trop Dis. 2008;2(9): e291.
5. Haider BA, Humayun Q, Bhutta ZA. Effect of administration of antihelminthics for soil transmitted helminths during pregnancy. Cochrane Database Syst Rev. 2009(2): CD005547

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2. Taylor-Robinson DC, Jones AP, Garner P. Deworming drugs for treating soil-transmitted intestinal worms in children: effects on growth and school performance. Cochrane Database Syst Rev. 2007(4:): CD000371. PMID: 17943740
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Chapter 3 A critical appraisal of soil-transmitted helminth control strategies

3.1 Chapter context

Largely since 2013, an international paradigm shift has been occurring with introduction of large-scale NTD control and elimination programmes in many developing countries. These programmes target chemotherapy towards multiple NTDs simultaneously, and are principally advocated to eliminate some NTDs, and reduce morbidity from STH. However, for STH, disease control from chemotherapy alone is not possible; these programmes have therefore been criticised for their lack of sustainability, with almost certain STH resurgence if the programmes cease. There is increasing pressure for the scientific community to provide supporting evidence for the benefits of such large-scale investment. Therefore, research into sustainable strategies for STH, including integrated disease control strategies, is crucial.

In this chapter, two publications are presented, addressing objective 2 of the thesis. The first is a review that aims to determine the evidence for optimal approaches for STH control. It explores the evidence for chemotherapy with anthelmintic drugs, and for WASH. It articulates the lack of solid evidence for long-term impact of chemotherapy and the difficulties in establishing an unequivocal case for WASH. Two integration strategies are then critically appraised. Given current global context, the manuscript focuses on chemotherapy-oriented integrated NTD control, with particular emphasis on the features of

STH that are affected in this multiple-disease integration environment. Finally, an assessment of broadening of chemotherapy-based NTD integration to "multi-component integration" that includes sustainable interventions (primarily integrating chemotherapy with WASH) is provided. Whilst multi-component integrated control may be an effective approach to sustainably reduce STH transmission, there is an urgent need for evidence to prove the feasibility of this approach. The review highlights some of these evidence requirements and the need for innovative trial designs in order to try and bridge this challenging evidence gap.

This review is intended to stimulate debate regarding integrated control activities, across the research and implementation and policy development communities. It is an Invited Opinion, and has been published with *Trends in Parasitology*. The search strategy and selection criteria for this narrative review are as follows. English language literature on MEDLINE database was searched (to October 2015) according to the following criteria: ("*Necator americanus*" or "*Ancylostoma*" or "*Ascaris lumbricoides*" or "*Trichuris trichiura*" or hookworm or "soil-transmitted helminth") and (albendazole or mebendazole or deworm or chemotherapy or water or sanitation or hygiene or access or control or integration). Article abstracts were reviewed and literature retrieved if there was reference to chemotherapy or WASH for STH control, appraisal of STH control, NTD control and elimination approaches. Reference lists of identified articles were cross-checked and additional publications selected from the WHO website. The article utilises 84 references from international published literature. Publications from 2010 onwards were prioritised.

For this paper, the PhD Candidate was responsible for 90% of the conception, 100% of the literature collection, and 90% of the analysis, interpretation, drafting and writing of the paper. Clements A was responsible for 10% of the conception and analysis. Clements A, Nery S, McCarthy J, Gray D and Soares Magalhães R were collectively responsible for 10% of the interpretation and writing of the paper.

The second publication presented in this chapter is a viewpoint published in *PLoS Neglected Tropical Diseases* in 2014. This is intended to be a thought-provoking article that challenges the current chemotherapy-oriented approach employed for the control of STH and schistosomiasis globally. This article was written as a result of concerns about the short-term, unsustainable approach to current helminth control programmes which, as stated above, focus primarily on repeated deworming of individuals without adequate consideration of sustainable sanitation and hygiene improvements. This paper also challenges the WHO guidelines for scaling down of deworming programmes, which use infection indicators (prevalence) to trigger scale-down, rather than WASH indicators, which better reflect the risk of resurgence.

For this paper, the PhD Candidate was responsible for 80% of the conception, 90% of the research, 60% of the interpretation, and 80% of the drafting and writing of the paper. Clements A and Gray D were responsible for 20% of the conception, 5% of the writing and drafting and 10% of the interpretation. Savage G was responsible for 10% of the research, 15% of the writing and drafting, and 20% of the interpretation. Atkinson J, Soares

Magalhães R, Nery S, McCarthy J, Velleman Y, Wicken J, Traub R, Williams G and Andrews R were responsible for 10% of the interpretation.

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Opinion

A Critical Appraisal of Control Strategies for Soil-Transmitted Helminths

Suzy J. Campbell,[1,*] Susana V. Nery,[1] James S. McCarthy,[2,3] Darren J. Gray,[1,2] Ricardo J. Soares Magalhães,[4,5] and Archie C.A. Clements[1]

Interventions that lead to reductions in soil-transmitted helminths (STHs) include chemotherapy with anthelmintic drugs and improvements in water, sanitation, and hygiene (WASH). In this opinion article we aim to determine the evidence for optimal approaches for STH control. First we explore the evidence for the above interventions. We then appraise two integration strategies: current chemotherapy-oriented integrated neglected tropical disease (NTD) control and expanded 'multicomponent integration', which includes integrated chemotherapy, WASH, and other intervention strategies. While multicomponent integrated control may be an effective approach to sustainably reduce STH transmission, there is a need for evidence to prove the feasibility of this approach.

The Need for Optimal Soil-Transmitted Helminth Control Strategies

The STHs – *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), and *Necator americanus*, *Ancylostoma duodenale*, and *Ancylostoma ceylanicum* (hookworms) – infect more than 1 billion people and cause an estimated disease burden of more than 5 million disability-adjusted life years (DALYs) [1] (Figure 1). STH infections impair nutrient utilization causing anemia, particularly in young children [2], and may disturb cognitive development [3]. STHs are linked to stunted growth in children and to low birth weight, which predisposes to stunted growth [4]. This contributes to a vicious cycle of poor health whereby STH infections both result from and contribute to poverty in endemic communities [5].

Chemotherapy (see Glossary) and **WASH** (Box 1) improvements are recognized interventions for reducing STH infections. Chemotherapy is the principal way to achieve rapid, substantial reductions in STH prevalence and intensity [6,7]. WASH is believed to be required to break STH transmission cycles [8] but existing evidence is sparse. Achieving adequate and sustained WASH is a complex and expensive long-term objective in the poorest communities [9].

In this opinion article, we review evidence for the impact of chemotherapy and WASH on STH infections and then critically appraise integration strategies. Given the current global context, we focus broadly on **chemotherapy-based integrated NTD control** and elimination; the features of STH that are affected by this broader integration environment are emphasized. We assess extending chemotherapy-based NTD integration with interventions to sustainably reduce NTDs, including STHs. This could include interventions such as WASH, vaccines, and vector control strategies and interventions against zoonotic NTDs. We term this '**multicomponent integrated control**'. To conclude we recommend research directions that may establish evidence of sustainable benefits from STH chemotherapy programs integrated with WASH.

Trends

Chemotherapy and improving water, sanitation, and hygiene (WASH) are recognized interventions against soil-transmitted helminths (STHs). There is strong evidence that chemotherapy rapidly reduces STH burdens. There is insufficient evidence that chemotherapy benefits long-term health or that WASH impacts any STH outcome. Carefully designed trials are needed to strengthen this evidence base.

Integrated neglected tropical disease (NTD) control and elimination simultaneously reduces the burden of multiple diseases and can achieve high treatment coverage. This will have limited success in STH control due to rapid reinfection rates.

Integration should focus on primary prevention and intersectoral cooperation and permanent infrastructure that may reduce STH transmission. Evaluation is needed to prove that integrated NTD control contributes to health-system strengthening.

[1]Research School of Population Health, College of Medicine, Biology, and Environment, The Australian National University, Canberra, Australia
[2]QIMR Berghofer Medical Research Institute, Brisbane, Australia
[3]School of Public Health, University of Queensland, Brisbane, Australia
[4]Children's Health and Environment Program, Queensland Children's Medical Research Institute, University of Queensland, Brisbane, Australia

[5]School of Veterinary Science, University of Queensland, Gatton, Australia

*Correspondence: suzanne.campbell@anu.edu.au (S.J. Campbell).

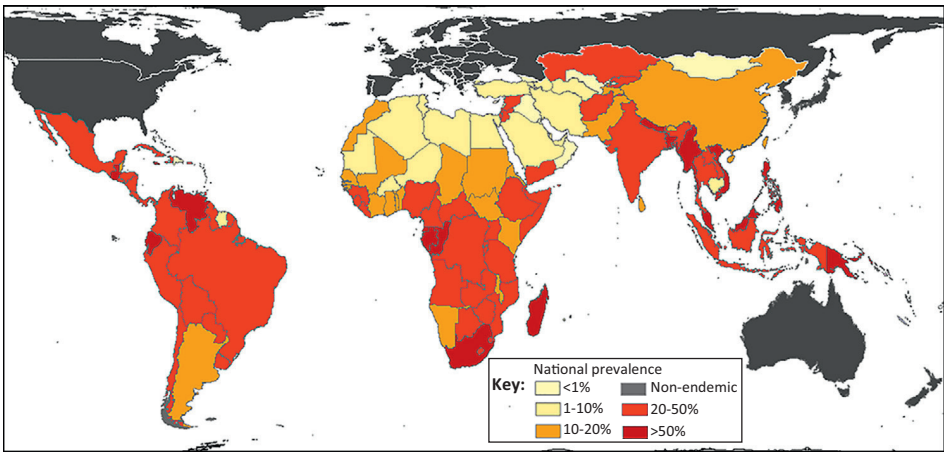

Figure 1. Global Distribution of Soil-Transmitted Helminths, 2010. Data from the *Global Atlas of Helminth Infection* were sourced to derive global estimates of soil-transmitted helminths (*Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus*, and *Ancylostoma duodenale*) [83]. Reproduced, with permission, from [83].

Chemotherapeutic STH Control

Chemotherapy reduces STH prevalence and infection intensity through rapid parasite clearance from the human host [10]. Five drugs (albendazole, levamisole, mebendazole, pyrantel, and ivermectin) are on the WHO model list of essential drugs for STH control [11]. The benzimidazoles (albendazole and mebendazole) have been given to millions of people, usually as single-dose tablets to schoolchildren in national helminth control programs [12]; however, their efficacy varies by drug, dose, helminth species, age, and infection intensity [6]. The cure rate of single-dose albendazole was recently reported as 98% for *A. lumbricoides*, 88% for hookworm, and 47% for *T. trichiura* [6]. For single-dose mebendazole, the reported cure rate is 95% for *A. lumbricoides*, 36% for *T. trichiura*, and 15% for hookworms [11]. Drug combinations involving benzimidazoles, levamisole, praziquantel, ivermectin, and oxantel pamoate [11,13–16] have presented opportunities for integrated NTD chemotherapy. More recently licensed drugs and veterinary drugs under investigation include nitazoxanide, tribendimidine, oxibendazole, triclabendazole, and oxantel–pyrantel [17,18]. *Bacillus thuringiensis* crystal proteins combined with nicotinic acetylcholine receptors *in vitro* may potentially be a potent combination anthelmintic [19]. While these drugs and combinations show promise, limited new drugs are being developed against STHs [6]. With a high burden of disease globally, research into the development of new and effective drugs is critically needed to broaden the base for chemotherapeutic STH control.

Advantages of Chemotherapy for STH-Associated Morbidity

Besides reduced STH prevalence and infection intensity, other effects on morbidity following chemotherapy are varied. Improvements in hemoglobin, weight, height, appetite, cognitive ability, physical fitness, and activity levels following chemotherapy in preschool and school-aged children have been reported [2,3,20–22] but other studies have found no effect on hemoglobin, iron status, or IgE level [23]. Treating pregnant women (of likely but not confirmed STH infection status in a highly endemic area) has been shown to improve maternal hemoglobin levels [24], improve infant birthweight, and reduce infant mortality at 6 months of age [25]. However Cochrane systematic reviews (most recently [26]) have repeatedly concluded that there was insufficient evidence to support improved morbidity outcomes. Studies are coming under intense scrutiny (http://blog.givewell.org/2015/07/24/new-deworming-reanalyses-and-cochrane-review/) [27,28] and may galvanize researchers to address evidence shortfalls. Despite this, chemotherapy is regarded as the most efficient and cost-effective way to reduce STH-associated morbidity [4].

Box 1. WASH: A Combination of Interventions and Philosophies

For epidemiologists, it is useful to consider WASH in terms of (i) infrastructure and (ii) behavioral components, as these require different quantitative or qualitative research. Infrastructural improvements are tangible, directly measurable investments within communities (e.g., installation of latrines, provision of access to a safe water supply), whereas hygiene (including health education) requires more sociological investigations into the aspects of behavior and culture that may be malleable to promote improvements in, or use of, WASH infrastructure. The two components are complementary and reliant on each other: infrastructure without behavioral change or vice versa will not be fruitful. This dichotomous conceptualization of WASH can explain a philosophical distinction that exists between the quantitative evidence-driven sector and the anthropological development sector that tends to focus on local ownership and community-tailored interventions, sometimes with little apparent segue back to health-related outcomes. Generating evidence for WASH benefits will be likely to require epidemiologists to undertake qualitative research to comprehensively investigate the parameters driving hygiene behaviors, as well as continuing quantitative analyses of WASH infrastructure.

Disadvantages of Chemotherapy

Chemotherapy programs only temporarily reduce STH transmission [29] because they cannot prevent reinfection. While WHO advocates repeated rounds of chemotherapy, in areas where chemotherapy programs have ceased infection prevalence and/or intensity has rapidly returned to baseline levels [30,31]. Studies have found STH infections in schoolchildren to continue after multiple rounds of treatment, sometimes with no reduction in prevalence or infection intensity [32,33]. This lack of sustained benefit considerably reduces program effectiveness. Without a break in STH transmission, no cessation dates can be set without risking resurgence.

Concerns of drug resistance have been expressed given the drug pressure that mass chemotherapy places on parasites [31]. Reduced efficacy of pyrantel, mebendazole, and albendazole against hookworms has been reported [34–37], as has extensive drug resistance in livestock helminth control programs [38]. Possible explanations for reduced efficacy include poor drug quality, reduced absorption and bioavailability, heavy infection intensity, variability of egg production and excretion, parasitological examination performed too soon after treatment, and poor strain susceptibility (tolerance) [39,40] rather than drug resistance. Strategies to reduce selection pressure involve approaches that promote refugia, including targeting treatment via school-based programs, or increasing treatment frequency to intervals greater than the helminth generation time [40]. However, these strategies may reduce the clinical effectiveness of STH control programs. Rotating drugs or using drug combinations may also delay drug resistance in parasites [11]. With limited drug alternatives, anthelmintic resistance could place the entire chemotherapy basis for STH control at risk.

Other concerns about chemotherapy include possible increased eczema risk in children whose mothers take anthelmintics during pregnancy [41]. Further, heavy reliance on drugs can lead to the introduction onto the market of poor-quality substitutes. In a recent investigation undertaken in Ethiopia, up to 45% of tested benzimidazole drugs did not meet pharmacopoeial acceptance criteria [42]. Where possible, drug quality must be monitored within country distribution systems.

Outputs of recent mathematical [8] and statistical [43] modeling studies indicate that chemotherapy of schoolchildren may suffice to break STH transmission cycles in specific epidemiological settings; for example, low-transmission environments with strong health systems and drug delivery mechanisms. In these settings elimination may be feasible [43]. However, other settings may require high-coverage, high-frequency, and broader community-based treatment [8,43], with additional WASH efforts in high-transmission settings (see below) [8,43]. While chemotherapy is important for STH control, when implemented alone it may have some negative consequences. For example, it has been argued that the focus on chemotherapy has been a disincentive to investments by the NTD sector in sustainable strategies such as WASH. This is exacerbated by insufficient epidemiological investigation of the effect of WASH on STH outcomes.

Glossary

Chemotherapy/preventive chemotherapy/deworming/drug administration: for STH control, these terms simply mean the provision of deworming tablets, but the terminology has been made complex. Both 'deworming' and 'chemotherapy' imply treatment of people infected with STH. 'Preventive chemotherapy' is highly misleading given that chemotherapy is unlikely to prevent infections across the community, or reinfections. 'Drug administration', which implies treating people regardless of infection status, would appear to be the most appropriate term. However, given that the most common term in the literature is 'chemotherapy', this term is used in this opinion article.

Integrated neglected tropical disease (NTD) chemotherapy: the integrated mass distribution of drugs against multiple NTDs.

Multicomponent integrated NTD control: the augmentation of integrated NTD chemotherapy with additional interventions (components) to reduce NTDs, including STHs. This transcends chemotherapy to include interventions such as WASH, vaccines, vector control strategies, and interventions against zoonotic NTDs.

Water, sanitation, and hygiene (WASH): the provision of access to a safe water supply, appropriately constructed sanitation infrastructure ensuring safe disposal of human excreta, and health education and promotion of hygiene (personal and household practices aimed at preserving cleanliness and health). This acronym is flexibly used in the literature. Some studies use the term to describe individual components (e.g., water access, sanitation) and others use the term to describe the integration of these components. In this opinion article, WASH is used as the umbrella term for the components of water, sanitation, and hygiene (including health education as a main conduit of hygiene promotion). We use the term 'integrated WASH' to describe WASH activities where the individual components cannot be considered separately.

Evidence of WASH Impact on STHs

WASH Infrastructure

Current evidence for the impact of WASH projects on STHs is observational and relatively sparse. In a systematic review [44], 54% reduced odds of any STH infection were reported with the use of treated water. Piped-water access was associated with reduced odds of *A. lumbricoides* (60%) and *T. trichiura* (43%) infection but no association was found between piped-water access and undifferentiated STHs. Additional recent associations have been reported between STH infections and drinking untreated water [45,46]. Providing access to fresh water in sufficient quantities enables people to practice safer hygiene behaviors, which is likely to reduce transmission.

In two systematic reviews [44,47] 38–46% reduced odds of *A. lumbricoides* infection and 39–42% reduced odds of *T. trichiura* infection were reported for latrine availability and usage. In one of these reviews [47], 40% reduced odds of hookworm infection was reported and in the other [44] 34% reduced odds of undifferentiated infection was reported associated with sanitation access. In a randomized controlled trial (RCT) of community and household latrine promotion and construction, latrine coverage increased (from 9% to 63% of households in intervention villages) but no reduction in STH prevalence was reported [48]. The lack of effect may be from either insufficient coverage or insufficient reduction of open defecation [48]. Highly responsive people will be reinfected if their neighbors continue open defecation.

Surprisingly few studies assess STH outcomes with 'improved' versus 'unimproved' water supplies or sanitation [as defined by the WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation (http://www.wssinfo.org/definitions-methods/watsan-categories/)]. Evidence is lacking on whether even adding a cement slab to an existing latrine reduces transmission [49]. Further, an improved latrine may transmit STH if it is poorly maintained, incorrectly used [50], or shared between households. STH infection is linked with low socioeconomic status, which presents a challenge for STH control; people knowledgeable about STH transmission may still be economically prevented from avoiding transmission [51].

WASH Behavior

In a systematic review [44] of observational studies, reduced odds of STH infection were reported for hand washing after defecation (53%) and soap use or availability (47%). Hand washing before eating and after defecating was associated with reduced odds of *A. lumbricoides* infection (62% and 55%, respectively) [44]. Two RCTs [52,53] provided strong evidence for the benefit of health education including hand washing after toileting. In one trial [52] a 90% higher mean score for STH knowledge was reported among children who received a hygiene promotion intervention, with almost 45% more intervention children hand washing after toileting, and 50% reduced STH prevalence compared with controls. In the second RCT [53], 58% reduced intensity of *A. lumbricoides* infection was reported among schoolchildren who had received a hygiene education program compared with controls. In an unblinded controlled trial [54], children who received a school-based health education package had significantly reduced hookworm reinfections and STH intensity, with improved knowledge of STH among teachers and the wider population. However, the results of two other RCTs indicated no significant differences in *A. lumbricoides* prevalence or school absenteeism between children who received health education and those who did not [55,56]. Post-trial nonrandomized analyses indicated more absenteeism among students with moderate-to-heavy *A. lumbricoides* infections and light hookworm infections during study follow-up. The inability to blind many hygiene studies makes impact assessment very challenging [57].

Using shoes to protect feet from contaminated soil has been shown to be protective in two systematic reviews [44,58] and should be advocated as a STH control measure. However,

protection is likely to be partial, given that *N. americanus* larvae can penetrate any exposed area of skin and other species are also infectious through oral exposure [29]. Using untreated or inadequately treated night soil as fertilizer on food gardens is hazardous as it directly allows infective stages of STHs to enter the human food chain. Large percentages of eggs, particularly those of *A. lumbricoides* and *T. trichiura*, remain viable at ambient temperatures even after 1 year [59]. Composting of excreta or chemical treatment of sewage must be conducted before excreta can be used [40].

Integrated WASH Interventions

Evidence for integrated WASH, or for augmenting chemotherapy with WASH, is limited. In the only adequately powered RCT that has investigated a school-based integrated WASH program (hygiene promotion, water treatment and storage, and installation of latrines) plus albendazole, a significant reduction in *A. lumbricoides* reinfection was observed among schoolchildren who received the intervention compared with albendazole alone [60]. However, no significant effects on other STH species were observed [60]. In a nonrandomized multi-intervention study [61], different combinations of health education, chemotherapy, and latrine construction all significantly reduced STH prevalence and intensity, with the highest prevalence reductions achieved with chemotherapy combined with sanitation and health education. While a systematic review [50] found more than 60% reduced STH prevalence with combined chemotherapy, sanitation, and health education compared with sanitation and health education alone, the underlying studies were observational, potentially suffering from confounding or bias.

A major additional benefit of WASH is its potential for reducing a range of diseases, particularly those causing diarrhea. Improving sanitation is hypothesized to improve health iteratively by contributing to improved economic development, which in turn bolsters sanitation infrastructure in a cycle of positive economic change [62]. However, improving WASH is enormously challenging. Financial and logistic difficulties impair community-wide WASH programs. Further, WASH is subject to cultural and environmental influences; for example, differing water-table depths and variations in community acceptance [49]. Finally, the benefits of introducing WASH in terms of reduced infection levels may be delayed by decades [29].

Integrated Chemotherapy for NTD Control and Elimination

With effective advocacy from the WHO, bilateral donors, and pharmaceutical companies, integrated NTD chemotherapy promotes the integration of separate mass drug administration programs targeting multiple NTDs including lymphatic filariasis, onchocerciasis, schistosomiasis, trachoma, and STHs [10]. This approach is based on the tenet that these diseases can be controlled or eliminated via large-scale chemotherapy [8,10] motivated by the desire to simultaneously achieve multiple-drug coverage in endemic areas. This has been facilitated by major philanthropic investment and large-scale pharmaceutical drug donations in one of the broadest public health campaigns ever attempted. Integrated NTD chemotherapy programs have been proposed to increase health benefits [63], be cost-effective and accessible [10], and contribute to drug delivery efficiencies, reduced program duplication, and, ultimately, health-system strengthening [63]. This entire approach is based on a drug delivery system.

The evolution of integrated NTD chemotherapy is as follows. STH chemotherapy traditionally utilized a 'vertical' health-service integration model, characterized by donor aid and disease-oriented outcomes, to circumvent the generally scarce availability of resources in endemic countries (Table 1) [64]. However, in recent years there has been a strong impetus to integrate NTD control and elimination programs into national health strategies. Largely driven by the WHO and international donors, this first commenced for STHs in 2001, with a push for national STH and schistosomiasis plans in 2002 [10]. Over succeeding years this has grown into more comprehensive integrated NTD chemotherapy strategies, primarily through World Health

Table 1. Vertical and Horizontal Integration and Service Delivery Models

	Vertical Integration	Horizontal Integration
Features	Top-down integration tied to specific donor priorities (and reporting) Tend to be disease or outcome specific Interventions not fully integrated into health systems	Delivery of health services through public-financed health systems as primary health care Usually under the auspices of the national government (usually Ministry of Health or equivalent)
Advantages	Useful for developing countries that do not have health-system infrastructure to support horizontal programs Can be kept separate from public sector intervention (which may include political interference) Results-driven approach tends to be realized due to intensive activity; this is often vital to, and can perpetuate, continuation of donor funding	More cost-effective and therefore more sustainable than vertical integration Most useful when countries face multiple concurrent disease challenges and need to reach a large population of people (including those who cannot access private providers)
Disadvantages	Can create siloed services and inefficient use of human and financial resources Often high cost, with diversion of resources from other areas of the health system, affecting long-term sustainability Approach does not lend itself to integration into the broader health system Funding for a specific priority can lead to wholesale neglect of other disease priorities	Often has complex service delivery and management, which can make attainment of health goals difficult Existing infrastructure and an established health system are essential for success Not independent from other health programs; therefore, can be affected by changing priorities and politics (including conflict)

Assembly resolution 66.12 [12] in 2013, which called for expanded NTD interventions to reach agreed global targets (http://www.who.int/neglected_diseases/NTD_RoadMap_2012_Fullversion.pdf). The WHO has supported countries to develop strategic 'NTD master plans' tailored to national NTD control and elimination priorities [10]. These plans have included an assessment of the within-country NTD epidemiology and coendemicity, national objectives and actions, and budgetary issues [10].

Depending on existing country capabilities, this approach can still be undertaken using a vertical implementation strategy. However, many countries are including both vertical and horizontal elements (Figure 2), harnessing the key benefits of achieving sustainability and health-system strengthening. By incorporating horizontal components, this NTD approach represents an attractive but challenging paradigm shift. It not only seeks to redefine NTD control and elimination but extends further, into fermenting necessary reforms of the wider primary health-care system in accordance with the founding principles defined in the original Alma-Ata Declaration (http://www.who.int/publications/almaata_declaration_en.pdf) and urged in the 2008 World Health Report (http://www.who.int/whr/2008/en/). This approach will ensure that NTDs remain a priority in the global health agenda and will contribute to the agenda of addressing how 'universal coverage' is implemented as part of primary health care [12]. Further, after the vertically oriented approach of the Millennium Development Goals (MDGs), a more horizontal approach better aligns with the Sustainable Development Goals (SDGs) (https://sustainabledevelopment.un.org/?menu=1300), with the focus on intersectoral cooperation presenting additional opportunities for health-system strengthening.

Implementation of integrated NTD chemotherapy programs has required considerable national and international scale-up of funding, drug donations, human and other resource deployment, in-country technical expertise, and political and programmatic will. Prevalence mapping has been encouraged to determine disease distributions (usually across several endemic NTDs to identify subnational foci [65]) and enable in-country resource prioritization [65,66]. This approach

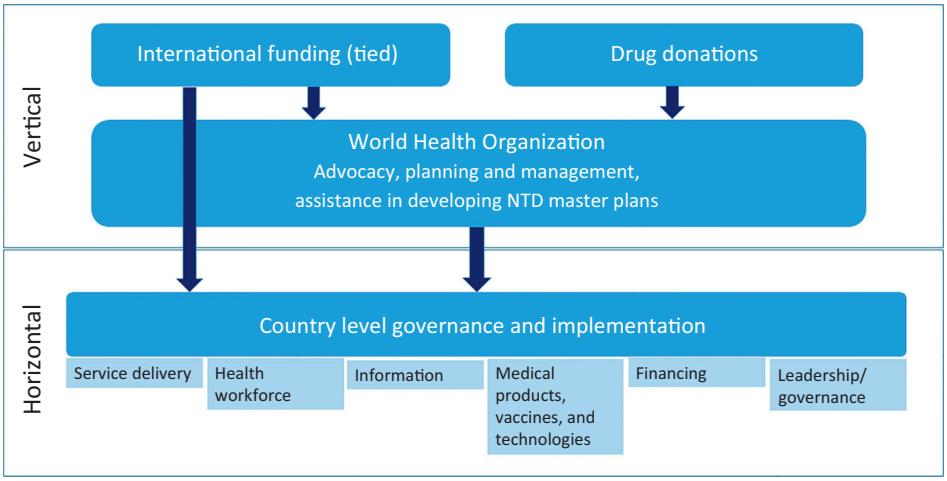

Figure 2. Schematic Diagram of Integrated Neglected Tropical Disease (NTD) Control and Elimination Programs, Highlighting Vertical and Horizontal Components. Large-scale international funding, pharmaceutical drug donations, and direction from the WHO form the vertical basis of this approach. At the country level, the implementation and management of tailored NTD master plans (usually managed by the Ministry for Health) may require vertical elements but where possible should utilize existing health-system infrastructure according to the capabilities of the country. NTD programs are one of a suite of health programs implemented within the broader health system. This diagram is highly simplified; it does not show the range of government tiers, public–private partnerships, non-government activities, or other aspects that may be present in many primary health-care systems. Adapted from [84].

requires developing or enhancing diagnostic capabilities and surveillance systems to contribute accurate and timely data. Extensive operational resources are required to integrate control and elimination activities into existing health-care or school infrastructure, ideally involving community participation with consideration of equity and access issues. Utilizing school infrastructure has been encouraged to enhance sustainability and to reach vulnerable populations at high coverage, with added benefits of providing health education and increasing school enrolment and attendance [10]. If reach is broadened beyond schoolchildren, delivery may also occur via health centers, aid posts, and outreach clinics and can be structured as health packages that include antenatal care, immunizations, vitamin A distribution, maternal health checks, and health education [10,61,67].

Evidence for Reach of Integrated NTD Chemotherapy

Most evidence for integrated NTD chemotherapy programs is reported as treatment coverage or disease reduction; this is evidence for chemotherapy program reach, not evidence for 'integration' *per se*. Integrated chemotherapy has been proposed to improve coverage in school-age children and to achieve large reductions in morbidity [12]. Coverage statistics are increasing each year; for STHs, over 338 million schoolchildren received chemotherapy in 2012 alone [12]. It is unclear how much this represents integrated NTD control versus albendazole distribution only. In terms of disease reduction, countries are starting to eliminate specific diseases, including onchocerciasis in Colombia and Ecuador [12], and have reduced many others, including lymphatic filariasis, leprosy, trachoma, schistosomiasis and STHs [68].

Strong advocacy for integrated NTD chemotherapy has facilitated the development of NTD master plans in over 70 countries [12]. However, with few exceptions [69,70], there is little evidence for what is, or is not, effective at a national or program level. Evaluations of community-directed treatment strategies [71–74] have provided information on success factors and enablers, including costs, stakeholder participation, community commitment, impact of

incentives, and the supply chain for intervention materials. Further evidence on how countries address resource allocation, technical upskilling, and integration into existing health initiatives is required. It is imperative that structurally sound monitoring and evaluation activities be built in and implemented from the outset. These need to incorporate ongoing surveillance and monitoring of disease and treatment outcomes, operational efficiencies, and programmatic measures of success and to ultimately demonstrate improvement in health indicators [12]. NTD master plans provide an important opportunity to develop an evidence base not only to inform on the benefits of NTD integration programs but also to effect broader primary health-care reform.

Challenges with NTD Integration

Integrated chemotherapy does not address the disadvantages of chemotherapy for STHs raised previously. Current epidemiological data provide little prospect of scaling down STH deworming programs without resurgence. Even if drug donations are assured, the lack of sufficient drug distribution mechanisms in many countries is a further risk to integrated NTD chemotherapy. This is likely to reflect varying country health-system capabilities, within-country access, and political and economic situations. Limited mechanisms to deliver drugs affects coverage (35% global coverage for STHs reported in 2012 [12]). It is unclear whether reported coverage includes retreatments recommended in highly endemic areas. Most schoolchildren remain at risk of STH reinfection and preschoolers and adults may be missed altogether, with the consequence that this strategy will have little effect on STH control.

Given country-level resources, any declining international funding impetus could jeopardize the global NTD initiative. Further, integrated NTD chemotherapy represents one of a suite of initiatives being advocated on the basis of universal coverage (including the WHO's 'End TB' and malaria elimination campaigns). In some countries this may facilitate sufficient infrastructure to integrate NTD activities and strengthen health systems. However, in others trying to simultaneously address all strategies may increase health-system fragility. Increased competition for resources for concurrent activities may lead to program failure if this cannot be successfully managed. This could adversely affect in-country political support, particularly in conflict-affected countries. At the very least, strong governance and cross-program cooperation is required.

Intersectoral Integration Including WASH: Multicomponent Control

Multicomponent integrated control augments drug-based integrated NTD chemotherapy. It focuses on integrating known beneficial interventions ('components') to cumulatively reduce NTD transmission and reinfection more than is possible with one intervention alone. This approach could include integrating chemotherapy with WASH, vaccines (when available), vector control, and interventions against zoonotic NTDs such as *Ancylostoma ceylanicum*, *A. caninum*, *Ascaris suum*, and *Schistosoma japonicum*. Vaccines in particular might be a long-term mechanism to achieve hookworm control [75]. Multicomponent integration could establish a degree of permanent infrastructure and sustainability, further enhance existing resource utilization, and allow flexible tailoring to community needs. An intersectoral approach involving private sector, government (including 'One Health', animal health, and non-health ministries), and non-government organizations is more likely to be successful. Critically, this requires redefining health priorities in a broader primary prevention context. Multicomponent integration needs to be carefully planned and implemented as it is both complex and expensive.

For STHs, multicomponent integration in Seychelles provides evidence that integrating chemotherapy and WASH can reduce STH prevalence and intensity and, importantly, be effectively incorporated into the existing primary health-care system [76]. The success of the Rockefeller Foundation hookworm control strategy in the southern USA is also credited with integrating chemotherapy and sanitation accompanied by economic development [77]. Broader than

STHs, multicomponent integrated schistosomiasis control programs have reduced the incidence of schistosomiasis and achieved local elimination in some settings [78–80]. The Chinese approach in particular emphasizes chemotherapy, WASH, agricultural mechanization, and fencing of water buffaloes [79–81], with reported reductions in schistosomiasis infection, *S. japonicum*-infected snails, and STHs [81]. NTD control measures can be integrated with malaria control and elimination initiatives (taking advantage of the activity of ivermectin against malaria vectors or possibly bundling with separate malaria interventions such as insecticide-treated net distributions) [60,61,65]. The SAFE (surgery, antibiotics, facial cleanliness, environmental improvements) strategy of trachoma elimination programs and the treatment of animals to control transmission of *Trypanosoma rhodesiense* [82] are additional examples of multicomponent integrated NTD control.

It is surprising that evidence for integrated chemotherapy and WASH is not stronger. Few RCTs have been attempted. This is likely to reflect difficulties in conducting RCTs containing WASH elements: randomization can conflict with program implementation and there are ethical challenges in designing studies entailing withholding an intervention from a community in need. However, such trials are possible. Some RCTs are utilizing a delayed intervention approach whereby control communities receive the WASH intervention at trial completion [51,58]. There is an urgent need to conduct further appropriately structured trials [9], such as carefully constructed longitudinal analyses or stepped-wedge RCTs, to evaluate the impact of integrated chemotherapy and WASH on STH outcomes. Given the resource constraints highlighted previously for both chemotherapy and WASH activities, integrating WASH with chemotherapy may seem to impose impossible additional requirements. However, increased global WASH impetus is occurring, with SDG Goal 6 aimed at ensuring the availability and sustainable management of water and sanitation for all (https://sustainabledevelopment.un.org/?menu=1300). Significant international effort to address this will be required. This may bring opportunities if the NTD sector is prepared to invest in multicomponent, intersectoral interventions.

Concluding Remarks

This opinion article highlights gaps in evidence and challenges in establishing sustainable benefits in health outcomes from STH chemotherapy programs and WASH, implemented either separately or together, and critically appraises integrated approaches for STH control. There is clearly a lack of evidence regarding the effect of WASH on STH outcomes. Carefully designed experimental trials are required to establish evidence to support the benefit of such programs and should be a priority (see Outstanding Questions). However, given the complexity and challenges of trial design and execution and, further, the lack of unequivocally improved health outcomes thus far seen in large-scale programs, additional strategies such as mixed-methods epidemiological analyses may be required to investigate alternative approaches. Meanwhile, lack of proven benefit is an insufficient argument for not investing in WASH. For chemotherapy, research into developing new and effective drugs against STHs should be a priority.

The other main evidence gap relates to the effectiveness of integrated NTD chemotherapy programs. This stands in contrast to this being proposed as a key platform from which sustainable multicomponent NTD interventions could be launched. Development of NTD master plans has placed the international community at a nexus for determining optimal approaches for NTD control. Evaluation of country-integrated NTD chemotherapy programs will provide evidence, so crucially needed in the health sector, for effective integration. Where such plans can add WASH elements, this will help address the evidence gap where it is needed the most: the evidence for integrated multicomponent strategies. Multicomponent integration would enable holistic NTD control that addresses STHs in particular. It would also build an intersectoral primary prevention base, facilitating additional health-system strengthening and ultimately contributing to the attainment of universal coverage.

Outstanding Questions

What metrics can be used to quantify STH-related morbidity and are these feasible to measure in research trials? To strengthen evidence for an impact of chemotherapy on morbidity outcomes, we need to move beyond measuring STH prevalence and the intensity of infection.

What trial designs will be most likely to assess benefits of WASH? What elements of WASH have the greatest impact on STH?

What are the best indicators for assessing progress toward STH control and elimination targets and how will we know when they are achieved?

How will different program aims (e.g., NTD elimination vs NTD control) affect STHs and what are the long-term implications for STHs if NTD control and elimination programs cease on attainment of non-STH disease-reduction goals?

What indicators are required to assess the effectiveness of integrated NTD programs and are these indicators feasible in all settings?

What are the enablers and barriers to effective program integration that countries are experiencing? Are these country, locality or culturally specific? Is there system flexibility to overcome challenges?

How can we most effectively approach intersectoral cooperation to establish primary prevention goals?

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PLOS | NEGLECTED TROPICAL DISEASES

Policy Platform

Water, Sanitation, and Hygiene (WASH): A Critical Component for Sustainable Soil-Transmitted Helminth and Schistosomiasis Control

Suzy J. Campbell[1], Georgia B. Savage[2], Darren J. Gray[1,3], Jo-An M. Atkinson[1], Ricardo J. Soares Magalhães[1], Susana V. Nery[1], James S. McCarthy[1,4,5], Yael Velleman[6], James H. Wicken[2], Rebecca J. Traub[7], Gail M. Williams[1], Ross M. Andrews[8], Archie C. A. Clements[1]*

1 School of Population Health, University of Queensland, Brisbane, Queensland, Australia, **2** WaterAid Australia, Melbourne, Victoria, Australia, **3** Molecular Parasitology Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia, **4** Clinical Tropical Medicine Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia, **5** Department of Infectious Diseases, Royal Women's and Children's Hospital, Brisbane, Queensland, Australia, **6** WaterAid, London, United Kingdom, **7** School of Veterinary Sciences, University of Queensland, Gatton, Queensland, Australia, **8** Menzies School of Health Research, Charles Darwin University, Darwin, Northern Territory, Australia

Introduction

Soil-transmitted helminths (STH) and schistosomes are parasites that affect the world's poorest people, causing losses of up to 39 million and 70 million disability adjusted life years (DALYs) respectively [1,2]. The World Health Organization (WHO) is at the forefront of developing policy for the control of STH and schistosomiasis, advocating for chemotherapy as the cornerstone of control, with the objective of reducing infection-associated morbidity [1,3,4]. Global uptake of chemotherapy with albendazole or mebendazole for STH and praziquantel for schistosomiasis has significantly increased and remains the principal control strategy. It is cost-effective [5] and reduces STH [6] and schistosome [7] infections in human hosts.

However, a fundamental limitation of chemotherapy for STH and schistosomiasis control is that it does not kill immature worms and cannot prevent reinfection. Chemotherapy-based control programmes have a temporary effect on transmission [8]. Indeed, studies have shown that infection prevalence and intensity can rapidly return to baseline levels soon after chemotherapy programmes are ceased. One factor is that the ability of helminth eggs and/or larvae to survive for extended periods in the environment [9] creates a source for rapid reinfection following chemotherapy [9]. A second is that small sections of the population usually remain out of reach of chemotherapy programmes, subgroups that frequently have a disproportionately heavy burden of infection, thereby serving as a reservoir for reinfection. Thus, longer-term effectiveness of chemotherapy in interrupting transmission is dependent on maintenance of regular retreatment. Many helminth control programmes rely on donated drugs [3], so there is a degree of uncertainty around their sustainability in the long term. In endemic areas, once mass treatment is stopped, disease prevalence can return to pretreatment levels within 18–24 months [10–12]. For schistosomiasis, cessation of chemotherapy can also result in more severe rebound of immunopathology [13].

The most frequently used chemotherapeutic drug, albendazole, does not have 100% efficacy [14]; therefore, chemotherapy programmes will not cure all treated individuals. Additionally, helminth control programmes have predominantly focused on specific risk groups (primarily schoolchildren) rather than the whole community, despite evidence in many communities that prevalence may be high in other groups [15], for example, preschool children [16]. A shift in approach to community-wide chemotherapy, or at least to include preschoolers as a target population, could potentially have a great impact on further reducing STH infections, particularly in settings where there is high prevalence in nonschool groups or where many children do not go to school.

Even where there are continuous control programmes, there is some evidence of declining uptake due to fear of treatment and poor communication about the chemotherapy process [17]. There is also the potential that mass drug administration may result in drug resistance, as is occurring in livestock helminth control programmes [18–20]. Humphries et al. (2011) believe that, given the current treatment pressure, it will only be a matter of time before drug resistance is seen in STH species that infect humans [21]. Controversially, recent reviews indicate that, on the basis of measures of infection-associated morbidity (such as improvements in nutrition, haemoglobin levels, school attendance, and school performance), there is insufficient reliable evidence to justify contemporary chemotherapy programmes [22,23]. We do, however, recognise that in developing country settings, where multiple disease and health-related interactions are likely to take place, it is difficult to associate nonspecific morbidity indicators to STH or schistosomiasis. Other issues that are not yet resolved with regards to chemotherapy

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* E-mail: archie.clements@anu.edu.au

include potential teratogenic effects of benzimidazole drugs and associations with eczema in children following maternal chemotherapy during pregnancy [24]. Thus, whilst chemotherapy is necessary to rapidly reduce the burden and morbidity of helminth infections, we argue that by itself it is an unsustainable strategy for helminth control and for reaching control and elimination targets. This highlights the essential role of interventions aimed at reducing environmental exposure, which chemotherapy alone does not address.

The provision of access to WASH, being a safe water supply, appropriately constructed sanitation infrastructure that ensures safe disposal of human excreta, and the promotion of hygiene (defined as personal and household practices such as hand-washing, bathing, and management of stored water in the home, all aimed at preserving cleanliness and health), is critical. WASH is a necessary but undervalued tool for helminth prevention and control, aiming to provide long-term improvements in people's wellbeing. Interventions that include WASH have been shown to be highly effective in reducing the environmental exposure to, and transmission of, eggs and larvae for STH [25] and schistosomes [26]. A 29% decrease in *Ascaris lumbricoides* prevalence and as much as a 77% reduction in schistosomiasis prevalence has been observed following implementation of improved water and/or sanitation facilities [25]. A recent study in three African countries estimated that the population attributable fraction (PAF) of schistosomiasis due to no piped water was 47–71% [27].

Areas with poor sanitation coverage often experience a high burden of disease from STH and schistosomiasis (Figure 1). WASH implementation can be complex and comprised of a large set of "hardware" (e.g., toilets, latrines, sewage treatments, and provision of safe water) [16] and "software" (e.g., behaviour change promotion and community resource management) elements, many of which are, strictly speaking, outside the official service delivery remit of the health system. Challenges for implementing WASH can include cost, lack of health professional involvement [28], lack of local government involvement and local public-private partnerships for latrine and infrastructure development [29], lack of advocacy [30], inappropriate choice of technology, poor operation and maintenance, inadequate revenue collection, lack of adequate and equitable financial investment from both government and international donors [31], and the lack of perception in many rural communities of the importance of improved excreta disposal practices [32]. This requires genuine cross-sectoral collaboration and political will; investment in WASH in developing countries contributes to practically all of the Millennium Development Goals (MDGs) [28], and should not be overlooked for helminth control simply because chemotherapeutic interventions exist that require a seemingly lower financial and logistical commitment.

Helminth Control Guidelines and the Neglect of WASH

For many years, authors [6,8] have argued that the effects of chemotherapy can only be sustainable if integrated with improvements in health promotion, hygiene, and sanitation. This has been recognised and advocated for in the World Health Assembly (WHA) resolutions on STH and schistosomiasis, as well as the recent resolution on NTDs. These foundational policy guidelines clearly highlight the importance of WASH as a fundamental component of helminth control and elimination [33–35]; however, as discussed below, WASH is not embraced in subsequent disease-specific control guidelines (e.g., STH and schistosomiasis). A longer-term view of effectiveness and sustainability of control efforts requires integrating interventions to reduce transmission and reinfection. Yet interventions such as WASH have been slow to be incorporated into control programmes. It is for this reason that the parties to the London Declaration on NTDs are seeking more coordinated access to clean water and basic sanitation, improved living conditions, vector control, health education, and stronger health systems in endemic areas [36].

The WHO published guidelines for the prevention and control of STH and schistosomiasis infections in 2002 [1] and recently produced updated guidelines entitled "Helminth control in school-age children: a guide for managers of control programmes, 2nd edition" [3], specifically targeting STH and schistosomiasis. This second document acknowledges the importance of WASH and provides advice that helminth control programmes need to comprehensively include WASH, with the definitive statement, "The only definitive solution for eliminating schistosomiasis and STH infections is improvement in environmental conditions and a change in risk behaviours" [3]. However, chemotherapy is prioritised as the "first-line rapid control measure," while improved water and sanitation and health education should be only "implemented according to the epidemiological situation and the availability of resources" [3]. No clear definition of what is meant by "epidemiological situation" in this context is provided. Our concern is that these last two statements will have the unintended effect of delaying action on WASH in favour of chemotherapy, without interrupting the vicious cycle of disease transmission. The guidelines could be enhanced by inclusion of comprehensive recommendations for implementing WASH hardware and software, citing methods and examples such as the Community-Led Total Sanitation (CLTS) approach, which has now been successfully implemented in over 20 countries [37], sanitation marketing, and other approaches that focus on creating demand for sanitation and changing unhealthy behaviours.

Of significant concern regarding the current WHO guidelines is that they contain no recommended control activities where prevalence of STH infection below 20% is identified at baseline [3]. Instead, following the chemotherapy focus of the document, "Affected individuals should be treated [for STH] on a case-by-case basis" (Table 2.3 in [3]); however, no suggestions for identifying these individuals are proposed. Such an approach needs to be supported by rigorous epidemiological evidence that clearly demonstrates benefits to the community concerned and appropriate mitigation of the risk of cross-infection into uninfected individuals. STH and schistosomes are extremely difficult to eliminate in communities where poverty and inadequate water and sanitation prevail, due to their high transmission potential [38]. Lack of specifying control activities in this scenario represents, at the very least, a missed opportunity for recommending WASH activities, particularly given the level of morbidity likely to be experienced in a community with 20% STH prevalence.

An additional area of the WHO guidelines that warrants close scrutiny are decision trees in the annexes, which recommend reducing frequency of chemotherapy after five to six years, based solely on measurements of prevalence. For prevalence of STH or schistosomiasis below 1%, the WHO guidelines indicate, "morbidity is under control with low risk of re-emergence," although serology for schistosomiasis is recommended with positive cases continuing to receive chemotherapy [3]. It is unclear whether serology is intended for all schoolchildren in this scenario, and additionally there is no evidence to indicate that risk of re-emergence of disease is not a problem at this threshold

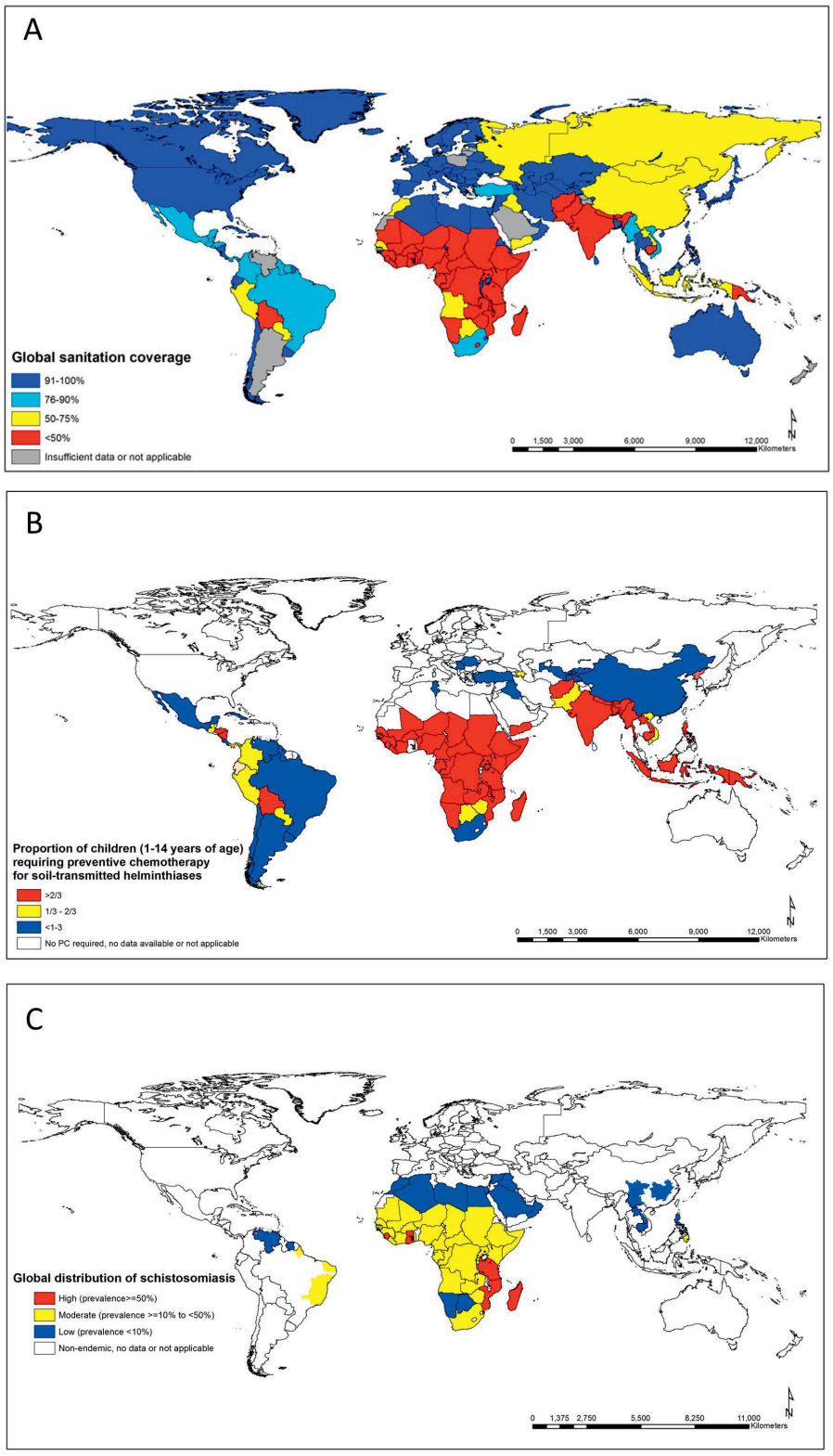

Figure 1. Consistencies in the global need for improved WASH and parasite control. (a) Global sanitation coverage (adapted from [51]). (b) Global requirements for chemotherapy for STH (adapted from [3]). (c) Global distribution of schistosomiasis (adapted from [3]).
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level, particularly if WASH is not adequate. We propose that WASH indicators be added to the decision trees, to provide sounder guidance for programme managers in their decision-making about helminth control programmes. It would also more comprehensively mitigate risk of resurgence of STH and schistosomes, as it would address necessary environmental improvements for control, as well as demonstrate longer-term, sustainable benefits to the communities concerned.

The WHO guidelines published in 2002 [1] were the first such document of its kind. It admirably articulated a large volume of technical information to assist programme managers develop prevention and control strategies. The more recent version, however, does not seem to have progressed considerably from the earlier version. Rather, the recognition in the 2002 version that resources must not be diverted prematurely in countries where morbidity has been significantly reduced but transmission continues [1] mitigates risk more appropriately than the current second edition guidelines. We believe there is a strong justification for a further revision to be undertaken.

Getting the Indicators Right

The current WHO guidelines use prevalence of infection as the most emphasised indicator of the success of worm control programmes, whilst the "condition of latrines and the quality of water supplies in schools may also be monitored if their improvement is one of the objectives of the programme" [3]. Use of prevalence is insufficient as it does not place emphasis on using interventions that have a more sustainable impact. Given the reinfection rate of STH and schistosomes, being guided by prevalence rates alone is high risk. As the WHO guidelines correctly point out, remaining "parasites maintain transmission capacity despite intense drug pressure, and this is predictive of a rapid return to high levels of prevalence if the [chemotherapy] intervention is interrupted" [3]. Intensity of infection (as measured by number of eggs in stool/urine) is markedly different within various groups of the community, such as different age groups and sex [39]. Thus, prevalence can easily mask the high transmission potential of a relatively small number of individuals. Hygiene activities are included with indicators for monitoring numbers of hygiene education programmes conducted, although these would not sufficiently measure hygiene behavioural change.

We recommend that, at the very least, corresponding WASH access indicators be included in any revised versions of WHO helminth control guidelines. These could include the MDG seven indicators of (i) proportion of the population using an improved drinking water source and (ii) proportion of the population using an improved sanitation facility [40], with "improved" water and sanitation defined by the WHO-UNICEF Joint Monitoring Programme for Water Supply and Sanitation [41]. These are the most developed and consistently used WASH indicators. Many national health surveys are collecting data on some of these indicators; thus the addition of these indicators should not involve adding completely novel indicators into helminth control programmes. We acknowledge that there has been some criticism of the MDG indicators with regards to equity, specifically, that the MDGs target the richer proportions of each country's population, rather than those at greatest need. This has not been resolved, and there has been a general call to develop more equitable indicators beyond 2015 [42]. However, based on current approaches, these indicators appear the most suitable at this time for ensuring that WASH is addressed in conjunction with chemotherapy.

There should also be guidance on appropriate implementation provided in the second edition WHO guidelines. Such guidance should encourage best-practice sanitation and hygiene promotion approaches relevant to the context in the programme location. The CLTS approach, which avoids the use of hardware subsidies and "latrinification" (construction of latrines for households without commensurate efforts to ensure safe sanitary practices and ownership and adequate maintenance of latrines) is one potential approach, alongside other emerging approaches such as sanitation marketing, which focuses on creation of demand for household investment sanitation hardware in order to allow progressive improvement away from basic latrines. Guidance should also specifically encourage improved coordination and planning across sectors, such as the participation of WASH agencies in national NTD task forces. It is known that sanitation does not become effective until it is used by a high percentage of the population [25,43], with coverage of properly built, used, and maintained sanitation required to be 90% to have an effect on STH transmission [44]. If insufficient proportions of people in a community have access to sanitation, even those who have latrines will still be at risk of infection [45], particularly if there is latrine access at local schools or institutions but not within the community, or vice versa. For this reason, we advocate for universal access to WASH to be considered in MDG planning beyond 2015. In the interim, setting WASH access indicators in any revised version of WHO helminth control guidelines is a crucial next step that will help to tackle the disease burden caused by STH and schistosomiasis. An additional and significant benefit of high community WASH access would be its impact on controlling other excreta-borne pathogens, including viruses, bacteria, and protozoa [46].

There is very little literature that indicates direct WASH impact on helminth control. We believe there is an urgent need to conduct epidemiological research, including appropriately structured intervention trials [47] and mathematical modelling studies [48,49], to evaluate the effect of integrated interventions on helminth infections and infection-associated morbidity. Existing evidence is already strong enough to support complementing drug-based interventions with the provision of WASH for all [50], but more work can be done to determine intervention thresholds for the selected WASH indicators to be incorporated into decision trees such as those presented in the annexes of the WHO guidelines.

Conclusion

Progress towards achieving global control of helminths crucially depends on sustainable solutions that move beyond treating symptoms towards reducing exposure. With that in mind, it is necessary to augment chemotherapy with WASH and other interventions such as health promotion to achieve a cumulative impact of preventing reinfection and providing the greatest and most sustainable gains for helminth control and elimination. We believe that a strong justification exists to revise the WHO guidelines in the face of the abovementioned shortcomings. Such revision will result in a much-enhanced document that covers the full spectrum of short- and longer-term interventions for more holistic STH and schistosomiasis control. Impact indicators for WASH, in addition to disease-related indicators such as prevalence of infection, should define the success of a control programme and guide decisions as to when such programmes should cease. This would ensure current gains in helminth control are built upon beyond the current dependence on chemotherapy.

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Chapter 4 Analysis of soil-transmitted helminth prevalence and risk factors for infection

4.1 Chapter context

Extremely limited information on STH is previously reported for Timor-Leste. Even GBD data extrapolate prevalence information from neighbouring Indonesia (1). This chapter therefore makes an important contribution to global epidemiological STH knowledge, by presenting the first detailed report of community-based STH prevalence, and the first detailed investigation of risk factors, in Timor-Leste. These data will be able to be used by the Global Atlas of Helminth Infection (GAHI) (245) to provide some prevalence statistics for Timor-Leste.

Given the associations with WASH as highlighted in Chapters 1 and 3, it is important to determine the WASH- and poverty-associated risk factors in STH-endemic communities. Whilst these data are observational, they provide an essential baseline to the RCT. Baseline knowledge not only of STH prevalence, but also existing WASH infrastructure and behaviours, will be crucial to evaluate the effectiveness of the integrated trial intervention.

Very few studies have investigated such a comprehensive range of WASH risk factors, adjusted for poverty. This analysis therefore also contributes considerably to broad knowledge of WASH risk factors. Further, this is one of very few identified studies, in any setting, which investigates risk factors for STH prevalence in age-stratified analyses. This is

important because differential impacts of 'risky' behaviours may be a major underlying reason for different infection dynamics of STH species, particularly *A. lumbricoides* which tend to be most prevalent in young children, and *N. americanus*, which tend to be most prevalent in adolescence and young adulthood (20). It has long been postulated that this is largely due to the hygiene and playing habits of young children (246), followed by greater tendency to use latrines (a possible focal point for hookworm infection) as people mature.

This analysis uses contemporary coprodiagnostic techniques for detection of STH and intestinal protozoa, and advanced epidemiological methods to investigate risk factors within the community. This chapter is in press at the *International Journal for Parasitology*.

4.2 Research objective

To determine prevalence of STH and protozoa, and investigate WASH and demographic associations with STH and protozoa infections, in the study population at baseline.

Rationale for methods

Following is a brief overview and rationale for the epidemiological and statistical methods used, providing additional information to the methods section in the attached manuscript.

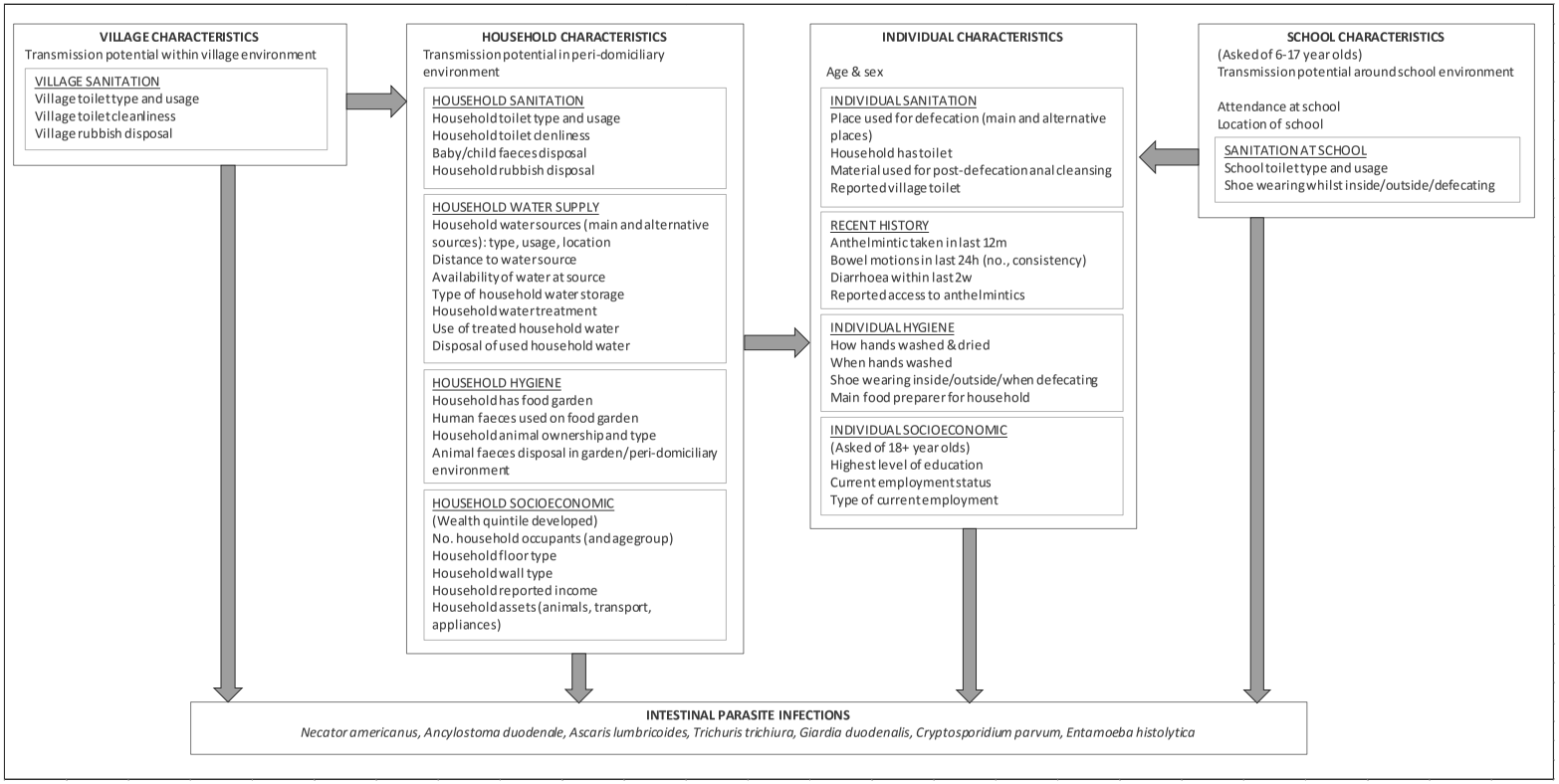
All individuals resident in participating villages were invited to participate. Children aged less than 12 months were excluded. To determine denominator population, all villagers were enrolled on participant registers regardless of their subsequent participation, with neighbours providing basic household numbers for any households that were not present. Additionally, for baseline surveying field staff were present for several days per site. This enabled people to continue to be enrolled if they had been absent on a previous day. Villagers had to be resident within the village to be eligible for the study. People in the same family were identifiable by use of household identifier numbers; that is, the unique identifier for each participant was comprised of a village (V), and household (H), and individual (P) code in the format VV-HH-PP, so that at any time data investigators could determine the village of residence, the household of residence, and the personal identification number of any individual.

A conceptual framework for the risk factor analysis is provided at Figure 4.1. Variables were categorised into 'domains' according to variable type, as a mechanism to manage a large range of variables. The domains were as follows: general (e.g. participant ID, age); individual recent history (e.g. recent diarrhoea); individual anthropometry (e.g. children's heights and weights); individual hygiene (e.g. handwashing practices); individual sanitation (e.g. defecation practices); school-related (six to 17 years); individual socioeconomic (18 years and over; e.g. level of education); household sanitation (e.g. presence and type of latrine); household water supply; household hygiene; household socioeconomic; village sanitation; parasitology. Analyses were undertaken firstly within, then across domains. Analyses were undertaken separately for age groups of preschool-aged children, school-

aged children and adults, because of known different STH risk factor profiles in these different age groups (reviewed in Chapter 1). Participants were excluded if they had not provided a stool sample for parasitological assessment, and if they had not answered an individual questionnaire.

The WHO usually defines school-aged children as those between five and 14 years of age, however note that the exact ages of school enrolment can vary slightly between different countries (247). In Timor-Leste, age of school commencement is six years; therefore this is used as the lower bound of the school-aged population in all analyses.

Figure 4.1: Conceptual framework for domains of variables included in risk factor analysis



Model selection and building

For risk factor analysis, infected versus uninfected individuals were analysed where diagnosis was made by PCR. Outcomes were individual STH species, hookworms (undifferentiated), STH (undifferentiated), and protozoa species. The outcome for each individual follows a Bernoulli distribution, because each outcome variable can only take the value of infection or no infection (248). Therefore, the distribution for an individual needs only one parameter (p), which is the probability of one of these two outcomes occurring. Since the probability of all events must sum to one, the probability of the event not occurring is therefore (1-p) (248). The distribution of the outcome for the sample is binomial, which is the sum of n independent Bernoulli distributions, where n is the sample size. The binomial distribution has parameters n and p. The outcome variable is modelled as the natural logarithm of the odds of infection, or the logit (249). In analyses, coefficients were exponentiated to be reported as odds ratios.

As reported in Chapter 1, STH are known to cluster within individuals, households and villages. This violates the key assumption of independence of observations in standard logistic regression and, if ignored, can lead to overestimation of statistical significance. Therefore, modelling needs to account for the hierarchical levels of correlation. Random effects, or more specifically mixed effects (as fixed effects are also included), modelling allows the inclusion of individuals nested within households, which are nested within villages, thereby correctly accounting for correlation among outcomes at the different levels.

Variables to be included in the analyses were informed by literature and considered to be associated with the outcome, based on the conceptual framework (Figure 4.1). The key variables of age, sex and socioeconomic status were determined necessary to include in all models, regardless of their statistical significance, due to their known importance. Because of the large number of potential risk factors, it was not appropriate to include all of these in a single model, therefore a model building strategy was established to include only those variables which were significant at the 10% level. Whilst a 5% level of statistical significance was used, variables with *P*-values of between 5% and 10% were also retained, so as not be be completely reliant on statistical significance, and to also consider clinical meaning. This was considered important given that the *P*-value has a continuous range between 0 and 1, and is dichotomised at 0.05.

The approach to model building was as follows:

(i) in univariable analyses a high significance cut-off of $P<0.2$ was used to avoid accidental exclusion of false positives;

(ii) in multivariable analysis a cut-off of $P<0.1$ was used for retention of variables, to consider epidemiological meaning; and

(iii) final reporting of multivariable models was according to a statistical significance of $P<0.05$.

Using the "melogit" command in STATA, univariable analysis was conducted initially to determine which risk factors would be analysed further. Variables with $P<0.2$ on univariable analyses were included in a forward stepwise regression. Variables were

excluded from the multivariable model if $P<0.1$ on the Wald test. Age, sex and socioeconomic status were entered as covariates, and household and village as random intercept effects. Likelihood ratio tests comparing the mixed effects model to a standard logistic regression model confirmed that the mixed effects model was substantially better ($P<0.0001$ for all STH), justifying use of this modelling strategy.

For this analysis the PhD Candidate was responsible for 90% of the conception, and 90% of the analysis, interpretation, drafting and writing of the paper. Clements A was responsible for 10% of the conception. D'Este C provided statistical advice and was responsible for 10% of the analysis. Clements A, Nery S, D'Este C, Gray D, McCarthy J, Traub R, Andrews R, Vallely A, Amaral S and Williams G were collectively responsible for 10% of the interpretation and writing of the paper.

WATER, SANITATION AND HYGIENE RELATED RISK FACTORS FOR SOIL-TRANSMITTED HELMINTH AND *GIARDIA DUODENALIS* INFECTIONS IN RURAL COMMUNITIES IN TIMOR-LESTE

Suzy J Campbell[a*], Susana V Nery[a], Catherine A D'Este[a], Darren J Gray[a,b,c], James S McCarthy[c,d], Rebecca J Traub[e], Ross M Andrews[f], Stacey Llewellyn[d], Andrew J Vallely[g], Gail M Williams[c], Salvador Amaral[a], Archie CA Clements[a]

A. Research School of Population Health, College of Medicine, Biology and Environment, The Australian National University, 62 Mills Rd, Acton, ACT 2601, Australia (suzanne.campbell@anu.edu.au; susana.nery@anu.edu.au; catherine.deste@anu.edu.au; darren.gray@anu.edu.au; salvadorcoro@yahoo.com; director.RSPH@anu.edu.au)
B. Molecular Parasitology Laboratory, QIMR Berghofer Medical Research Institute, 300 Herston Rd, Brisbane QLD 4006, Australia
C. School of Public Health, University of Queensland, Herston Rd, Brisbane QLD 4006, Australia (gail.williams@uq.edu.au)
D. Clinical Tropical Medicine Laboratory, QIMR Berghofer Medical Research Institute, 300 Herston Rd, Brisbane QLD 4006, Australia (james.mccarthy@qimrberghofer.edu.au; stacey.llewellyn@qimrberghofer.edu.au)
E. Faculty of Veterinary and Agricultural Science, The University of Melbourne, Parkville VIC 3010, Australia (rebecca.traub@unimelb.edu.au)

F. Menzies School of Health Research, Charles Darwin University, Ellengowan Dr, Casuarina NT 0810, Australia (ross.Andrews@menzies.edu.au)

G. Kirby Institute, University of New South Wales, Wallace Wurth Building, High St, Kensington NSW 2052, Australia (avallely@kirby.unsw.edu.au)

*Corresponding Author: Suzy Campbell, Global Health Division, Research School of Population Health, The Australian National University, 62 Mills Rd, Acton, ACT 2601, Australia. Email: suzanne.campbell@anu.edu.au, Phone: (+61) 2 6125 0538, Fax: (+61) 2 6125 5608.

[1]

[1] Note: Supplementary data associated with this article.

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

Abstract

Background: There is little evidence on prevalence or risk factors for soil-transmitted helminth (STH) infections in Timor-Leste. This study describes the epidemiology, water, sanitation and hygiene (WASH) and socioeconomic risk factors, of STH and intestinal protozoa among communities in Manufahi District, Timor-Leste. Methods: As part of a cluster randomised controlled trial (RCT), a baseline cross-sectional survey was conducted across 18 villages, with data from six additional villages. Stool samples were assessed for STH and protozoal infections using quantitative PCR (qPCR) and questionnaires administered to collect WASH and socioeconomic data. Risk factors for infection were assessed using multivariable mixed-effects logistic regression, stratified by age group (preschool, school-aged and adult). Findings: Overall, STH prevalence was 69% (95% Confidence Interval (CI) 67%-71%), with *Necator americanus* being most common (60%; 95% CI 58%-62%) followed by *Ascaris* spp. (24%; 95% CI 23%-26%). *Ascaris*-*N. americanus* co-infection was common (17%; 95% CI 15%-18%). *Giardia duodenalis* was the main protozoan identified (13%; 95% CI 11%-14%). Baseline WASH infrastructure and behaviours were poor. Although risk factors varied by age 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. Interpretation: In this first assessment of community-based prevalence and associated risk factors in Timor-Leste, STH infections were highly

prevalent, indicating a need for STH control. Few associations with WASH were evident, despite WASH being generally poor. In our RCT we will investigate implications of improving WASH on STH infection in impoverished communities. Funding: Australian National Health and Medical Research Council (NHMRC) Partnership project in collaboration with WaterAid Australia.

Key words

Soil-transmitted; helminth; hookworm; *Necator americanus*; *Ascaris lumbricoides*; *Giardia*; prevalence; risk factor

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 Asia's poorest countries, ranked 133rd of 187 countries on the 2015 Human Development Index (UNDP, 2015). 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 UNICEF reported that only 18% of rural Timorese communities had improved sanitation (UNICEF, 2013). A recent school-based national survey (*N*=2198) revealed a 29% STH infection prevalence in school-aged children, 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, 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 among 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 among people who have access to piped water, use available latrines, and wash hands before eating and after defecation, and reduced odds of hookworm infection among 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, A. suum, T. trichiura* and *T. suis* have been recovered from domestic animals, highlighting their potential mechanical role in STH dissemination (Olsen et al., 2001; Traub et al., 2002; Traub et al., 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; Traub et al., 2009), being male, and being aged less than 12-15 years (Mohammed Mahdy et al., 2008).

This cross-sectional analysis is the first assessment of community-based STH and intestinal protozoal prevalence, and associated risk factors, in Timor-Leste. It is 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. Material and methods

2.1 Ethical approval and consent

The study protocol was approved by the University of Queensland 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 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 surveying conducted between May 2012–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 polymerase chain reaction (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; 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) 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 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. Univariable analyses were undertaken for each helminth separately, co-infections, any STH, and *G. duodenalis*. Because of anticipated risk factor differences among preschool-aged children (PSAC), school-aged children (SAC), and adults, analyses were performed separately for these age groups. Univariable regression models were undertaken for each risk factor; variables were excluded from further analysis if $P>0{\cdot}2$, except for age in years, sex and socioeconomic quintile, which were considered core variables and were included in all analyses.

Multivariable mixed-effects logistic regression models were developed using forward stepwise variable addition to the core model, with household and village level random effects to account for clustering. Variables were grouped by domains, and were added to the model and retained if $P<0{\cdot}1$ on the Wald test, within, and subsequently across domains of variables, with stepwise selection applied as required to remove variables iteratively, until the most parsimonious adjusted model for each outcome was achieved. Interactions were not investigated. Whilst a 5% level of statistical significance was used, variables with *P*-values between 5% and 10% were also retained in the final model, so as not to be completely reliant on statistical significance, and to also consider clinical meaning. The original study aimed to recruit 2880 people based on power requirements for the RCT (Nery et al., 2015); post-hoc calculations were performed to determine the power to detect risk factor effects within age groups. Detectable associations assuming 80% power, with a

5% significance level, were between odds ratio 1.4-7.5 depending on age group and parasite.

3. Results

From 24 villages, 2827 eligible people were present at baseline. Of these, 2152 participants (1038 males, 1114 females) completed an individual questionnaire and provided a stool sample and were included in this analysis (Table 1). There was no evidence of systematic differences between responders and non-responders for whom data were available, when assessed by age group, sex, and village of residence. Few households reported having WASH infrastructure, or ownership of many assets. Reported deworming within previous 12 months was very low (4·5%).

3.1 Prevalence of infection

Overall, prevalence of undifferentiated STH was 69% (95% Confidence Interval (CI) 67%-71%), comprising *Necator americanus* 60% (95% CI 58%-62%), undifferentiated *Ascaris* spp. 24% (95% CI 23%-26%), *Ancylostoma* spp. 4·7% (Figure 1), and *T. trichiura* 0·33%. One *Strongyloides stercoralis*-positive individual was identified. *G. duodenalis* was the most common protozoan identified, at 13% prevalence (95% CI 11%-14%), with less than 0·1% prevalence each for *Entamoeba* and *Cryptosporidium*. Co-infections were common, with *Ascaris-N. americanus* predominating (17%; 95% CI 15%-18%), followed by *N. americanus-Ancylostoma* (3·9%). Due to low prevalence, no regression analyses were undertaken for *Ancylostoma*, *T. trichiura*, *S. stercoralis*, or protozoal species other than *G. duodenalis*. STH infection prevalence was significantly higher in males than females (53%

vs. 47%; $P<0{\cdot}0001$). *N. americanus* prevalence was observed to increase during childhood, and throughout adulthood (Figure 2). *Ascaris* prevalence increased during childhood and declined in adolescence and adulthood. *G. duodenalis* prevalence declined with age. Prevalence of *Ascaris* and *N. americanus* was significantly higher among poorer households compared to wealthier households ($P<0{\cdot}0001$).

3.2 Factors associated with infection

Univariable analyses showed a broad range of WASH and demographic factors had $P<0.2$ for models with individual species infections as well as non-differentiated STH infections (Tables 2-4, Supplementary Tables S1-S5) and were thus included in multivariable analyses. Many effects did not remain in the final models.

3.3 Adjusted factors associated with N. americanus infection

Age was a risk factor for infection amongst PSAC, reducing in effect and significance in SAC, and being non-significant in adulthood. Sex was not associated with *N. americanus* infection among PSAC, but being male was associated with higher odds of *N. americanus* infection in SAC and even greater odds by adulthood (Table 2). Similarly, household socioeconomic status was not a risk factor for infection in PSAC, but among SAC although there was no trend with decreasing socioeconomic quintile, the second wealthiest quintile was associated with high odds compared to the wealthiest quintile; in adults, there was a gradient of generally increasing odds of infection with increasing relative poverty, being significant for all quintiles except the second wealthiest. For PSAC, having one SAC in the household was a risk factor, but more than one was not. For SAC, not having a household

food garden was a risk factor. For SAC and adults, *Ascaris* co-infection was a risk factor. For adults, having a tubewell or borehole, or sharing a piped main water supply, was protective compared to using an unprotected main water source. A protective gradient was found for increasing level of education, however this was only statistically significant for those at the highest education level compared to those who never attended school. Having one or more PSAC in the household were also protective factors.

3.4 Adjusted factors associated with Ascaris infection

Age was strongly associated with *Ascaris* infection in children, although the effect varied from increased odds of infection per year of age in PSAC, to decreasing odds with year of age in SAC; age was not significant in adults (Table 3). For PSAC, the only other factor associated with infection was a protective effect from the main household water supply not running for at least one month per year, although household rubbish disposed of by other methods was only marginally non-significant (P=0.052). Unexpectedly, having no toilet, or a pit latrine without a slab, at school were protective factors for SAC; handwashing without soap had higher odds of infection relative to handwashing with soap but was marginally non-significant. Among adults, *Ascaris* infection was unexpectedly associated with piped main water supply to the yard compared to water from an unprotected spring. Having one SAC in the house compared to none was marginally protective. No associations were found between *Ascaris* infection and sex or socioeconomic status across any age group.

3.5 Adjusted factors associated with G. duodenalis infection

Adjusted risk factors for *G. duodenalis* infection showed no patterns across age groups

(Table 4). For PSAC no risk factors investigated were significant. For SAC, increasing age was protective, as was having a pit latrine without a slab at school (compared to pit latrine with a slab). For adults, *N. americanus* co-infection was protective. No association was found between *G. duodenalis* infection and sex or socioeconomic status across any age group.

4. Discussion

To our knowledge, this is the first reported community-based survey of STH and intestinal protozoa, or risk factor analysis, undertaken in Timor-Leste. We report very high prevalence (69%) of STH in this community. This is considerably higher than found in a recent national school-based survey, which reported 18% prevalence for hookworms and 8% for *Ascaris* from schools in Manufahi District (Martins et al., 2012). This difference is likely largely attributed to our use of qPCR, a highly sensitive diagnostic method, rather than microscopic-based screening. Additionally, we included non-school aged people who had high STH prevalence. However, care needs to be taken with generalisability of these estimates to different areas of Timor-Leste because our sampling strategy was based on a RCT design whereby villages were not randomly selected. Manufahi District is a rural, predominantly agricultural region; selected villages selected shared these features, however were purposively selected based on having less than 50% coverage with household latrines (Nery et al., 2015), which may mean that they were more impoverished. In this area, high STH endemicity occurred in conjunction with poor existing WASH, resulting in favourable conditions for transmission.

Whilst broad ranges of *Ascaris* and *N. americanus* prevalence are reported across the Malay archipelago (Jex et al., 2011), our *T. trichiura* prevalence was low, particularly given diagnostic sensitivity of qPCR. This has important implications for chemotherapeutic control because the cure rate for single-dose albendazole is lowest for *T. trichiura* (Vercruysse et al., 2011), hence single dosage may suffice to substantially reduce STH infection levels in the study area. We report similar prevalence of *G. duodenalis* to rates identified by PCR in rural Malaysia (16%; Anuar et al., 2014). The very low prevalence of *E. histolytica* and *C. parvum* is notable; as is *S. stercoralis*, with one unpublished report of a single *S. stercoralis* infection from Timor-Leste prior to our study (Reeve, 2010).

Improper disposal of human excreta is considered a major driver of STH transmission in endemic settings (Strunz et al., 2014). However relatively few sanitation indicators emerged as risk factors for infection, again most likely due to uniformly poor sanitation infrastructure and ubiquitous open defecation. For SAC, existence of a school pit latrine with a slab was associated with greater odds of *Ascaris* and *G. duodenalis* infection than either a pit latrine without slab or, for *Ascaris*, no school latrine at all. This may be due to poor quality school latrines, inadequate maintenance of school latrines, which could then contribute to transmission (Asaolu and Ofoezie, 2003), or functionality issues such as toilets being in disrepair, having a full pit or other deterrents to correct usage. An alternative explanation is that because the school latrine question was asked of SAC; although these questions used pictorial representations of latrine types, there could have been misunderstanding of school toilet type. School toilets could not be interviewer verified, and for children under 12 the answer was provided by a guardian (who may have

no knowledge of the type of latrine at school). The school latrine result therefore must be interpreted with caution.

Two systematic reviews reported handwashing with soap as protective against *Ascaris* (Fung and Cairncross, 2009), and STH generally (Strunz et al., 2014). However, we found few associations between hygiene behaviours and STH infection. This could be due to a low level of these behaviours across the community, coupled with inadequate availability of improved water sources, which means there may be little opportunity to practice hygienic behaviour. Alternatively this could reflect self-reporting rather than actual behaviour. No associations were found between any species and handwashing times (e.g. before meals or after defecation).

Generally, we found few consistent associations between access to safe water sources and STH infections in our multivariable analyses. This likely reflects homogeneously poor access to improved water sources in study communities, limiting our ability to find major associations, and additionally the indirect nature of STH transmission through water. Although *N. americanus* larvae in water can be infective if swallowed, their longevity in water is decreased. Unsurprisingly, among adults, use of a tubewell or borehole, and of piped water, were protective against *N. americanus* infection; these are considered "improved" water sources by the WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation (JMP) (WHO/UNICEF, 2010). However for adults, odds of *Ascaris* infection unexpectedly increased with piped water use to the yard compared to an unprotected spring, which would be generally considered higher risk. There could have

been incorrect interpretations of pictorial information of water source type. Further, participants interpreted use of hollow bamboo stems to supply water to the household vicinity as "piped water". Alternatively, this may reflect contamination at the water source, as spring-boxes (designed to be protective) are uncommon. Confounding by unmeasured variables is an additional explanation. The protective association between irregular availability of main water supply and *Ascaris* infection in PSAC may indicate a greater tendency within households to treat or boil water when main water is not available, thus conferring protection. Alternatively, secondary water sources may have less contamination. We collected data on household water treatment, however not on frequency nor length of time it was boiled.

Whilst there is a known link between STH, poverty and WASH (Brooker et al., 2004; Traub et al., 2004), poverty associations with STH life cycle are indirect and the relative and separate contributions of poverty and WASH factors remain unclear. We aimed to determine the associations of WASH risk factors after controlling for poverty. As with any PC-derived wealth score, its applicability is relevant to our study population only. Further, we deliberately excluded some variables that would ordinarily be incorporated into a wealth score, such as presence of household latrines and household water supply, because we wanted to investigate these factors separately. As sensitivity analyses, we investigated removal of household flooring from the socioeconomic quintile and incorporated it in multivariable models (results not shown); household flooring was not significantly associated with any species infection in the sensitivity analyses. Therefore, whilst an

earthen floor provides a direct transmission path for *N. americanus* via skin penetration, household flooring appears to be an indirect, poverty-associated, risk factor. Relative poverty was significant only in *N. americanus* models. This, and the lack of association between poverty and *Ascaris* infection, differs from other Asian studies (Karagiannis Voules et al., 2015; Kounnavong et al., 2011), possibly reflecting lower socioeconomic status of study participants relative to these other locations.

The poverty association with *N. americanus* infection is particularly important given our study villages were all socioeconomically poor, with 92% of households reporting living on less than $US2 per day. This figure may itself be underestimated by reporting bias. Thus this is a significant effect on a relative scale of poverty within a generally impoverished setting; this likely underscores poverty as one of the most critical, but least modifiable, risk factors. Prioritisation of strategies that contribute towards alleviating poverty need to be implemented and sustained in this area.

Interestingly, a protective association of *N. americanus* infection on *G. duodenalis* infection was only mildly non-significant in multivariable analysis. Previous studies have reported an increase in *G. duodenalis* infections following deworming (Blackwell et al., 2013; Rousham, 1994). The possibility of antagonistic effects between these two parasites warrants further research.

Ascaris followed anticipated prevalence trends by age, with maximum prevalence ocurring at six to 11 years, and reducing into adulthood. The increased prevalence of *N. americanus* persisting into older age groups is consistent with data from other countries in Asia (Gandhi et al., 2001; Bethony et al., 2002; Karagiannis Voules et al., 2015).

Results of our age-specific multivariable analyses were in accord with well-described associations between age, sex, *Ascaris* and *N. americanus* infection (Brooker et al., 2004). We found links between PSAC age and both *N. americanus* and *Ascaris* infection, with effects weakening in higher age groups to become non-significant in adulthood. Whilst there was no sex association with *Ascaris* infection, for *N. americanus* infection there was no significant association with being male in PSAC, but a significant association in SAC, further increasing in adulthood to have the highest significance and effect size. Age was significant for *G. duodenalis* in SAC only; sex was non-significant. Differences in age and sex effects by stratum might reflect different age- and sex-related exposure patterns (e.g., important school-related, household composition, or occupational exposures), host immune responses, or longevity of different parasite species (e.g., *N. americanus* can live in individuals for up to 18 years, resulting in a greater time-accumulation effect for this species (Beaver, 1988)).

The major limitation of our study was that behaviours as exposure measures were self-reported. There may have been inadequate understanding, even with pictorial charts, of definitions of latrines or water source types, which may have contributed to some of the unexpected results found. Guidelines that inform accurate, consistent measurements of

WASH for epidemiological studies, would be valuable. Power calculations also indicated that there was reduced power to detect risk factor effects within age-stratified analyses, with some likelihood of type two error. However, age-stratified analyses were prioritised because of the known different age-helminth profiles. The differences in risk factors for each age group are borne out by these stratified analyses: whilst some risk factors were consistent across age groups, others were not: some had odds ratios greater than one, some less than one, and not all effects were in the same direction.

Use of semi-quantitative qPCR data is relatively new for STH diagnosis, and is considered more sensitive and specific than microscopy-based techniques (Llewellyn et al., 2016). Possibly, with detection of light-intensity infections that could have been missed by microscopy, associations with related covariates may have been weaker which could mean that WASH relationships may have been hidden by light infections.

Despite these limitations, this study has particular strength in that we used a highly sensitive and specific PCR-based diagnostic technique undertaken by trained laboratory personnel to assess STH prevalence. Using PCR we were also able to identify genera of hookworm.

Whilst we report risk factors for an area of low WASH infrastructure prior to interventions, this study makes a significant contribution to evidence of STH risk factors, as very few studies have investigated these in age-stratified analyses. This is important given the different age-associated prevalences of STH species, particularly *Ascaris* and hookworm,

and the fact that many interventions (mass drug administration and WASH programmes) can be targeted at specific population groups, including school-attending children, or the whole community.

In this first reported study of community-level prevalence and risk factors for gastrointestinal parasites in Timor-Leste, we found high endemicity using qPCR, justifying implementation of STH control strategies. STH control programmes must be underpinned with comprehensive knowledge of local STH epidemiology in order to target resources most effectively. This analysis demonstrates that there are many potential village, household, and individual level improvements which could protect residents against STH infection. This detailed investigation also contributes to the evidence of a range of WASH and poverty-associated risk factors on STH prevalence.

Author contributions

SJC and ACAC designed the analysis. SVN was responsible for data collection and entry. SVN and SJC conducted data cleaning and management. SL analysed qPCR specimens. SJC conducted data analysis, with statistical advice from CADE. SJC drafted the manuscript, and ACAC, DJG, SVN and CADE contributed suggestions to the analysis. All authors have contributed to interpretation of findings, and have approved the final manuscript.

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Table 1 Selected baseline characteristics of study participants (*N*=2152)

Personal characteristics	**Preschool; aged 1<6 years (*N*=393) n (%)**	**School-aged; aged 6<18 years (*n*=668) n (%)**	**Adults; aged ≥18 years (*n*=1090) n (%)**
Any STH prevalence	197 (50)	472 (71)	817 (75)
Ascaris spp. prevalence	109 (28)	207 (31)	210 (19)
N. americanus prevalence	132 (34)	407 (61)	759 (70)
Ancylostoma spp. prevalence	18 (4.6)	27 (4·0)	57 (5·2)
Any STH co-infection	56 (14)	158 (24)	205 (19)
N. americanus-Ancylostoma spp. co-infection	13 (3·3)	23 (3·4)	48 (4·4)
Ascaris-N. americanus co-infection	48 (12)	146 (22)	164 (15)
Ascaris-Ancylostoma spp. co-infection	9 (2·3)	11 (1·7)	12 (1·1)
Any protozoa prevalence	101 (26)	113 (17)	60 (5·5)
G. duodenalis prevalence	100 (26)	111 (17)	57 (5·2)
Male sex	204 (52)	338 (51)	496 (46)
Reported taking anthelmintic in previous 12 months	43 (11)	35 (5·3)	19 (1·8)
Uses soap/ash to wash hands	275 (70)	502 (75)	848 (78)
Always wears shoes inside home	88 (23)	283 (43)	559 (52)
Always wears shoes outside home	120 (31)	424 (64)	774 (71)
Always wears shoes when toileting	134 (35)	441 (67)	786 (73)
Uses unhygienic toilet	330 (85)	516 (78)	881 (81)
Currently attends school	-	593 (91)	-
Pit latrine with slab at school	-	262 (45)	-
Pit latrine without slab at school	-	170 (29)	-
Other toilet type at school	-	34 (5·9)	-
No answer/no toilet at school	-	112 (19)	-
Never attended school	-	-	451 (44)
Not finished primary school	-	-	207 (20)
Completed primary but not secondary school	-	-	245 (24)
Completed secondary school or higher	-	-	118 (12)
Agricultural occupation	-	-	772 (92)
Non-agricultural occupation	-	-	69 (8·2)
Not employed	-	-	139 (13)
Household characteristics	**n (%) from household questionnaires (*N*=594)**		
Pit latrine with slab	50 (8·4)		
Pit latrine without slab	77 (13)		
Other toilet type	5 (0·8)		
No household toilet/ no answer	462 (78)		
Improved household water source	106 (18)		
1-4 people in household	294 (50)		
5-9 people in household	280 (48)		
10 or more people in household	14 (2·4)		
Village characteristics	**n (%) from village questionnaires (*N*=24)**		
Pit latrine with slab	1 (4·2)		
Pit latrine without slab	1 (4·2)		
Toilet type not specified	1 (4·2)		
No village toilet	21 (88)		
Village toilet assessed as unclean	3 (100)		

Notes: Seven observations for *T. trichiura* and one observation for *S. stercoralis* are not listed in table. Numbers may not sum to total sample due to missing data. Parasitological outcomes determined by PCR, types of household latrines observed by interviewer, remaining data are self-reported. "Always wearing shoes" was contrasted to sometimes/never wearing shoes. "Unhygienic toilet" defined as any people who did not use a hygienic toilet (this included people who used a mixture of hygienic and non-hygienic toilets; hygienic toilets defined as use of a house/school/village/neighbour toilet and nothing else). "Improved" household water source as defined by JMP to include piped water into dwelling or yard, public tap or standpipe, tubewell or borehole, protected dug well, protected spring (WHO/UNICEF, 2010). Household and village characteristics report number of the households or villages with the characteristic e.g., number of villages that have a village (i.e. public) pit latrine with slab = 1. Village cleanliness derived from interviewer assessment of toilet according to specified criteria that were subsequently recategorised as "toilet is clean/toilet is unclean", whereby presence of any "unclean" observation (e.g. urine and faeces presence on seat or floor) indicated "uncleanliness" of toilet.

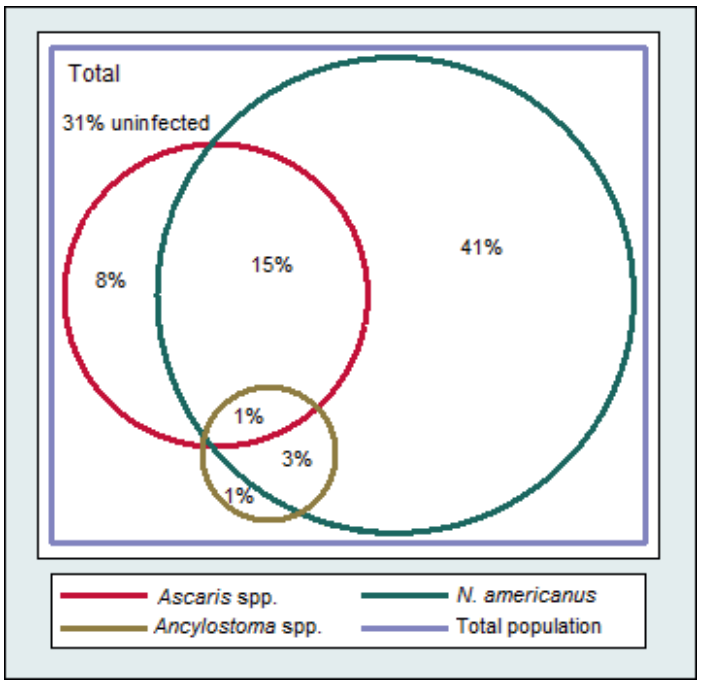

Figure 1 Proportional Venn diagram of infection status with the three most prevalent STH species for *N*=2152 who provided stool and questionnaire

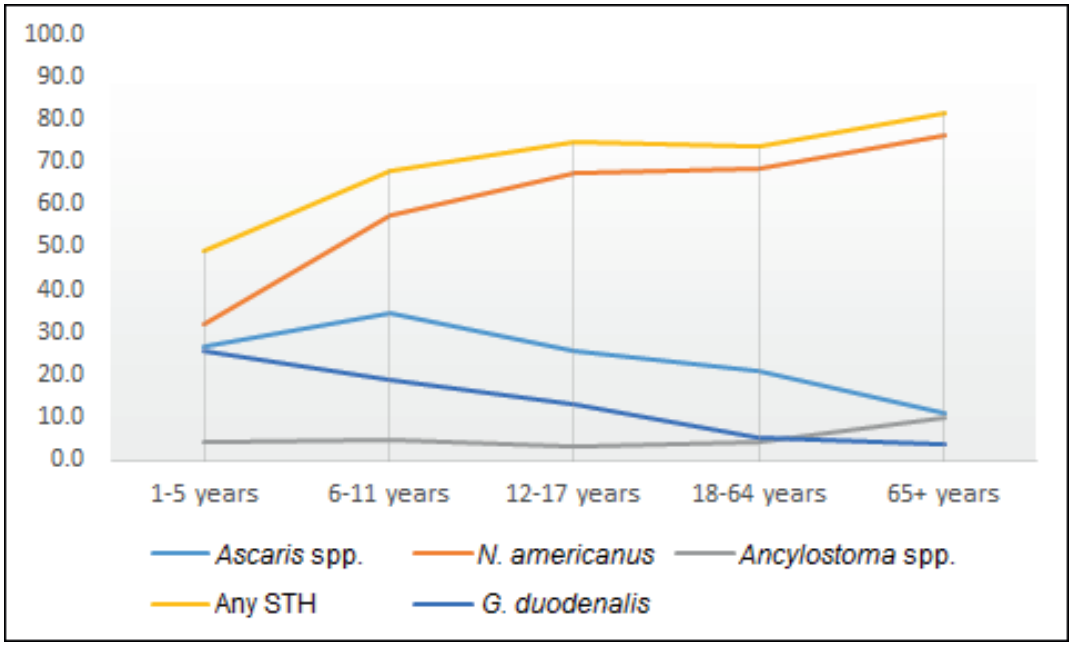

Figure 2 Prevalence of STH and *G. duodenalis* by age group

Table 2 Multivariable logistic regression models for *N. americanus* infection (*N*=2152)

Parameter	Preschool; aged <6 years (*n*=393) AOR	(95% CI)	*P*	School-aged; aged 6<18 years (*n*=668) AOR	(95% CI)	*P*	Adults; aged ≥18 years (*n*=1090) AOR	(95% CI)	*P*
Domain: General									
Age in years	2·0	(1·5-2·6)	<0·0001	1·1	(1·0-1·2)	0·013	1·0	(1·0-1·0)	0·128
Male sex	1·7	(0·89-3·1)	0·109	2·7	(1·8-4·1)	<0·0001	4·2	(2·8-6·3)	<0·0001
Domain: Individual socioeconomic									
Not finished primary school							0·82	(0·47-1·5)	0·499
Completed primary but not secondary school							0·79	(0·44-1·4)	0·409
Completed secondary school or higher							0·36	(0·18-0·72)	0·004
Domain: Household water supply									
Main water supply: piped water to yard				1·1	(0·28-4·6)	0·850	1·3	(0·50-3·4)	0·596
Main water supply: piped water shared				0·42#	(0·07-2·5)	0·341	0·22	(0·07-0·73)	0·013
Main water supply: tubewell/borehole				0·20	(0·03-1·2)	0·075	0·39	(0·16-0·95)	0·039
Main water supply: unprotected dug well				2·0#	(0·17-24·0)	0·583	0·27#	(0·01-6·5)	0·418
Main water supply: protected spring				1·1#	(0·17-6·8)	0·950	2·1#	(0·45-10·0)	0·341
Main water supply: surface water				1·3	(0·62-2·8)	0·470	1·1	(0·68-1·8)	0·700
Domain: Household hygiene									
Household has a food garden				0·43	(0·20-0·90)	0·026			
Domain: Household socioeconomic									
1 person aged <5 years in household							0·54	(0·33-0·89)	0·016
2 or more people aged <5 years in household							0·50	(0·29-0·88)	0·017
1 person aged 5-17 years in household	4.0	(1·2-12·9)	0·022						
2 or more people aged 5-17 years in household	1·9	(0·73-4·9)	0·191						
Socioeconomic quintile 4	1·1	(0·36-3·3)	0·892	3·0	(1·4-6·3)	0·004	1·6	(0·85-2·9)	0·149
Socioeconomic quintile 3	0·60	(0·18-2·0)	0·397	1·2	(0·58-2·5)	0·609	2·8	(1·5-5·3)	0·002
Socioeconomic quintile 2	1·5	(0·50-4·6)	0·470	1·0	(0·47-2·1)	0·997	3·4	(1·8-6·6)	<0·0001
Socioeconomic quintile 1 (poorest)	1·6	(0·52-5·1)	0·406	1·9	(0·86-4·3)	0·112	3·2	(1·6-6·2)	0·001
Domain: qPCR									
Ascaris infection				1·8	(1·0-3·1)	0·049	1·9	(1·1-3·3)	0·024

Notes: (i) See supplementary material for a complete list of all species regression models and risk factors investigated. (ii) AOR, adjusted odds ratio; CI, confidence interval; *P*, Wald test. (iii) # Insufficient observations (<10 observations) in subgroup of variable; result should be interpreted cautiously. (iv) Individual socioeconomic variables only analysed for adults. (v) Age (continuous), sex and socioeconomic quintile were included in all multivariable regression models as covariates. (vi) Water supply variables follow JMP definitions (WHO/UNICEF, 2010). (vii) Reference categories are as follows: **General domain:** female sex. **Individual socioeconomic domain:** never went to school. **Household water supply domain:** main water supply being an unprotected spring. **Household hygiene domain:** household does not own food garden. **Household socioeconomic domain:** no people aged <5 years in household/no answer; no people aged 5-17 years in household/no answer; socioeconomic quintile 5 (wealthiest). **qPCR domain:** no *Ascaris* infection.

Table 3 Multivariable logistic regression models for *Ascaris* infection (*N*=2152)

Parameter	Preschool; aged <6 years (*n*=393) AOR	(95% CI)	*P*	School-aged; aged 6<18 years (*n*=668) AOR	(95% CI)	*P*	Adults; aged ≥18 years (*n*=1090) AOR	(95% CI)	*P*
Domain: General									
Age in years	1·3	(1·1-1·6)	0·012	0·86	(0·78-0·95)	0·002	1·0	(0·98-1·0)	0·540
Male sex	1·6	(0·80-3·0)	0·190	1·7	(0·93-3·0)	0·084	0·72	(0·49-1·1)	0·093
Domain: Individual hygiene									
Does not use soap/ash to wash hands				2·3	(1.0-5·5)	0·056			
Domain: School related									
School toilet: No toilet				0·17	(0·05-0·62)	0·007			
School toilet: Pit latrine without slab				0·19	(0·05-0·65)	0·009			
School toilet: Other toilet type				2·0	(0·27-14·7)	0·493			
Domain: Household sanitation									
Household rubbish disposed of by burning only	1·3	(0·54-3·0)	0·589						
Household rubbish disposed of by other method	0·37	(0·14-1·0)	0·052						
Domain: Household water supply									
Main water supply: piped water to yard							7·0	(2·1-23·4)	0·002
Main water supply: piped water shared							0·62	(0·11-3·6)	0·590
Main water supply: tubewell/borehole							0·06#	(<0·01-2·2)	0·124
Main water supply: protected spring							1·7#	(0·39-7·7)	0·473
Main water supply: surface water							1·6	(0·84-3·1)	0·150
Main water supply not running at least 1 week/month	0·84#	(0·17-4·0)	0·824						
Main water supply not running at least 1 month/year	0·26	(0·08-0·88)	0·030						
Domain: Household socioeconomic									
1 person aged <5 years in household							1·6	(0·98-2·6)	0·063
2 or more people aged <5 years in household							1·4	(0·83-2·5)	0·196
1 person aged 5-17 years in household							0·54	(0·30-0·98)	0·044
2 or more people aged 5-17 years in household							1·0	(0·64-1·6)	0·991
Socioeconomic quintile 5 (wealthiest)	0·45#	(0·11-1·9)	0·268	1			1		
Socioeconomic quintile 4	1·2	(0·35-4·3)	0·755	0·54	(0·17-1·7)	0·280	0·50	(0·23-1·1)	0·078
Socioeconomic quintile 3	0·96	(0·30-3·1)	0·948	0·87	(0·28-2·7)	0·809	0·88	(0·45-1·7)	0·709
Socioeconomic quintile 2	1·3	(0·46-3·9)	0·591	0·83	(0·28-2·5)	0·745	0·84	(0·42-1·7)	0·614
Socioeconomic quintile 1 (poorest)	1			0·60	(0·18-2·0)	0·406	1·2	(0·60-2·5)	0·579

Notes: (i) See supplementary material for a complete list of all species regression models and risk factors investigated. (ii) AOR, adjusted odds ratio; CI, confidence interval; *P*, Wald test. (iii) # Insufficient observations (<10 observations) in subgroup of variable; result should be interpreted cautiously. (iv) School-related variables only analysed for school-aged children. (v) Age (continuous), sex and socioeconomic quintile were included in all multivariable regression models as covariates. (vi) Water supply variables follow JMP definitions (WHO/UNICEF, 2010). (vii) Definitions: "Other school toilet type" includes flush toilet or septic tank (low observation numbers). "Household rubbish disposed of by other method" includes disposing it into a bin, a river, burying it or composting it. (viii) Reference categories are as follows: **General domain:** female sex. **Individual hygiene domain:** uses soap/ash to wash hands. **School related domain:** school toilet being a pit latrine with slab. **Household sanitation domain:** household rubbish disposed of in bush only. **Household water supply domain:** main water supply being an unprotected spring; main water supply always running. **Household socioeconomic domain:** no people aged <5 years in household/no answer; no people aged 5-17 years in household/no answer; socioeconomic quintile 5 (wealthiest). Socioeconomic quintile 1 (poorest) is the reference category for preschool children, due to low observation numbers in the wealthiest quintile for this age group.

Table 4 Multivariable logistic regression models for *G. duodenalis* infection (*N*=2152)

Parameter	Preschool; aged <6 years (*n*=393) AOR	(95% CI)	*P*	School-aged; aged 6<18 years (*n*=668) AOR	(95% CI)	*P*	Adults; aged ≥18 years (*n*=1090) AOR	(95% CI)	*P*
Domain: General									
Age in years	0·89	(0·76-1·1)	0·163	0·87	(0·79-0·95)	0·002	0·98	(0·96-1·0)	0·073
Male sex	0·91	(0·56-1·5)	0·709	1·2	(0·76-2·0)	0·386	0·91	(0·46-1·8)	0·774
Domain: School related									
School toilet: No toilet				1·2	(0·59-2·5)	0·623			
School toilet: Pit latrine without slab				0·45	(0·22-0·93)	0·031			
School toilet: Other toilet type				2·1#	(0·74-6·0)	0·163			
Domain: Household water supply									
Secondary water source used	1·6	(0·92-2·9)	0·093						
Domain: Household socioeconomic									
Socioeconomic quintile 4	1·2	(0·52-2·7)	0·689	0·98	(0·45-2·1)	0·955	1·4	(0·42-4·5)	0·599
Socioeconomic quintile 3	1·1	(0·47-2·6)	0·820	1·2	(0·53-2·6)	0·698	0·97	(0·29-3·2)	0·958
Socioeconomic quintile 2	0·69	(0·29-1·6)	0·386	0·81	(0·37-1·8)	0·592	0·76	(0·21-2·7)	0·678
Socioeconomic quintile 1 (poorest)	1·4	(0·62-3·2)	0·420	0·70	(0·30-1·6)	0·410	0·95	(0·28-3·3)	0·941
Domain: qPCR									
N. americanus infection							0·48	(0·21-1·1)	0·069

Notes: (i) See supplementary material for a complete list of all species regression models and risk factors investigated. (ii) AOR, adjusted odds ratio; CI, confidence interval; *P*, Wald test. (iii) # Insufficient observations (<10 observations) in subgroup of variable; result should be interpreted cautiously. (iv) School-related variables only analysed for school-aged children. (v) Age (continuous), sex and socioeconomic quintile were included in all multivariable regression models as covariates. (vi) Definitions: "Other school toilet type" includes flush toilet or septic tank (low observation numbers). (vii) Reference categories are as follows: **General domain:** female sex. **School related domain:** school toilet being a pit latrine with slab. **Household water supply domain:** no secondary water source used. **Household socioeconomic domain:** socioeconomic quintile 5 (wealthiest). **qPCR domain:** no *N. americanus* infection.

Supplementary tables

Supplement to Campbell SJ, Nery SV, D'Este CA, Gray DJ, McCarthy JS, Traub RJ, Andrews RM, Llewellyn S, Vallely A, Williams G, Amaral S, Clements ACA. Water, sanitation and hygiene related risk factors for soil-transmitted helminth and *Giardia duodenalis* infections in rural communities in Timor-Leste.

Notes for all supplementary tables:

(i) Supplementary material shows logistic regression models for all risk factors investigated, for soil-transmitted helminth (STH) or protozoa infection status, stratified by age group, according to the following:

S1: *Necator americanus* infection (univariable results)
S2: *Ascaris* infection (univariable results)
S3: Undifferentiated hookworm infection (univariable and multivariable results)
S4: Undifferentiated STH infection (univariable and multivariable results)
S5: *Giardia duodenalis* infection (univariable results)

(ii) All models conducted as mixed-effects logistic regression modelling to account for clustering.
(iii) Variables were not included in univariable analysis if they had insufficient numbers in any age group.
(iv) OR, odds ratio; CI, confidence interval; *P*, Wald test; AOR, adjusted odds ratio.
(v) Odds ratios in bold had $P<0{\cdot}2$ in univariable regression and were therefore entered in multivariable regression models.
(vi) *** $P<0{\cdot}01$, ** $P<0{\cdot}05$ in univariable analysis.
(vii) IO, insufficient observations (<10 in cell) in variable to enable inclusion in univariable regression model.
(viii) # Insufficient observations (<10 observations) in subgroup of variable; result should be interpreted cautiously.
(ix) School-related variables only analysed for schoolchildren.
(x) Individual socioeconomic variables and pregnancy status only analysed for adults.
(xi) Age (continuous), sex and socioeconomic quintile included in all multivariable regression models as covariates.
(xii) Water supply variables defined in accordance with the WHO/UNICEF Joint Monitoring Programme (JMP) for Water Supply and Sanitation (see: http://www.wssinfo.org/definitions-methods/watsan-categories/).
(xiii) Univariable results reported, but multivariable analysis did not include the following variables as they were used to build a socioeconomic index: number of household dogs, number of household pigs, number of household chickens, number of household cows, number of household horses, household flooring, household income level, household vehicle ownership, household electricity supply, household appliance ownership. These are indicated as NA=not applicable where results were significant in univariable analysis. Household wall construction not included in socioeconomic index due to limited variability of responses.
(xiv) Multivariable analysis did not include the following variables: age group (categorical; age as a continuous variable was used), hookworm infection (due to overlap with *N. americanus* infection), protozoa infection (due to overlap with *G. duodenalis* infection); these are indicated as NA=not applicable, where results were significant in univariable analysis.
(xv) Definitions: "washes hands at other times" includes after cleaning babies' bottoms/ disposing of children's faeces, before food preparation, before eating/feeding children, after contact with animals, after contact with soil or dirt, after coming inside house. "Used unhygienic toilet" any people who did not use a hygienic toilet (this included people who used a mixture of hygienic and non-hygienic toilets; hygienic toilets defined as use of a house/school/village/neighbour toilet and nothing else). "Cleans self by other method after toileting" includes cleaning self with newspaper, corn cobs or stones. "Other school toilet type" includes flush toilet or septic tank (low observation numbers). "Other household toilet type" includes hanging latrines (low observation numbers). "Household rubbish disposed of by other method" includes disposing it into a bin, a river, burying it or composting it. "Household owns other animals" includes chickens, cows or horses. "Household walls of high-grade construction" includes tin or brick construction, "low-grade construction" is wood (including manufactured wood), bamboo, straw or weatherboard. "Other household appliances" include refrigerator or computer. "Village rubbish disposed of by other method" includes burying it or disposing of it in the river.
(xvi) Unless otherwise indicated, reference categories are as follows: **General domain:** lowest age group in the stratum; female sex. **Individual hygiene domain:** uses soap/ash to wash hands; washes hands after defecation only; always wears shoes inside house; always wears shoes outside house; always wears shoes when toileting. **Individual sanitation domain:** uses hygienic toilet only; household has no toilet; cleans self with leaves only after toileting; village has no public toilet. **School related domain:** does not attend school; school located in same bairro (local sub-village) to where individual lives; always wears shoes inside classroom; always wears shoes outside

classroom; always wears shoes when toileting at school; school toilet being a pit latrine with slab; does not use school toilet. **Individual socioeconomic domain:** never went to school; employed; agricultural occupation. **Household sanitation domain:** household toilet being a pit latrine with slab; household toilet observed (by interviewer) to be clean; household toilet is not shared with another household; household rubbish disposed of in bush only. **Household water supply domain:** main water supply being an unprotected spring; main water supply located in household compound; distance to main water supply less than 15 minutes; main water supply always running; household water being stored in an uncovered container; no secondary water source used; secondary water supply used was unimproved (according to JMP definitions), or no answer provided; secondary water source located in village (but not household compound); household water is not boiled. **Household hygiene domain:** household does not own food garden; human faeces not used on food garden; household owns dogs or pigs; no dogs owned or no answer; no pigs owned or no answer; no chickens owned or no answer; no cows owned or no answer; no horses owned or no answer. **Household socioeconomic domain:** no people aged <5 years in household/no answer; no people aged 5-17 years in household/no answer; no people aged 18-64 years in household/no answer; no people aged ≥65 years in household/no answer; 1-4 people in household; household has earthen flooring; household has walls of low-grade construction; household income less than USD $1/day; household does not own bicycle or motor vehicle/ no answer; household has no electricity; household owns no appliances/no answer; socioeconomic quintile 5 (wealthiest). Socioeconomic quintile 1 (poorest) is the reference category for preschool children in the *Ascaris* model, due to low observation numbers in the wealthiest quintile for this age group. **Village domain:** no village toilet; village rubbish disposed of in bushes only. **qPCR domain:** no *Ascaris* infection; no *N. americanus* infection; no *Ancylostoma* infection; no hookworm infection; no STH infection; no *G. duodenalis* infection; no protozoa infection. **Individual recent history domain:** non-pregnant female; no deworming treatment taken within last 12 months; no current diarrhoea symptoms; no diarrhoea in last two weeks; no access to anthelmintics.

S1: Univariable logistic regression results for *N. americanus* infection (*N*=2152); full list of risk factors investigated

Parameter	Preschool; aged <6 years (*n*=393) OR	(95% CI)	School-aged; aged 6<18 years (*n*=668) OR	(95% CI)	Adults; aged ≥18 years (*n*=1090) OR	(95% CI)
Domain: General						
Age in years	**2·0*****	(1·5-2·7)	**1·1****	(1·0-1·2)	**1·0*****	(1·0-1·0)
Age group 1 to 5 years	1					
Age group 6 to 11 years	**3·6*****	(1·4-9·3)	1			
Age group 12 to 17 years			**1·6****	(1·1-2·5)		
Age group 18-64 years					1	
Age group 65+ years					**1·8****	(1·1-2·8)
Male sex	1·4	(0·84-2·2)	**3·0*****	(1·9-4·6)	**4·4*****	(3·0-6·5)
Domain: Individual hygiene						
Does not use soap/ash to wash hands	0·74	(0·39-1·4)	1·3	(0·75-2·2)	1·1	(0·69-1·6)
Washes hands after defecation and at other times	1·4	(0·73-2·8)	0·91	(0·52-1·6)	0·80	(0·37-1·7)
Washes hands at other times only (but not after defecation)	1·3	(0·58-2·9)	0·84	(0·42-1·7)	1·0	(0·45-2·5)
Sometimes/never wears shoes inside house	0·79	(0·43-1·5)	1·0	(0·63-1·6)	1·3	(0·89-1·9)
Sometimes/never wears shoes outside house	0·86	(0·49-1·5)	1·2	(0·74-2·0)	**1·6****	(1·1-2·4)
Sometimes/never wears shoes when toileting	0·84	(0·47-1·5)	0·97	(0·58-1·6)	**1·4**	(0·95-2·1)
Domain: Individual sanitation						
Used unhygienic toilet	1·1	(0·52-2·4)	0·83	(0·45-1·5)	**1·4**	(0·89-2·2)
Household has toilet	0·76	(0·37-1·5)	1·2	(0·65-2·2)	**0·70**	(0·46-1·1)
Cleans self with water and hand only after toileting	0·67	(0·35-1·3)	0·96	(0·47-2·0)	0·72	(0·42-1·2)
Cleans self by other method after toileting	0·71	(0·34-1·5)	0·90	(0·49-1·6)	**0·61****	(0·41-0·92)
Village has public toilet	IO		1·2	(0·39-3·7)	1·4	(0·63-3·0)
Domain: School related						
Attends school			0·73	(0·35-1·5)		
School in same aldeia (sub-village larger than bairro)			1·7	(0·65-4·6)		
School in same village (broader area than aldeia)/subdistrict/district			1·5	(0·54-4·1)		
Sometimes/never wears shoes inside classroom			1·3	(0·62-2·6)		
Sometimes/never wears shoes outside classroom			1·5	(0·72-3·1)		
Sometimes/never wears shoes when toileting at school			1·3	(0·63-2·9)		
School toilet: No toilet			1·6	(0·64-3·8)		
School toilet: Pit latrine without slab			**0·62**	(0·30-1·3)		
School toilet: Other toilet type			0·86	(0·27-2·7)		
Uses school toilet			0·99	(0·34-2·9)		
Domain: Individual socioeconomic						
Not finished primary school					**0·66**	(0·42-1·0)
Completed primary but not secondary school					**0·59****	(0·39-0·89)
Completed secondary school or higher					**0·34*****	(0·20-0·58)
Unemployed					**0·48*****	(0·30-0·77)
Non-agricultural employment					**0·50****	(0·26-0·97)
Domain: Household sanitation						
No household toilet/no answer	1		1·1	(0·50-2·4)	**1·8**	(0·95-3·4)
Household toilet: Pit latrine with slab	0·38#	(0·10-1·4)	1		1	
Household toilet: Pit latrine without slab	1·1	(0·47-2·7)	1·6	(0·57-4·6)	1·5	(0·70-3·3)
Household toilet: Other toilet type	#		#		0·90#	(0·14-6·0)

Toilet observed to be dirty	IO		1·2	(0·36-4·0)	1·3	(0·67-2·7)
Household toilet is shared	IO		2·0	(0·50-7·7)	0·61	(0·29-1·3)
Household rubbish disposed of by burning only	1·1	(0·61-2·1)	1·0	(0·56-1·8)	1·2	(0·73-1·8)
Household rubbish disposed of by other method	1·4	(0·71-2·9)	0·83	(0·45-1·5)	0·92	(0·59-1·4)
Domain: Household water supply						
Main water supply: piped water to yard	2·1#	(0·42-10·3)	0·67	(0·17-2·6)	**2·1**	(0·78-5·7)
Main water supply: piped water shared	#		0·32#	(0·05-1·9)	**0·30**	(0·09-1·0)
Main water supply: tubewell/borehole	0·72#	(0·08-6·6)	**0·15****	(0·03-0·81)	**0·32****	(0·12-0·87)
Main water supply: unprotected dug well	#		1·0#	(0·09-12·4)	0·44#	(0·04-5·6)
Main water supply: protected spring	1·9#	(0·20-19·0)	0·69#	(0·11-4·3)	1·9#	(0·44-8·1)
Main water supply: surface water	1·6	(0·70-3·5)	1·1	(0·50-2·2)	1·1	(0·64-1·7)
Main water supply located elsewhere	IO		**2·3**	(0·99-5·2)	1·2	(0·69-2·1)
Distance to main water supply: 15 min or more	1·5	(0·79-2·9)	**1·7**	(0·93-3·0)	1·2	(0·75-1·8)
Main water supply not running at least 1 week/month	0·95#	(0·29-3·1)	0·88	(0·33-2·4)	1·2	(0·53-2·9)
Main water supply not running at least 1 month/year	1·1	(0·45-2·4)	0·65	(0·31-1·4)	**0·51****	(0·30-0·85)
Water stored in covered container only	**1·7**	(0·84-3·4)	**1·6**	(0·87-2·8)	1·3	(0·84-2·0)
Secondary water source used	1·3	(0·62-2·6)	0·70	(0·37-1·3)	0·94	(0·60-1·5)
Secondary water source: in household compound	IO		0·58#	(0·08-4·1)	2·5#	(0·64-10·1)
Secondary water source: in neighbouring village			0·68	(0·14-3·3)	0·87	(0·30-2·5)
Household water is boiled	0·67	(0·35-1·3)	0·80	(0·46-1·4)	0·85	(0·56-1·3)
Domain: Household hygiene						
Household has a food garden	1·8	(0·79-4·0)	**0·54**	(0·26-1·1)	0·95	(0·55-1·6)
Human faeces used on food garden	IO		IO		1·1	(0·33-3·6)
Household owns no animals/no answer	0·39#	(0·03-5·9)	0·49#	(0·08-3·1)	0·74#	(0·19-2·9)
Household owns other animals	0·66	(0·30-1·5)	1·2	(0·57-2·5)	1·2	(0·71-2·1)
1-5 dogs	1·7	(0·68-4·4)	1·3	(0·58-2·8)	0·96	(0·55-1·7)
6 or more dogs	2·9#	(0·56-15·2)	1·6	(0·47-5·1)	0·72	(0·29-1·8)
1-5 pigs	1·2	(0·41-3·4)	1·0	(0·38-2·7)	1·1	(0·53-2·1)
6 or more pigs	0·73	(0·22-2·5)	1·3	(0·41-3·9)	0·68	(0·31-1·5)
1-5 chickens	0·80	(0·27-2·4)	1·3	(0·47-3·5)	0·79	(0·38-1·6)
6 or more chickens	0·62	(0·20-1·9)	1·2	(0·44-3·4)	**0·45****	(0·21-0·96)
1-5 cows	1·2	(0·59-2·6)	**0·62**	(0·36-1·1)	**0·75**	(0·49-1·2)
6 or more cows	0·56#	(0·17-1·9)	**2·0**	(0·79-4·9)	0·85	(0·43-1·7)
1 horse owned	1·5	(0·70-3·3)	1·6	(0·80-3·1)	0·83	(0·51-1·4)
2 or more horses owned	0·52#	(0·18-1·5)	1·5	(0·71-3·2)	0·97	(0·56-1·7)
Domain: Household socioeconomic						
1 person aged <5 years in household	0·57	(0·24-1·4)	0·72	(0·42-1·3)	**0·50*****	(0·33-0·76)
2 or more people aged <5 years in household	0·67	(0·29-1·6)	1·1	(0·64-2·0)	**0·49*****	(0·31-0·78)
1 person aged 5-17 years in household	**4·1*****	(1·6-10·6)	2·2#	(0·49-9·8)	0·98	(0·59-1·6)
2 or more people aged 5-17 years in household	**2·7****	(1·3-5·6)	1·0	(0·51-2·1)	0·88	(0·59-1·3)
No people aged 18-64 years in household/no answer			2·9#	(0·56-15·0)	1	
1 person aged 18-64 years in household			1		0·90	(0·33-2·5)
2 or more people aged 18-64 years in household			1·4	(0·50-4·0)	0·66	(0·32-1·3)
1 person aged 65+ years in household			2·4	(0·89-6·5)	0·70	(0·36-1·4)
2 or more people aged 65+ years in household			1·6	(0·61-4·2)	1·3	(0·72-2·3)
5-9 people in household	**2·0****	(1·0-4·0)	0·79	(0·43-1·4)	0·78	(0·55-1·1)
10 or more people in household	2·6#	(0·72-9·6)	0·94	(0·32-2·8)	0·58	(0·22-1·6)
Household has wooden flooring	**0·54**	(0·25-1·2)	1·3	(0·68-2·4)	0·82	(0·53-1·3)

Household has cement/tile flooring	**0·55**	(0·23-1·4)	**0·59**	(0·29-1·2)	**0·55****	(0·32-0·95)
Household has walls of high-grade construction	IO		0·73	(0·30-1·8)	0·84	(0·41-1·7)
Household income more than or equal to USD $1/day	0·72	(0·39-1·3)	0·90	(0·56-1·5)	0·98	(0·68-1·4)
Household owns bicycle only	0·60#	(0·21-1·7)	1·2	(0·45-3·3)	**0·43****	(0·21-0·88)
Household owns motor	0·65#	(0·24-1·7)	**0·57**	(0·27-1·2)	**0·42*****	(0·23-0·76)
Household has electricity	0·89	(0·44-1·8)	0·70	(0·37-1·3)	0·85	(0·55-1·3)
Household owns radio only	0·83	(0·47-1·5)	0·89	(0·53-1·5)	**0·65****	(0·44-0·96)
Household owns television only	0·20	(0·02-2·0)	**0·34**	(0·11-1·0)	**0·41**	(0·14-1·2)
Household owns other appliances	0·59#	(0·17-2·0)	**0·16*****	(0·06-0·43)	**0·29*****	(0·14-0·61)
Socioeconomic quintile 4	0·98	(0·40-2·4)	**2·9*****	(1·4-6·1)	**1·5**	(0·89-2·6)
Socioeconomic quintile 3	0·74	(0·29-1·9)	1·3	(0·64-2·7)	**2·5*****	(1·4-4·4)
Socioeconomic quintile 2	1·5	(0·61-3·5)	1·1	(0·52-2·2)	**2·7*****	(1·5-4·7)
Socioeconomic quintile 1 (poorest)	1·8	(0·73-4·5)	**2·1**	(0·96-4·6)	**2·9*****	(1·6-5·1)
Domain: Village						
Village toilet type: Pit latrine without slab	IO		0·66	(0·05-8·1)	1·4	(0·38-5·5)
Village toilet type: Pit latrine with slab			1·0#	(0·08-13·4)	0·95	(0·24-3·7)
Village rubbish disposed of by burning only	1·6	(0·16-15·4)	0·78	(0·08-8·5)	**0·45**	(0·15-1·3)
Village rubbish disposed of by other method	0·69	(0·24-2·0)	0·68	(0·23-2·0)	0·83	(0·47-1·5)
Domain: qPCR						
Ascaris infection	1·3	(0·70-2·5)	**1·6**	(0·94-2·8)	**1·4**	(0·89-2·3)
G. duodenalis infection	1·0	(0·57-1·8)	0·60	(0·35-1·0)	**0·42****	(0·20-0·85)
Protozoa infection	1·0	(0·59-1·8)	0·59	(0·34-1·0)	**0·48****	(0·24-0·95)
Domain: Individual recent history						
Pregnant female					0·60	(0·24-1·5)
Not female/not reproductive age					**4·6*****	(3·1-6·9)
Deworming treatment taken within last 12 months	IO		1·4	(0·49-4·1)	IO	
3 or more bowel motions in last 24 hours	0·24**#	(0·06-0·96)	IO		IO	
Diarrhoea during last 2 weeks	IO		1·0	(0·48-2·1)	0·80	(0·43-1·5)
Access to anthelmintics	**0·43**	(0·14-1·4)	0·65	(0·26-1·6)	0·75	(0·34-1·7)

S2: Univariable logistic regression results for *Ascaris* infection (*N*=2152); full list of risk factors investigated

Parameter	Preschool; aged <6 years (*n*=393) OR	(95% CI)	School-aged; aged 6<18 years (*n*=668) OR	(95% CI)	Adults; aged ≥18 years (*n*=1090) OR	(95% CI)
Domain: General						
Age in years	**1·3****	(1·1-1·6)	**0·91****	(0·84-0·98)	**0·99****	(0·98-1·0)
Age group 1 to 5 years	1					
Age group 6 to 11 years	**2·7**	(0·78-9·1)	1			
Age group 12 to 17 years			**0·53****	(0·32-0·89)		
Age group 18-64 years					1	
Age group 65+ years					**0·46*****	(0·26-0·83)
Male sex	1·5	(0·77-2·8)	**1·6**	(0·99-2·7)	**0·68****	(0·46-0·99)
Domain: Individual hygiene						
Does not use soap/ash to wash hands	1·0	(0·41-2·5)	**2·2****	(1·0-4·5)	1·0	(0·58-1·8)
Washes hands after defecation only	1		1		1·4#	(0·51-3·6)
Washes hands after defecation and at other times	1·3	(0·52-3·2)	0·78	(0·39-1·5)	1	
Washes hands at other times only (but not after defecation)	1·1	(0·38-3·2)	0·55	(0·23-1·3)	1·3	(0·80-2·1)
Sometimes/never wears shoes inside house	0·70	(0·29-1·7)	0·76	(0·42-1·4)	0·91	(0·60-1·4)
Sometimes/never wears shoes outside house	0·78	(0·35-1·7)	1·1	(0·62-2·0)	1·1	(0·70-1·8)
Sometimes/never wears shoes when toileting	0·52	(0·21-1·3)	0·88	(0·48-1·6)	0·86	(0·53-1·4)
Domain: Individual sanitation						
Used unhygienic toilet	2·0	(0·61-6·8)	1·1	(0·50-2·6)	**1·7**	(0·91-3·1)
Household has toilet	0·97	(0·36-2·6)	0·77	(0·34-1·7)	**0·59**	(0·34-1·0)
Cleans self with water and hand only after toileting	0·94	(0·42-2·1)	0·57	(0·21-1·6)	0·93	(0·44-2·0)
Cleans self by other method after toileting	1·3	(0·49-3·2)	0·99	(0·44-2·2)	0·85	(0·49-1·5)
Village has public toilet	IO		IO		1·2	(0·42-3·5)
Domain: School related						
Attends school			0·54	(0·21-1·4)		
School in same aldeia (sub-village larger than bairro)			0·76	(0·18-3·2)		
School in same village (broader area than aldeia)/subdistrict/district			0·79	(0·18-3·4)		
Sometimes/never wears shoes inside classroom			1·1	(0·47-2·7)		
Sometimes/never wears shoes outside classroom			1·3	(0·54-3·1)		
Sometimes/never wears shoes when toileting at school			0·93	(0·36-2·4)		
School toilet: No toilet			**0·22****	(0·06-0·76)		
School toilet: Pit latrine without slab			**0·27****	(0·08-0·89)		
School toilet: Other toilet type			2·1	(0·32-14·2)		
Uses school toilet			1·4	(0·31-6·1)		
Domain: Individual socioeconomic						
Not finished primary school					1·2	(0·68-2·0)
Completed primary but not secondary school					1·6	(0·93-2·6)
Completed secondary school or higher					1·1	(0·54-2·2)
Unemployed					**1·8**	(0·99-3·2)
Non-agricultural employment					1·1	(0·52-2·5)
Domain: Household sanitation						
Household toilet: Pit latrine without slab	IO		4·0	(0·89-18·3)	0·79	(0·29-2·2)
Household toilet: Other toilet type			1·5#	(0·03-66·0)	0·57#	(0·08-3·9)
No household toilet/no answer			2·5	(0·79-7·8)	1·4	(0·65-3·2)

Toilet observed to be dirty			2·2	(0·20-23·1)	2·1	(0·59-7·4)
Household toilet is shared	IO		IO		0·57	(0·18-1·8)
Household rubbish disposed of by burning only	1·4	(0·59-3·3)	0·85	(0·40-1·8)	0·78	(0·45-1·3)
Household rubbish disposed of by other method	**0·45**	(0·17-1·2)	**0·49**	(0·22-1·1)	0·84	(0·49-1·4)
Domain: Household water supply						
Main water supply: piped water to yard	1·6	(0·21-12·3)	1·6	(0·27-9·2)	**5·5****	(1·6-18·2)
Main water supply: piped water shared	7·1#	(0·38-130·7)	1·8#	(0·17-17·7)	0·45	(0·08-2·7)
Main water supply: tubewell/borehole	#	#	0·08#	(<0·01-4·5)	0·09#	(<0·01-4·6)
Main water supply: protected spring	1·8#	(0·05-63·2)	0·15#	(<0·01-2·6)	1·5#	(0·34-6·9)
Main water supply: surface water	0·86	(0·28-2·6)	0·77	(0·28-2·1)	**1·6**	(0·85-3·0)
Main water supply located elsewhere	1·7	(0·36-7·7)	**0·36**	(0·11-1·2)	0·83	(0·40-1·7)
Distance to main water supply: 15 min or more	2·2	(0·84-5·6)	1·7	(0·77-3·7)	0·93	(0·53-1·6)
Main water supply not running at least 1 week/month	0·64#	(0·13-3·2)	0·96	(0·28-3·2)	0·66#	(0·25-1·7)
Main water supply not running at least 1 month/year	**0·31**	(0·09-1·0)	1·4	(0·56-3·3)	0·66	(0·34-1·3)
Water stored in covered container only	0·91	(0·36-2·3)	0·61	(0·28-1·3)	0·78	(0·46-1·3)
Secondary water source used	0·65	(0·25-1·7)	0·77	(0·35-1·7)	0·89	(0·53-1·5)
Improved secondary water source					1·6	(0·46-5·5)
Household water is boiled	0·48	(0·19-1·2)	1·6	(0·75-3·6)	0·80	(0·47-1·4)
Domain: Household hygiene						
Household has a food garden	0·83	(0·27-2·5)	1·9	(0·71-5·2)	1·4	(0·72-2·7)
Human faeces used on food garden	IO		0·33	(0·04-2·9)	**3·8**	(0·74-19·3)
Household owns no animals/no answer	0·08#	(<0·01-2·5)	1·1#	(0·09-13·8)	0·94#	(0·21-4·3)
Household owns other animals	**2·0**	(0·74-5·6)	1·3	(0·52-3·4)	1·3	(0·73-2·3)
1-5 dogs	1·2	(0·36-3·7)	0·78	(0·29-2·1)	0·99	(0·54-1·8)
6 or more dogs	0·22#	(0·02-2·3)	0·65#	(0·13-3·2)	0·72#	(0·23-2·3)
1-5 pigs	0·95	(0·23-3·8)	1·1	(0·26-4·2)	0·66	(0·32-1·4)
6 or more pigs	0·46#	(0·07-2·9)	0·62#	(0·11-3·5)	0·99	(0·37-2·7)
1-5 chickens	1·2	(0·26-5·1)	0·94	(0·28-3·2)	1·1	(0·53-2·1)
6 or more chickens	0·74	(0·15-3·7)	0·89	(0·24-3·2)	0·90	(0·42-2·0)
1-5 cows	0·52	(0·17-1·6)	0·68	(0·32-1·5)	0·93	(0·55-1·6)
6 or more cows	1·2#	(0·25-5·6)	1·0	(0·29-3·6)	0·54#	(0·19-1·5)
1 horse owned	0·52	(0·17-1·6)	0·56	(0·24-1·3)	0·70	(0·39-1·2)
2 or more horses owned	0·28#	(0·06-1·4)	0·50	(0·20-1·3)	**0·48****	(0·24-0·94)
Domain: Household socioeconomic						
1 person aged <5 years in household	0·56	(0·16-2·0)	1·5	(0·76-3·1)	**1·7****	(1·1-2·8)
2 or more people aged <5 years in household	0·62	(0·18-2·1)	0·73	(0·35-1·5)	**1·5**	(0·87-2·5)
No people aged 5-17 years in household/no answer	1		1·0#	(0·13-8·2)	1	
1 person aged 5-17 years in household	0·53#	(0·15-1·8)	1		**0·51****	(0·28-0·93)
2 or more people aged 5-17 years in household	1·3	(0·52-3·1)	2·0	(0·78-5·0)	1·1	(0·68-1·7)
1 person aged 18-64 years in household	IO		0·59#	(0·08-4·5)	1·7	(0·54-5·1)
2 or more people aged 18-64 years in household			0·84	(0·19-3·8)	1·3	(0·55-2·9)
1 person aged 65+ years in household	IO		0·41#	(0·10-1·7)	0·68#	(0·27-1·7)
2 or more people aged 65+ years in household			0·50	(0·15-1·7)	0·94	(0·51-1·7)
5-9 people in household	1·9	(0·72-4·7)	1·2	(0·55-2·5)	1·1	(0·76-1·7)
10 or more people in household	0·98#	(0·15-6·5)	0·41	(0·11-1·5)	0·97#	(0·34-2·8)
Household has wooden flooring	0·65	(0·21-2·0)	0·77	(0·35-1·7)	0·90	(0·52-1·5)
Household has cement/tile flooring	0·70#	(0·19-2·7)	0·50	(0·18-1·4)	1·2	(0·59-2·6)
Household has walls of high-grade construction	IO		1·3	(0·35-4·5)	1·5	(0·63-3·5)

Household income more than or equal to USD $1/day	1·2	(0·51-2·6)	0·87	(0·46-1·6)	0·89	(0·58-1·4)
Household owns bicycle only	1·9#	(0·37-9·7)	0·73#	(0·12-4·7)	1·2#	(0·36-4·3)
Household owns motor	1·5	(0·40-5·4)	0·95	(0·31-2·9)	**1·8**	(0·86-3·6)
Household has electricity	0·68	(0·25-1·9)	1·3	(0·53-3·1)	1·1	(0·62-1·9)
Household owns radio only	0·98	(0·43-2·2)	1·1	(0·58-2·2)	1·0	(0·65-1·6)
Household owns television only	1·2#	(0·10-13·1)	1·9#	(0·36-9·9)	1·3	(0·24-6·9)
Household owns other appliances	0·70#	(0·11-4·5)	1·8	(0·43-7·4)	0·46#	(0·11-1·9)
Socioeconomic quintile 5 (wealthiest)	0·36#	(0·08-1·7)	1		1	
Socioeconomic quintile 4	0·76	(0·20-2·9)	0·69	(0·25 - 1·9)	**0·50**	(0·23-1·1)
Socioeconomic quintile 3	0·78	(0·23-2·7)	1·2	(0·45 - 3·2)	0·89	(0·46-1·8)
Socioeconomic quintile 2	0·98	(0·32-3·0)	0·97	(0·36 - 2·6)	0·86	(0·43-1·7)
Socioeconomic quintile 1 (poorest)	1		0·79	(0·28 - 2·2)	1·2	(0·58-2·3)
Domain: Village						
Village toilet type: Pit latrine without slab	IO		IO		1·8	(0·04-72·4)
Village toilet type: Pit latrine with slab					0·16#	(<0·01-8·3)
Village rubbish disposed of by burning only	0·24#	(<0·01-20·2)	0·04#	(<0·01-3·0)	0·04#	(<0·01-1·5)
Village rubbish disposed of by other method	0·52	(0·07-3·7)	0·33	(0·05-2·1)	**0·35**	(0·08-1·5)
Domain: qPCR						
N. americanus infection	1·3	(0·60-2·6)	**1·6**	(0·89-2·8)	1·1	(0·72-1·8)
Ancylostoma infection	IO		2·1	(0·61-7·3)	0·98	(0·43-2·2)
Hookworm infection	1·2	(0·58-2·5)	**1·6**	(0·87-2·8)	1·1	(0·72-1·8)
G. duodenalis infection	0·98	(0·45-2·1)	1·0	(0·52-2·1)	1·3	(0·61-2·6)
Protozoa infection	0·92	(0·43-2·0)	1·1	(0·56-2·2)	1·3	(0·63-2·6)
Domain: Individual recent history						
Pregnant female					0·93	(0·35-2·5)
Not female/not reproductive age					**0·63****	(0·43-0·93)
Deworming treatment taken within last 12 months	2·2	(0·46-10·2)	0·92	(0·20-4·3)	IO	
Current diarrhoea symptoms	IO		IO		1·2	(0·46-3·0)
Diarrhoea during last 2 weeks	IO		0·52	(0·18-1·5)	1·5	(0·75-3·1)
Access to anthelmintics	IO		0·74	(0·21-2·6)	1·0	(0·35-3·0)

S3: Univariable and multivariable logistic regression results for any hookworm infection (*N*=2152); full list of risk factors investigated

	Preschool; aged <6 years (*n*=393)					School-aged; aged 6<18 years (*n*=668)					Adults; aged ≥18 years (*n*=1090)				
	Univariable		Multivariable			Univariable		Multivariable			Univariable		Multivariable		
Parameter	**OR**	**(95% CI)**	**AOR**	**(95% CI)**	***P***	**OR**	**(95% CI)**	**AOR**	**(95% CI)**	***P***	**OR**	**(95% CI)**	**AOR**	**(95% CI)**	***P***
Domain: General															
Age in years	**1·9***	(1·5-2·5)	1·9	(1·4-2·4)	<0·0001	**1·1***	(1·0-1·2)	1·1	(1·0-1·2)	0·010	**1·0***	(1·0-1·0)	1·0	(1·0-1·0)	0·079
Age group 1 to 5 years	1														
Age group 6 to 11 years	**3·4***	(1·3-8·7)				1									
Age group 12 to 17 years						**1·8***	(1·2-2·8)								
Age group 18-64 years											1				
Age group 65+ years											**1·9***	(1·2-3·0)			
Male sex	1·3	(0·80-2·1)	1·5	(0·83-2·7)	0·181	**2·9***	(1·9-4·4)	2·6	(1·7-4·1)	<0·0001	**4·5***	(3·1-6·6)	4·2	(2·8-6·3)	<0·0001
Domain: Individual hygiene															
Did not use soap/ash to wash hands	0·85	(0·45-1·6)				1·2	(0·71-2·1)				1·1	(0·70-1·6)			
Washes hands after defecation and at other times	1·4	(0·71-2·7)				0·91	(0·52-1·6)				0·82	(0·38-1·8)			
Washes hands at other times only (but not after defecation)	1·3	(0·59-2·9)				0·84	(0·42-1·7)				1·2	(0·50-2·7)			
Sometimes/never wears shoes inside house	0·77	(0·41-1·4)				0·95	(0·60-1·5)				1·3	(0·90-1·9)			
Sometimes/never wears shoes outside house	0·86	(0·49-1·5)				1·2	(0·72-1·9)				**1·6***	(1·1-2·4)			
Sometimes/never wears shoes when toileting	0·84	(0·48-1·5)				0·93	(0·56-1·6)				**1·4**	(0·92-2·1)			
Domain: Individual sanitation															
Used unhygienic toilet	1·2	(0·56-2·7)				0·82	(0·45-1·5)				**1·4**	(0·90-2·2)			
Household has toilet	0·69	(0·34-1·4)				1·2	(0·65-2·2)				**0·70**	(0·46-1·1)			
Cleans self with water and hand only after toileting	0·67	(0·35-1·3)				1·0	(0·49-2·1)				0·70	(0·41-1·2)			
Cleans self by other method after toileting	0·69	(0·33-1·5)				0·93	(0·51-1·7)				**0·65****	(0·43-0·98)			
Village has public toilet	IO					1·2	(0·39-3·6)				1·3	(0·59-2·8)			
Domain: School related															
Attends school						0·75	(0·36-1·6)								
School in same aldeia (sub-						1·7	(0·64-4·5)								

village larger than bairro)												
School in same village (broader area than aldeia)/subdistrict/district			1·5	(0·54-4·1)								
Sometimes/never wears shoes inside classroom			1·2	(0·60-2·5)								
Sometimes/never wears shoes outside classroom			1·4	(0·70-3·0)								
Sometimes/never wears shoes when toileting at school			1·3	(0·61-2·8)								
School toilet: No toilet			1·6	(0·64-3·7)								
School toilet: Pit latrine without slab			0·63	(0·31-1·3)								
School toilet: Other toilet type			1·1	(0·34-3·4)								
Uses school toilet			1·0	(0·35-2·9)								
Domain: Individual socioeconomic												
Not finished primary school								**0·67**	(0·43-1·0)	0·87	(0·49-1·5)	0·620
Completed primary but not secondary school								**0·60** **	(0·40-0·91)	0·84	(0·47-1·5)	0·543
Completed secondary school or higher								**0·34** ***	(0·20-0·58)	0·38	(0·19-0·75)	0·005
Unemployed								**0·51** ***	(0·32-0·82)			
Non-agricultural employment								**0·48** **	(0·26-0·92)			
Domain: Household sanitation												
Household toilet: Pit latrine without slab	IO		1·7	(0·60-4·8)				1·6	(0·74-3·4)			
Household toilet: Other toilet type			#					0·90 #	(0·14-6·0)			
No household toilet/no answer			1·1	(0·50-2·5)				**1·8**	(0·97-3·5)			
Toilet observed to be dirty	IO		1·2	(0·37-4·2)				1·3	(0·63-2·6)			
Household toilet is shared	IO		2·2	(0·56-8·8)				0·56	(0·26-1·2)			
Household rubbish disposed of by burning only	1·0	(0·54-1·9)	1·1	(0·58-2·0)				1·1	(0·72-1·8)			
Household rubbish disposed of by other method	1·3	(0·67-2·7)	0·84	(0·46-1·5)				0·94	(0·61-1·5)			
Domain: Household water supply												
Main water supply: piped water to yard	2·3#	(0·48-11·2)	0·69	(0·17-2·7)	0·83	(0·20-3·4)	0·801	**2·0**	(0·73-5·3)	1·2	(0·47-3·1)	0·698
Main water supply: piped water shared	0·74 #	(0·05-10·8)	0·33 #	(0·06-2·0)	0·33#	(0·05-2·0)	0·225	**0·28** **	(0·08-0·95)	0·21	(0·07-0·67)	0·009

Main water supply: tubewell/borehole	0·69 #	(0·08-6·3)	**0·15 ** **	(0·03-0·82)	0·16	(0·03-0·91)	0·039	**0·30 ** **	(0·11-0·80)	0·37	(0·16-0·89)	0·027
Main water supply: unprotected dug well	#		1·0#	(0·09-12·2)	1·7#	(0·11-25·4)	0·709	0·43 #	(0·03-5·5)	0·26#	(0·01-5·9)	0·400
Main water supply: protected spring	1·9#	(0·19-18·2)	0·71 #	(0·11-4·5)	0·75#	(0·11-5·2)	0·768	1·8#	(0·42-7·6)	2·0#	(0·43-9·0)	0·382
Main water supply: surface water	1·5	(0·69-3·4)	1·2	(0·57-2·5)	1·1	(0·49-2·3)	0·875	1·0	(0·62-1·7)	1·1	(0·70-1·8)	0·659
Main water supply located elsewhere	1·1	(0·40-2·9)	**2·3**	(1·0-5·3)				1·2	(0·70-2·1)			
Distance to main water supply: 15 min or more	1·3	(0·69-2·5)	**1·7**	(0·97-3·1)				1·1	(0·74-1·8)			
Main water supply not running at least 1 week/month	0·82 #	(0·25-2·6)	0·88	(0·33-2·4)				1·2	(0·50-2·7)			
Main water supply not running at least 1 month/year	0·86	(0·38-1·9)	0·67	(0·32-1·4)				**0·50 *** **	(0·30-0·83)			
Water stored in covered container only	**1·8**	(0·90-3·6)	**1·6**	(0·90-3·0)	1·9	(0·98-3·5)	0·057	1·3	(0·85-2·0)			
Secondary water source used	1·0	(0·50-2·0)	0·71	(0·38-1·4)				0·92	(0·59-1·4)			
Secondary water source: in household compound	IO		0·55 #	(0·08-4·0)				IO				
Secondary water source: in neighbouring village	IO		0·66	(0·13-3·3)				IO				
Household water is boiled	0·72	(0·38-1·4)	0·79	(0·45-1·4)				0·79	(0·52-1·2)			
Domain: Household hygiene												
Household has a food garden	1·9	(0·82-4·2)	**0·55**	(0·26-1·2)				0·98	(0·57-1·7)			
Human faeces used on food garden	IO		IO					1·1	(0·32-3·4)			
Household owns no animals/no answer	0·35 #	(0·02-5·2)	0·49 #	(0·08-3·1)				0·71 #	(0·19-2·8)			
Household owns other animals	0·71	(0·32-1·6)	1·3	(0·60-2·6)				1·3	(0·76-2·3)			
1-5 dogs	1·5	(0·61-3·7)	1·2	(0·54-2·6)				0·89	(0·50-1·6)			
6 or more dogs	3·6#	(0·72-18·0)	1·4	(0·43-4·7)				0·63	(0·25-1·6)			
1-5 pigs	1·1	(0·39-3·0)	1·0	(0·38-2·7)				1·1	(0·56-2·2)			
6 or more pigs	0·64	(0·19-2·1)	1·4	(0·44-4·3)				0·69	(0·31-1·5)			
1-5 chickens	0·70	(0·24-2·0)	1·3	(0·48-3·6)				0·74	(0·35-1·6)			
6 or more chickens	0·53	(0·17-1·6)	1·2	(0·44-3·4)				0·43 **	(0·20-0·91)	NA		

1-5 cows	1·2	(0·57-2·5)				0·60	(0·35-1·0)	NA	0·78	(0·51-1·2)			
6 or more cows	0·51#	(0·15-1·7)				1·8	(0·73-4·5)	NA	0·81	(0·41-1·6)			
1 horse owned	1·4	(0·62-2·9)				1·6	(0·80-3·1)		0·87	(0·53-1·4)			
2 or more horses owned	0·58#	(0·22-1·6)				1·7	(0·77-3·5)		0·95	(0·55-1·7)			
Domain: Household socioeconomic													
1 person aged <5 years in household	0·68	(0·29-1·6)				0·72	(0·42-1·3)		**0·50****	(0·33-0·76)	0·56	(0·34-0·91)	0·020
2 or more people aged <5 years in household	0·72	(0·31-1·7)				1·1	(0·61-2·0)		**0·49****	(0·31-0·78)	0·51	(0·29-0·90)	0·019
1 person aged 5-17 years in household	**3·6****	(1·4-8·9)	3·2	(1·1-9·9)	0·040	2·2#	(0·49-9·9)		0·97	(0·58-1·6)			
2 or more people aged 5-17 years in household	**2·6****	(1·2-5·4)	1·8	(0·75-4·5)	0·186	1·1	(0·52-2·2)		0·83	(0·56-1·2)			
No people aged 18-64 years in household/no answer	IO					2·9#	(0·56-15·2)		1				
1 person aged 18-64 years in household						1			0·99	(0·36-2·7)			
2 or more people aged 18-64 years in household						1·4	(0·50-4·1)		0·68	(0·34-1·4)			
1 person aged 65+ years in household	IO					2·4	(0·88-6·5)		0·70	(0·36-1·4)			
2 or more people aged 65+ years in household						1·6	(0·60-4·2)		1·3	(0·72-2·3)			
5-9 people in household	**2·0****	(1·0-3·9)				0·81	(0·44-1·5)		**0·77**	(0·54-1·1)			
10 or more people in household	2·5#	(0·68-8·8)				0·93	(0·31-2·8)		**0·51**	(0·19-1·4)			
Household has wooden flooring	0·54	(0·24-1·2)	NA			1·3	(0·70-2·5)		0·82	(0·52-1·3)			
Household has cement/tile flooring	0·49	(0·20-1·2)	NA			0·62	(0·31-1·3)	NA	0·55**	(0·32-0·95)	NA		
Household has walls of high-grade construction	IO					0·72	(0·30-1·8)		0·81	(0·39-1·7)			
Household income more than or equal to USD $1/day	0·66	(0·36-1·2)	NA			0·88	(0·54-1·4)		0·97	(0·68-1·4)			
Household owns bicycle only	IO					1·2	(0·43-3·2)		0·42**	(0·21-0·84)	NA		
Household owns motor						0·56	(0·26-1·2)	NA	0·41***	(0·22-0·73)	NA		
Household has electricity	0·95	(0·47-1·9)				0·69	(0·37-1·3)		0·87	(0·55-1·4)			
Household owns radio only	0·86	(0·49-1·5)				0·93	(0·56-1·6)		0·65**	(0·44-0·96)	NA		
Household owns television	0·20	(0·02-1·9)				0·33	(0·11-1·0)	NA	0·40	(0·13-	NA		

only	#											1·2)			
Household owns other appliances	0·57 #	(0·17-1·9)				0·16 ***	(0·06-0·43)	NA			0·30 ***	(0·14-0·64)	NA		
Socioeconomic quintile 4	1·1	(0·43-2·6)	1·19	(0·41-3·5)	0·757	**3·0***	(1·43-6·2)	0·66	(0·32-1·3)	0·238	**1·5**	(0·87-2·5)	1·56	(0·85-2·9)	0·148
Socioeconomic quintile 3	0·87	(0·34-2·2)	0·75	(0·24-2·4)	0·617	1·3	(0·64-2·7)	0·73	(0·31-1·7)	0·452	**2·6***	(1·5-4·6)	2·96	(1·6-5·6)	0·001
Socioeconomic quintile 2	1·5	(0·63-3·7)	1·6	(0·54-4·7)	0·396	1·0	(0·50-2·1)	0·5	(0·19-1·2)	0·127	**2·6***	(1·5-4·6)	3·3	(1·8-6·4)	<0·0001
Socioeconomic quintile 1 (poorest)	**1·9**	(0·75-4·7)	1·8	(0·58-5·4)	0·316	**2·1**	(0·94-4·6)	1·3	(0·56-3·0)	0·545	**2·9***	(1·7-5·3)	3·3	(1·7-6·5)	<0·0001
Domain: Village															
Village toilet type: Pit latrine without slab	IO					0·64	(0·05-7·7)				1·4	(0·35-5·3)			
Village toilet type: Pit latrine with slab						1·0#	(0·08-12·8)				1·0	(0·26-4·1)			
Village rubbish disposed of by burning only	1·5	(0·16-14·4)				0·82	(0·08-8·8)				**0·43**	(0·14-1·3)			
Village rubbish disposed of by other method	0·78	(0·27-2·2)				0·72	(0·25-2·1)				0·85	(0·48-1·5)			
Domain: qPCR															
Ascaris infection	1·3	(0·68-2·4)				**1·6**	(0·92-2·7)				**1·4**	(0·89-2·3)	1·94	(1·1-3·3)	0·015
G. duodenalis infection	1·0	(0·56-1·8)				0·61	(0·35-1·1)				**0·41** **	(0·20-0·83)			
Protozoa infection	1·0	(0·58-1·8)				0·60	(0·35-1·0)				**0·46** **	(0·23-0·93)	NA		
Domain: Individual recent history															
Pregnant female											0·59	(0·24-1·5)			
Not female/not reproductive age											**4·6*** **	(3·1-6·8)			
Deworming treatment taken within last 12 months	IO					1·4	(0·49-4·0)				IO				
Diarrhoea during last 2 weeks	IO					0·97	(0·47-2·0)				0·90	(0·48-1·7)			
Access to anthelmintics	0·55	(0·18-1·7)				0·69	(0·28-1·7)				0·72	(0·32-1·6)			

S4: Univariable and multivariable logistic regression results for undifferentiated soil-transmitted helminth species (*N*=2152); full list of risk factors investigated

	Preschool; aged <6 years (*n*=393)					School-aged; aged 6<18 years (*n*=668)					Adults; aged ≥18 years (*n*=1090)				
	Univariable		Multivariable			Univariable		Multivariable			Univariable		Multivariable		
Parameter	**OR**	**(95% CI)**	**AOR**	**(95% CI)**	***P***	**OR**	**(95% CI)**	**AOR**	**(95% CI)**	***P***	**OR**	**(95% CI)**	**AOR**	**(95% CI)**	***P***
Domain: General															
Age in years	**1·7***	(1·3-2·1)	1·7	(1·4-2·1)	<0·0001	**1·1**	(1·0-1·2)	1·1	(0·95-1·2)	0·349	**1·0***	(1·0-1·0)	1·0	(1·0-1·0)	0·099
Age group 1 to 5 years	1														
Age group 6 to 11 years	**2·5**	(0·93-6·8)				1									
Age group 12 to 17 years						**1·6**	(0·95-2·6)								
Age group 18-64 years											1				
Age group 65+ years											**1·8***	(1·2-3·0)			
Male sex	1·2	(0·75-2·0)	1·4	(0·81-2·4)	0·234	**4·3***	(2·4-7·7)	4·3	(2·3-8·2)	<0·0001	**3·5***	(2·4-5·0)	3·2	(2·2-4·8)	<0·0001
Domain: Individual hygiene															
Did not use soap/ash to wash hands	0·87	(0·47-1·6)				**2·1***	(1·1-4·2)	2·4	(1·0-5·4)	0·042	1·2	(0·77-1·8)			
Washes hands after defecation and at other times	1·4	(0·70-2·6)				0·95	(0·48-1·9)				0·68	(0·31-1·5)			
Washes hands at other times only (but not after defecation)	1·4	(0·67-3·1)				0·63	(0·27-1·5)				0·92	(0·38-2·2)			
Sometimes/never wears shoes inside house	0·73	(0·39-1·4)				0·75	(0·41-1·4)				1·2	(0·84-1·8)			
Sometimes/never wears shoes outside house	0·80	(0·46-1·4)				1·4	(0·76-2·6)				**1·7***	(1·1-2·5)			
Sometimes/never wears shoes when toileting	0·90	(0·51-1·6)				1·0	(0·56-2·0)				**1·4**	(0·93-2·2)			
Domain: Individual sanitation															
Used unhygienic toilet	1·3	(0·61-2·7)				0·63	(0·29-1·4)				**1·4**	(0·88-2·1)			
Household has toilet	0·82	(0·42-1·6)				1·4	(0·65-3·1)				**0·70**	(0·47-1·1)			
Cleans self with water and hand only after toileting	0·73	(0·40-1·3)				0·89	(0·36-2·2)				0·76	(0·44-1·3)			
Cleans self by other method after toileting	0·85	(0·43-1·7)				0·98	(0·46-2·1)				**0·69**	(0·45-1·1)			
Village has public toilet	**0·32**	(0·08-1·2)				0·90	(0·21-3·8)				0·98	(0·42-2·3)			
Domain: School related															
Attends school						0·51	(0·20-1·3)								
School in same aldeia (sub-village larger than bairro)						2·0	(0·49-8·0)								
School in same village						2·5	(0·58-10·5)								

(broader area than aldeia)/subdistrict/district											
Sometimes/never wears shoes inside classroom			0·90	(0·38-2·1)							
Sometimes/never wears shoes outside classroom			1·4	(0·55-3·6)							
Sometimes/never wears shoes when toileting at school			0·84	(0·33-2·2)							
School toilet: No toilet			0·90	(0·27-2·9)	0·79	(0·21-3·0)	0·727				
School toilet: Pit latrine without slab			**0·37** **	(0·15-0·93)	0·28	(0·10-0·80)	0·017				
School toilet: Other toilet type			0·85 #	(0·20-3·6)	0·67	(0·13-3·4)	0·628				
Uses school toilet			0·99	(0·24-4·1)							
Domain: Individual socioeconomic											
Not finished primary school								**0·71**	(0·45-1·1)	0·92	(0·53-1·6)	0·764
Completed primary but not secondary school								**0·72**	(0·47-1·0)	0·98	(0·56-1·7)	0·941
Completed secondary school or higher								**0·45** ***	(0·27-0·75)	0·52	(0·27-1·0)	0·051
Unemployed								**0·62** **	(0·38-0·99)			
Non-agricultural employment								0·67	(0·36-1·2)			
Domain: Household sanitation											
Household toilet: Pit latrine without slab	1·8	(0·50-6·7)	1·9	(0·50-7·6)				1·3	(0·64-2·8)			
Household toilet: Other toilet type	#		#					0·45 #	(0·08-2·7)			
No household toilet/no answer	1·8	(0·59-5·2)	1·0	(0·36-2·9)				**1·6**	(0·87-3·0)			
Toilet observed to be dirty	IO		0·61	(0·10-3·9)				1·4	(0·57-3·4)			
Household toilet is shared	**3·8**	(0·61-23·6)	1·3	(0·15-10·7)				0·50	(0·20-1·2)			
Household rubbish disposed of by burning only	1·0	(0·55-1·9)	0·85	(0·39-1·9)	0·90	(0·37-2·2)	0·821	1·1	(0·68-1·7)			
Household rubbish disposed of by other method	1·1	(0·55-2·1)	**0·45** **	(0·20-0·98)	0·35	(0·14-0·85)	0·021	0·87	(0·56-1·3)			
Domain: Household water supply											
Main water supply: piped water to yard	3·1#	(0·62-15·9)	1·1#	(0·09-12·3)	1·6#	(0·10-24·0)	0·740	3·8** #	(1·1-13·0)	3·7#	(1·0-13·3)	0·049
Main water supply: piped water shared	3·7#	(0·39-34·1)	1·0#	(0·07-14·0)	1·2#	(0·07-20·2)	0·921	**0·26**	(0·06-1·1)	0·24	(0·06-1·0)	0·055
Main water supply: tubewell/borehole	0·40 #	(0·04-4·3)	**0·05** **	(<0·01-0·57)	0·02	(<0·01-0·42)	0·013	**0·27** **	(0·09-0·82)	0·32	(0·10-1·0)	0·056

Main water supply: unprotected dug well	#					0·91 #	(0·04-19·1)	0·42#	(0·01-16·8)	0·643	0·50 #	(0·05-5·1)	0·36#	(0·02-6·5)	0·489
Main water supply: protected spring	1·3#	(0·12-13·3)				0·35 #	(0·04-3·4)	0·39#	(0·03-4·8)	0·460	1·3#	(0·32-5·4)	1·5#	(0·31-6·9)	0·632
Main water supply: surface water	1·2	(0·58-2·6)				1·3	(0·47-3·4)	2·3	(0·68-7·9)	0·179	1·1	(0·67-1·8)	1·4	(0·77-2·3)	0·302
Main water supply located elsewhere	1·2	(0·46-3·2)				0·89	(0·29-2·7)				1·2	(0·70-2·2)			
Distance to main water supply: 15 min or more	**1·7**	(0·89-3·4)	2·0	(0·92-4·2)	0·081	1·4	(0·67-3·0)				1·3	(0·82-2·0)			
Main water supply not running at least 1 week/month	0·89	(0·28-2·9)				0·76	(0·22-2·6)				0·84	(0·37-1·9)			
Main water supply not running at least 1 month/year	0·67	(0·28-1·6)				0·62	(0·22-1·7)				0·60	(0·35-1·0)			
Water stored in covered container only	1·4	(0·71-2·6)				1·1	(0·52-2·4)				1·1	(0·75-1·7)			
Secondary water source used	1·0	(0·51-2·1)				**0·43**	(0·18-1·0)	0·43	(0·16-1·17)	0·098	0·92	(0·57-1·5)			
Secondary water source: in household compound	IO					0·39 #	(0·03-4·4)				IO				
Secondary water source: in neighbouring village	IO					0·34	(0·04-2·7)				IO				
Household water is boiled	0·68	(0·36-1·3)				0·97	(0·47-2·0)				0·75	(0·50-1·1)			
Domain: Household hygiene															
Household has a food garden	1·4	(0·64-3·0)				0·60	(0·24-1·5)				0·91	(0·54-1·5)			
Human faeces used on food garden	IO					IO					1·7	(0·44-6·9)			
Household owns no animals/no answer	0·18 #	(0·01-2·3)				0·42 #	(0·04-4·2)				0·77 #	(0·20-2·9)			
Household owns other animals	0·91	(0·43-2·0)				1·4	(0·51-3·6)				1·3	(0·75-2·2)			
1-5 dogs	1·6	(0·64-3·9)				1·5	(0·52-4·0)				0·97	(0·56-1·7)			
6 or more dogs	1·9#	(0·36-10·6)				1·5	(0·33-6·9)				0·73	(0·30-1·8)			
1-5 pigs	1·1	(0·40-3·1)				0·89	(0·24-3·3)				1·0	(0·52-2·0)			
6 or more pigs	0·79	(0·23-2·7)				1·3	(0·29-5·9)				0·70	(0·32-1·5)			
1-5 chickens	1·0	(0·33-3·3)				1·8	(0·47-6·8)				0·64	(0·29-1·4)			
6 or more chickens	0·68	(0·21-2·2)				1·5	(0·39-5·9)				0·38 **	(0·17-0·85)	NA		
1-5 cows	0·96	(0·48-1·9)				0·47 **	(0·24-0·92)	NA			0·79	(0·52-1·2)			
6 or more cows	0·72	(0·25-2·1)				1·7	(0·57-5·4)				0·78	(0·41-			

1 horse owned	0·91	(0·43-1·9)				1·3	(0·53-3·1)				0·83	1·5) (0·51-1·3)			
2 or more horses owned	0·50	(0·20-1·3)	NA			1·1	(0·42-2·9)				0·88	(0·51-1·5)			
Domain: Household socioeconomic															
1 person aged <5 years in household	0·71	(0·31-1·6)				0·97	(0·47-2·0)				**0·55** ***	(0·37-0·82)	0·63	(0·39-1·0)	0·051
2 or more people aged <5 years in household	0·68	(0·30-1·5)				1·3	(0·58-2·7)				**0·59** **	(0·38-0·93)	0·68	(0·40-1·2)	0·163
No people aged 5-17 years in household/no answer	1					1·6#	(0·27-10·0)				1				
1 person aged 5-17 years in household	**2·2**	(0·96-5·0)				1					0·84	(0·51-1·4)			
2 or more people aged 5-17 years in household	**2·2** *	(1·2-4·2)				0·96	(0·39-2·4)				0·83	(0·57-1·2)			
1 person aged 18-64 years in household	IO					IO					0·77	(0·29-2·1)			
2 or more people aged 18-64 years in household											0·68	(0·34-1·4)			
1 person aged 65+ years in household	IO					2·4	(0·69-8·4)				0·86	(0·45-1·6)			
2 or more people aged 65+ years in household						1·1	(0·31-3·9)				1·3	(0·75-2·3)			
5-9 people in household	**1·9** *	(1·0-3·5)				0·76	(0·35-1·7)				0·81	(0·58-1·2)			
10 or more people in household	2·8#	(0·79-9·7)				0·77	(0·18-3·2)				0·60	(0·23-1·6)			
Household has wooden flooring	0·61	(0·28-1·3)				0·97	(0·43-2·2)				0·95	(0·61-1·5)			
Household has cement/tile flooring	0·66	(0·28-1·5)				0·44	(0·17-1·1)	NA			0·60	(0·36-1·0)	NA		
Household has walls of high-grade construction	IO					0·79	(0·24-2·6)				0·88	(0·44-1·8)			
Household income more than or equal to USD $1/day	0·96	(0·55-1·7)				0·83	(0·44-1·6)				1·1	(0·76-1·5)			
Household owns bicycle only	0·87	(0·32-2·4)				1·6	(0·43-5·65)				0·59	(0·30-1·1)	NA		
Household owns motor	1·0	(0·43-2·6)				0·34 **	(0·12-0·95)	NA			0·66	(0·37-1·2)	NA		
Household has electricity	0·78	(0·39-1·6)				0·64	(0·27-1·5)				0·94	(0·60-1·5)			
Household owns radio only	0·92	(0·52-1·7)				1·1	(0·57-2·2)				0·64 **	(0·44-0·94)	NA		
Household owns television only	0·45 #	(0·09-2·3)				0·25	(0·06-1·0)	NA			0·44	(0·15-1·2)	NA		
Household owns other appliances	0·57	(0·17-1·9)				0·13 ***	(0·04-0·44)	NA			0·32 ***	(0·16-0·63)	NA		
Socioeconomic quintile 4	1·5	(0·64-3·6)	1·01	(0·39-2·7)	0·982	**2·3**	(0·92-5·6)	0·92	(0·36-2·3)	0·861	1·3	(0·79-	1·42	(0·80-	0·231

											2·1)		2·5)	
Socioeconomic quintile 3	1·1	(0·44-2·7)	2·59	(0·91-7·4)	0·074	1·6	(0·63-4·0)	1·0	(0·32-3·1)	1·000	**1·8****	(1·1-3·1)	1·78	(0·96-3·3)	0·067
Socioeconomic quintile 2	1·5	(0·63-3·6)	1·73	(0·56-5·3)	0·343	1·1	(0·43-2·8)	0·58	(0·15-2·2)	0·418	**2·2****	(1·3-3·9)	2·44	(1·3-4·6)	0·007
Socioeconomic quintile 1 (poorest)	**1·8**	(0·73-4·7)	1·38	(0·53-3·6)	0·507	**3·4****	(1·12-10·5)	1·6	(0·53-5·0)	0·390	**2·5****	(1·4-4·4)	2·97	(1·5-5·8)	0·001
Domain: Village															
Village toilet type: Pit latrine without slab	IO					0·44	(0·01-16·4)				1·2	(0·18-7·3)			
Village toilet type: Pit latrine with slab						0·47#	(0·01-18·4)				1·1#	(0·17-7·0)			
Village rubbish disposed of by burning only	0·68	(0·04-12·1)				0·28	(<0·01-8·8)				**0·28**	(0·05-1·4)			
Village rubbish disposed of by other method	0·66	(0·18-2·4)				0·38	(0·08-1·8)				0·71	(0·33-1·5)			
Domain: qPCR															
G. duodenalis infection	0·92	(0·51-1·6)				0·57	(0·30-1·1)				0·55	(0·26-1·1)			
Protozoa infection	0·93	(0·52-1·7)				0·55	(0·30-1·1)				0·61	(0·30-1·2)			
Domain: Individual recent history															
Pregnant female											0·58	(0·24-1·4)			
Not female/not reproductive age											**3·4****	(2·3-5·0)			
Deworming treatment taken within last 12 months	0·50	(0·16-1·6)				1·2	(0·31-4·6)				IO				
Diarrhoea during last 2 weeks	**0·48**	(0·19-1·2)				**0·56**	(0·24-1·3)				1·0	(0·54-1·9)			
Access to anthelmintics	**0·36**	(0·12-1·1)				0·60	(0·19-1·9)				0·50	(0·22-1·2)			

S5: Univariable logistic regression results for *G. duodenalis* infection (*N*=2152); full list of risk factors investigated

Parameter	Preschool; aged <6 years (*n*=393) OR	(95% CI)	School-aged; aged 6<18 years (*n*=668) OR	(95% CI)	Adults; aged ≥18 years (*n*=1090) OR	(95% CI)
Domain: General						
Age in years	0·91	(0·78-1·1)	**0·91****	(0·84-0·98)	**0·98****	(0·95-1·0)
Age group 1 to 5 years	IO					
Age group 6 to 11 years			1			
Age group 12 to 17 years			**0·62**	(0·38-1·0)		
Age group 18-64 years					IO	
Age group 65+ years						
Male sex	0·95	(0·59-1·5)	1·1	(0·71-1·7)	0·70	(0·37-1·3)
Domain: Individual hygiene						
Did not use soap/ash to wash hands	0·94	(0·54-1·7)	0·82	(0·47-1·4)	0·75	(0·30-1·9)
Washes hands after defecation only	1		1		0·89#	(0·16-4·9)
Washes hands after defecation and at other times	1·3	(0·73-2·4)	0·87	(0·49-1·6)	1	
Washes hands at other times only (but not after defecation)	0·92	(0·46-1·8)	1·0	(0·49-2·1)	1·3	(0·57-3·0)
Sometimes/never wears shoes inside house	1·1	(0·61-2·0)	1·4	(0·85-2·4)	0·82	(0·40-1·7)
Sometimes/never wears shoes outside house	1·3	(0·77-2·3)	0·96	(0·58-1·6)	1·0	(0·47-2·2)
Sometimes/never wears shoes when toileting	1·4	(0·81-2·4)	1·2	(0·73-2·0)	0·83	(0·37-1·9)
Domain: Individual sanitation						
Used unhygienic toilet	1·2	(0·58-2·5)	0·76	(0·43-1·4)	IO	
Household has toilet	**1·6**	(0·88-2·8)	1·2	(0·72-2·1)	0·97	(0·41-2·3)
Cleans self with water and hand only after toileting	0·84	(0·47-1·5)	1·3	(0·64-2·6)	0·97#	(0·32-3·0)
Cleans self by other method after toileting	1·0	(0·54-1·9)	1·1	(0·58-1·9)	0·97	(0·43-2·2)
Village has public toilet	IO		**2·2**	(0·92-5·2)	IO	
Domain: School related						
Attends school			0·72	(0·34-1·5)		
School in same aldeia (sub-village larger than bairro)			1·3	(0·59-3·1)		
School in same village (broader area than aldeia)/subdistrict/district			1·5	(0·63-3·6)		
Sometimes/never wears shoes inside classroom			**2·0**	(1·0-4·2)		
Sometimes/never wears shoes outside classroom			1·2	(0·59-2·5)		
Sometimes/never wears shoes when toileting at school			1·5	(0·70-3·2)		
School toilet: No toilet			1·2	(0·60-2·2)		
School toilet: Pit latrine without slab			**0·49**	(0·25-0·97)		
School toilet: Other toilet type			1·4#	(0·53-3·7)		
Domain: Individual socioeconomic						
Not finished primary school					0·85#	(0·30-2·4)
Completed primary but not secondary school					1·3	(0·51-3·0)
Completed secondary school or higher					**2·8**	(1·0-7·8)
Domain: Household sanitation						
Household toilet: Pit latrine without slab	IO		1·6	(0·60-4·2)	IO	
Household toilet: Other toilet type			#			
No household toilet/no answer			1·1	(0·48-2·3)		
Household rubbish disposed of by burning only	1·1	(0·63-2·0)	0·89	(0·49-1·6)	1·2	(0·51-2·8)
Household rubbish disposed of by other method	1·4	(0·78-2·5)	0·79	(0·44-1·4)	0·96	(0·38-2·4)
Domain: Household water supply						

Main water supply: piped water to yard	1·0#	(0·34-3·2)	0·43#	(0·11-1·7)	1·5#	(0·26-8·5)
Main water supply: piped water shared	1·1#	(0·19-6·3)	0·86#	(0·18-4·1)	1·4#	(0·18-11·1)
Main water supply: tubewell/borehole	0·89#	(0·24-3·4)	0·32#	(0·06-1·7)	0·28#	(0·02-3·2)
Main water supply: protected spring	0·45#	(0·05-4·3)	1·1#	(0·17-6·5)	0·68#	(0·05-9·0)
Main water supply: surface water	1·2	(0·67-2·3)	1·2	(0·66-2·1)	0·47	(0·18-1·2)
Main water supply located elsewhere	0·78	(0·36-1·7)	IO		IO	
Distance to main water supply: 15 min or more	1·1	(0·63-1·8)	**0·64**	(0·37-1·1)	0·76	(0·33-1·7)
Main water supply not running at least 1 week/month	1·7#	(0·65-4·4)	1·2#	(0·46-3·3)	3·1#	(0·75-13·0)
Main water supply not running at least 1 month/year	1·3	(0·70-2·5)	1·3	(0·66-2·4)	1·3	(0·49-3·3)
Water stored in covered container only	1·0	(0·58-1·8)	0·94	(0·53-1·7)	0·68	(0·29-1·6)
Secondary water source used	**1·5**	(0·87-2·7)	1·4	(0·81-2·4)	**1·8**	(0·80-4·2)
Household water is boiled	1·0	(0·60-1·7)	1·2	(0·68-2·0)	0·86	(0·39-1·9)
Domain: Household hygiene						
Household has a food garden	0·93	(0·45-1·9)	1·3	(0·62-2·8)	IO	
Household owns no animals/no answer	2·1#	(0·32-13·7)	1·3#	(0·21-7·9)	IO	
Household owns other animals	1·6	(0·83-3·0)	0·80	(0·37-1·7)		
1-5 dogs	0·90	(0·41-2·0)	IO		IO	
6 or more dogs	0·51#	(0·09-2·9)				
No pigs owned/no answer	1		0·83#	(0·32-2·2)	IO	
1-5 pigs	**0·32*****	(0·15-0·72)	1			
6 or more pigs	**0·33****	(0·13-0·83)	**2·1****	(1·1-3·8)		
1-5 chickens	1		1		1	
No chickens owned/no answer	1·5#	(0·56-3·8)	0·77#	(0·25-2·4)	0·67#	(0·17-2·7)
6 or more chickens	0·90	(0·54-1·5)	**1·7****	(1·1-2·9)	0·98	(0·46-2·1)
1-5 cows	0·90	(0·51-1·6)	0·71	(0·41-1·2)	0·71	(0·29-1·7)
6 or more cows	0·42#	(0·13-1·3)	0·61	(0·24-1·6)	0·17#	(0·02-1·7)
1 horse owned	0·81	(0·42-1·5)	1·2	(0·62-2·2)	IO	
2 or more horses owned	1·3	(0·59-2·8)	0·58	(0·27-1·3)		
Domain: Household socioeconomic						
No people aged <5 years in household/no answer	0·78#	(0·32-1·9)	1		1	
1 person aged <5 years in household	1		1·0	(0·59-1·8)	1·3	(0·59-3·0)
2 or more people aged <5 years in household	1·3	(0·79-2·3)	1·1	(0·59-1·9)	0·91#	(0·35-2·3)
No people aged 5-17 years in household/no answer	1		0·47#	(0·09-2·6)	1	
1 person aged 5-17 years in household	1·2	(0·52-2·7)	1		1·8	(0·69-4·9)
2 or more people aged 5-17 years in household	1·4	(0·76-2·7)	0·85	(0·41-1·8)	1·4	(0·64-3·2)
2 or more people aged 65+ years in household	IO		IO		1·8	(0·67-4·7)
5-9 people in household	1·3	(0·67-2·3)	0·91	(0·49-1·7)	1·0	(0·51-2·0)
10 or more people in household	0·96#	(0·27-3·5)	1·7	(0·66-4·5)	0·56#	(0·05-6·3)
Household has wooden flooring	0·74	(0·37-1·5)	0·76	(0·41-1·4)	0·53	(0·20-1·4)
Household has cement/tile flooring	1·1	(0·53-2·4)	0·99	(0·48-2·1)	0·97	(0·34-2·8)
Household income more than or equal to USD $1/day	**0·71**	(0·42-1·2)	0·74	(0·45-1·2)	0·74	(0·36-1·5)
Household owns bicycle only	IO		0·60#	(0·20-1·8)	0·63#	(0·11-3·7)
Household owns motor			0·92	(0·42-2·0)	**3·0****	(1·1-8·4)
Household has electricity	1·2	(0·65-2·0)	1·6	(0·85-2·9)	2·2	(0·82-5·9)
Household owns radio only	1·1	(0·67-1·9)	**1·8****	(1·1-3·0)	1·6	(0·77-3·4)
Household owns television only	1·7#	(0·38-7·4)	1·4#	(0·46-4·5)	#	
Household owns other appliances	1·2#	(0·41-3·4)	1·5#	(0·58-3·7)	1·3#	(0·27-5·8)
Socioeconomic quintile 4	1·2	(0·54-2·6)	1·1	(0·53-2·3)	1·2	(0·40-3·5)

Socioeconomic quintile 3	1·1	(0·48-2·5)	1·3	(0·60-2·6)	0·80	(0·26-2·4)
Socioeconomic quintile 2	0·73	(0·32-1·7)	0·86	(0·41-1·8)	0·66#	(0·21-2·1)
Socioeconomic quintile 1 (poorest)	1·3	(0·60-2·9)	0·77	(0·35-1·7)	0·85	(0·27-2·6)
Domain: Village						
Village rubbish disposed of by burning only	0·89	(0·33-2·4)	0·93	(0·36-2·4)	0·97#	(0·12-7·7)
Village rubbish disposed of by other method	0·75	(0·41-1·4)	0·83	(0·46-1·5)	0·75	(0·25-2·2)
Domain: qPCR						
Ascaris infection	1·0	(0·59-1·8)	0·95	(0·56-1·6)	**1·7**	(0·80-3·7)
N. americanus infection	1·0	(0·60-1·7)	0·73	(0·45-1·2)	**0·43****	(0·21-0·90)
Hookworm infection	0·99	(0·59-1·7)	0·74	(0·45-1·2)	**0·42****	(0·20-0·88)
STH infection	0·92	(0·56-1·5)	0·72	(0·43-1·2)	0·58	(0·26-1·3)
Domain: Individual recent history						
Deworming treatment taken within last 12 months	1·0	(0·43-2·5)	IO		IO	
Access to anthelmintics	1·4	(0·61-3·0)	1·5	(0·66-3·6)	IO	

Chapter 5 Analysis of soil-transmitted helminth associations with child and community morbidity

5.1 Chapter context

It is problematic to use prevalence as an indicator of STH morbidity or transmission because large changes in average intensity may only be accompanied by small changes in prevalence (17). As STH do not multiply in the host, infection intensity depends on the time and extent of exposure (5). Strong direct correlations have been reported between intensity of STH infection and morbidity (71,79-82). As such, intensity of infection is a better indicator of STH transmission and morbidity than prevalence. In this analysis PCR-derived Ct-values of STH infection were used to investigate intensity of STH infection on morbidity outcomes. These specifically included community haemoglobin and anaemia, and standardised measures of anthropometry in children aged 1<18 years: stunting, wasting and being underweight.

To undertake this analysis, classes of infection intensity were determined using an algorithm that correlated Ct-values from qPCR to eggs per gram of faeces (epg) equivalents, determined by seeding experiments (239). This attempt is hypothesis-generating; few attempts at categorising qPCR into classes of infection intensity have been identified in the literature (250) and none for subsequent epidemiological analyses. The

categorised intensity of infection variable was then used in both this analysis, and in the final analysis (Chapter 6).

Chapter 2 has provided important morbidity context for this analysis. It is very important to investigate STH impacts on morbidity so as to maintain impetus for disease control activities; even in observational studies there is much that is still unknown. Very few studies have investigated intensity of infection on a scale of morbidity (as opposed to prevalence of morbidity). More efforts to investigate potentially different impacts by age are also crucial, as children of youngest ages have the greatest growth velocities, and impact of STH in these formative years is suspected to be highly deleterious. This analysis provides the first investigation of STH association with community haemoglobin and child morbidity outcomes in Timor-Leste. This was investigated using both an intensity of infection scale, and for child anthropometric indices, a level of severity scale.

This chapter makes an important contribution to knowledge of STH impact on community and child development outcomes in Timor-Leste, for which there is no prior literature. It also directly contributes to the research agenda of how to use PCR data to assess STH infection intensity. This is potentially much more powerful than using an expensive and advanced coprodiagnostic technique to simply measure prevalence. The material cost of processing samples and running both multiplex PCRs for these data has been estimated as AU$12.37 per sample (AU$6.05 per extraction; AU$3.16 per multiplex PCR) (239). The cost of qPCR, coupled with inability for onsite analysis, are issues affecting current applicability of qPCR in epidemiological studies, and are likely to require collaboration

with external institutions for analytical purposes in many developing countries. More work in this area is crucial. This chapter meets objective 4 of the thesis. This chapter is under review with *Parasites and Vectors*.

5.2 Research objectives

(i) To determine the impact of STH on haemoglobin concentration in community members, and anthropometric indices in children aged 1<18 years, using data from the study sample at baseline.

(ii) To determine cut-points for intensity of infection by analysing DNA concentration from quantitative PCR results.

Rationale for methods

Similar to the structure of the previous chapter, this section briefly provides the rationale for the statistical methods used, with additional information to the methods section in the attached manuscript.

Participants were excluded from models for which they had not provided the outcome (haemoglobin, height or weight) measurement, and if they had not provided a stool sample for parasitological assessment. For this reason, the study population in this analysis (N=2038) varies slightly from Chapter 4 (N=2152).

Model selection and building

Detail on z-score development is provided in the manuscript. For morbidity analyses, few people had severe anaemia, so a binary-coded anaemia outcome was used, and modelled using logistic regression. However, there were sufficient numbers of severely stunted, wasted and underweight children to allow multinomial categorisation of these morbidity outcomes on a scale of severity: severe/moderate/normal. Modelling these measures as multinomial outcomes is important to investigate STH impact on severity of morbidity in this population.

The "mlogit" command is usually used in STATA for multinomial logistic regression; however, because of the additional need to account for the household and village random effects, generalised structural equation modelling (GSEM) was used. These are essentially multinomial logit models which additionally allow inclusion of mixed effects. These models use different syntax to other STATA regression models, they are computationally intensive, and interpretationally more complex. This analysis used a three-level nested multinomial outcome model (Figure 5.1). The base outcome was assigned as the "normal growth" version of the morbidity variable. As other outcome categories are measured back to this, coefficients are log(relative risks). All output was therefore exponentiated so that results can be interpreted as relative risks (important: these are relative to the **base** outcome).

For variable selection, in addition to descriptive methods (see Appendix 1), the distribution of residuals for continuous explanatory variables was assessed. Because age was shown to follow a non-linear relationship with morbidity outcomes, a categorised age group variable was used. Analyses were further stratified by age groups, ideally with the aim of analysing preschool children morbidity impacts separately from school-aged children. However, infection numbers were too low when models were stratified in this way, so children aged 1<18 years and adults 18 years and over were analysed separately in regression models. For all models, measures of model fit were examined using likelihood ratio tests, selecting models with lower *P*-values as this indicates a better model.

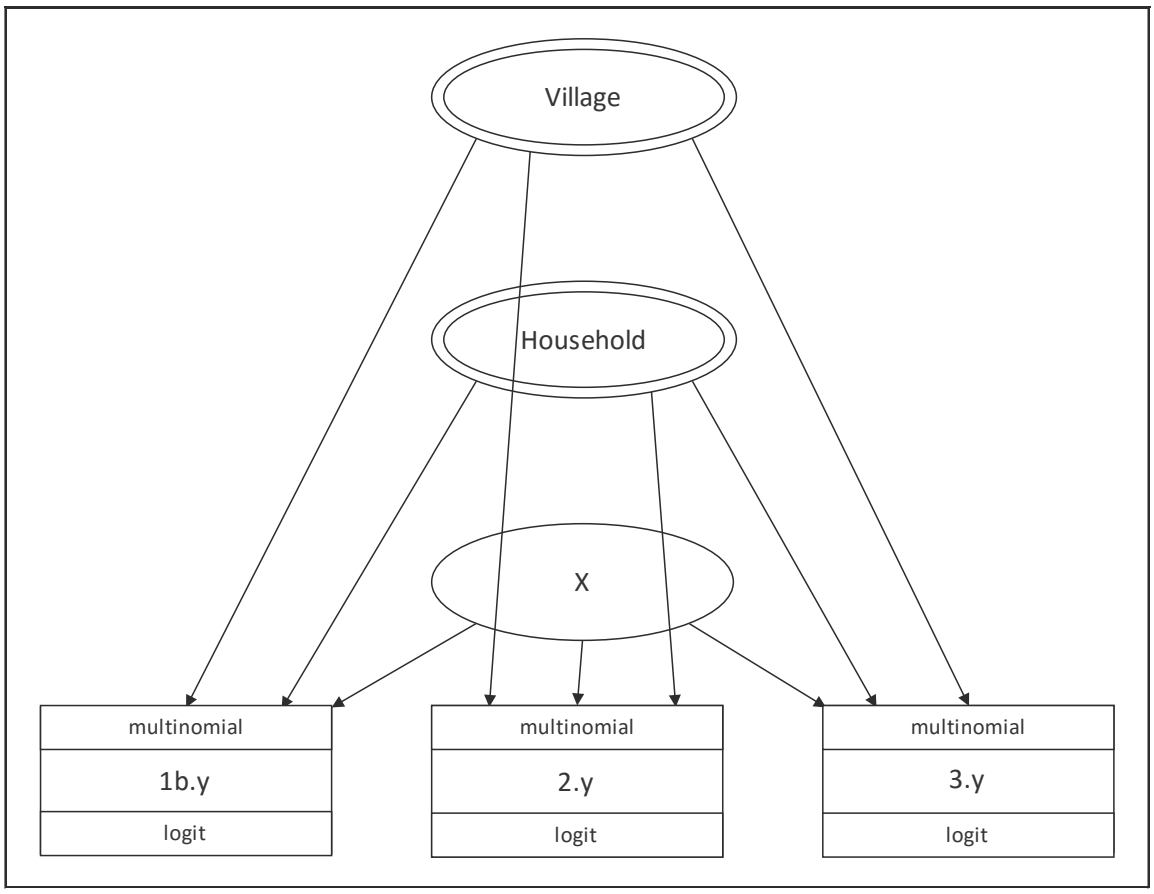

Figure 5.1: Path diagram for GSEM model of child anthropometry outcomes

Notes: y is the multinomial outcome, with different classes as indicated by 1,2,3. "b" indicates that 1.y is the reference outcome category (which could be omitted from the diagram but is shown for simplicity). X is an explanatory variable; any X's in the multivariable model will follow this same path. "Village" and "household" in double circles are latent random effects. The arrows (paths); indicate the direction in which one variable affects another. "Multinomial" and "logit" explain the "multinomial" family and use of the logit (mlogit) link. For example, for the outcome of stunting, 1b.y respresents the base category of normal-growth children, to which other stunting categories are compared. Diagram and notes adapted from (251).

For this analysis, the PhD Candidate was responsible for 90% of the conception, 90% of the analysis, and 90% of the interpretation and writing of the paper. Clements A was responsible for 10% of the conception. D'Este C provided statistical advice and was responsible for 10% of the analysis. Clements A, Nery S, D'Este C, Gray D, McCarthy J, Traub R, Andrews R, Vallely A and Williams G will collectively be responsible for 10% of the interpretation and writing of the paper.

Investigations into the association between soil-transmitted helminth infections, haemoglobin and child development indices in Manufahi District, Timor-Leste

Suzy J Campbell[a*], Susana V Nery[a], Catherine A D'Este[a], Darren J Gray[a,b,c], James S McCarthy[c,d], Rebecca J Traub[e], Ross M Andrews[f], Stacey Llewellyn[d], Andrew J Vallely[g], Gail M Williams[c], Archie CA Clements[a]

A. Research School of Population Health, College of Medicine, Biology and Environment, The Australian National University, 62 Mills Rd, Acton, ACT 2601, Australia (suzanne.campbell@anu.edu.au; susana.nery@anu.edu.au; catherine.deste@anu.edu.au; darren.gray@anu.edu.au; director.RSPH@anu.edu.au)
B. Molecular Parasitology Laboratory, QIMR Berghofer Medical Research Institute, 300 Herston Rd, Brisbane QLD 4006, Australia
C. School of Public Health, University of Queensland, Herston Rd, Brisbane QLD 4006, Australia (gail.williams@uq.edu.au)
D. Clinical Tropical Medicine Laboratory, QIMR Berghofer Medical Research Institute, 300 Herston Rd, Brisbane QLD 4006, Australia (james.mccarthy@qimrberghofer.edu.au; stacey.llewellyn@qimrberghofer.edu.au)
E. Faculty of Veterinary and Agricultural Science, The University of Melbourne, Parkville VIC 3010, Australia (rebecca.traub@unimelb.edu.au)
F. Menzies School of Health Research, Charles Darwin University, Ellengowan Dr, Casuarina NT 0810, Australia (ross.andrews@menzies.edu.au)
G. Kirby Institute, University of New South Wales, Wallace Wurth Building, High St, Kensington NSW 2052, Australia (avallely@kirby.unsw.edu.au)

*Corresponding Author: Suzy Campbell, Department of Global Health, Research School of Population Health, The Australian National University, 62 Mills Rd, Acton, ACT 2601, Australia. Email: suzanne.campbell@anu.edu.au, Phone: (+61) 2 6125 0538, Fax: (+61) 2 6125 5608

Investigations into the association between soil-transmitted helminth infections, haemoglobin and child development indices in Manufahi District, Timor-Leste

Abstract

Background: Timor-Leste has high prevalence of soil-transmitted helminth (STH) infections. High proportions of the population have been reported as being anaemic, and extremely high proportions of children as stunted or wasted. There have been no published analyses of the contributions of STH to these morbidity outcomes.

Methods: Using baseline cross-sectional data from 24 villages (18 villages enrolled in a cluster randomised controlled trial, and identically-collected data from six additional villages), analyses of the association between STH infections and community haemoglobin and child development indices were undertaken. Stool samples were assessed for STH using qPCR and participant haemoglobin, heights and weights were recorded. Questionnaires were administered to collect demographic and socioeconomic data. Intensity of infection was categorised using correlational analysis between qPCR cycle threshold values and eggs per gram of faeces equivalents, with algorithms generated from seeding experiments. Mixed-effects logistic and multinomial regression were used to assess the association between STH infection intensity classes and anaemia, and child stunting, wasting and underweight.

Results: Very high stunting (60%), underweight (60%), and wasting (20%) in children, but low anaemia prevalence (15%), were found in the study communities. STH were not significantly associated with morbidity outcomes. Male children and those in the poorest socioeconomic quintile were significantly more likely to be moderately and severely

stunted. Male children were significantly more likely than female children to be severely underweight. Increasing age was also a risk factor for being underweight. Few risk factors emerged for wasting in these analyses.

Conclusions: According to World Health Organization international reference standards, levels of child morbidity in this population constitute a public health emergency, although the international reference standards need to be critically evaluated for their applicability in Timor-Leste. Strategies to improve child development and morbidity outcomes, for example via nutrition and iron supplementation programmes, are recommended for these communities. Despite the apparent lack of an association from STH in driving anaemia, stunting, wasting and underweight, high endemicity indicates a need for STH control strategies. (Trial registration: Australian and New Zealand Clinical Trials Registry ACTRN12614000680662; retrospectively registered).

Key words

Soil-transmitted; helminth; *Necator americanus*; *Ascaris*; morbidity; haemoglobin; anaemia; stunting; wasting; underweight; PCR.

Background

Southeast Asia harbours one-third of the world's soil-transmitted helminths (STH) [1], and Timor-Leste is one of the poorest countries in the region [2]. Two recent cross-sectional studies (one school-based and the other community-based) identified moderate school-based STH prevalence (29%) [3], and high community-based (69%) [4] STH prevalence, in the Manufahi District of Timor-Leste, using different diagnostic techniques. Specifically, in

this area, prevalence of *Necator americanus* was 60%, *Ascaris* spp. 24%, *Ancylostoma* spp. 4.7%, and *Trichuris trichiura* 0.33%, with *Giardia duodenalis* the most common protozoan identified (13%) [4]. Inadequate water and sanitation infrastructure and hygiene behaviours in this area likely contribute to high STH endemicity [4], which in turn could contribute significantly to morbidity.

STH have previously been associated with anaemia, stunting and wasting [5-9]. The mechanism whereby hookworms contribute to reduced haemoglobin and more indirectly to poor growth and development outcomes is via blood loss and inflammation, with heavily-infected people at greatest risk of morbidity [10]. *A. lumbricoides* is not considered a contributor to blood loss (reviewed in [11]) and *T. trichiura* contributes to blood loss in heavy infection. Whilst STH have been shown to be associated with stunting and wasting [12, 13] the causal relationship is not clear.

Despite inability to establish causality with observational analyses, investigating the relationship between STH infection and haemoglobin concentration, and child anthropometric indices, is of considerable importance in Timor-Leste. Extremely high proportions of Timorese under five years of age are reported as stunted (50%), and wasted (11%) [14], yet knowledge of the contribution of STH to this is very limited, with no prior investigations identified. A cross-sectional survey in 2008 found 22% of children aged 24-59 months of age were anaemic [15]. Additionally, a demographic health survey in 2009-10 found 38% of Timorese children aged 6-59 months, and 21% of Timorese women aged 15-49 years were anaemic [16]. Since this time, risk factors for anaemia in women of

reproductive age have been investigated [17]. However, limited data on STH have prevented STH contributions from being assessed.

Using quantitative polymerase chain reaction (qPCR) for STH diagnosis and intensity of infection assessment [18], we aim to (i) determine classes of STH infection intensity from PCR-derived data, and (ii) provide the first analysis of morbidity associated with STH infections in Manufahi District, Timor-Leste. We used an algorithm to correlate cycle threshold (Ct) values from qPCR to eggs per gram of faeces (epg) equivalents, determined by seeding experiments [18]. The association between intensity of *N. americanus* and *Ascaris* spp. (as exposures) and mean haemoglobin concentrations and anaemia diagnosis (as outcomes) were then investigated for all community members, and associations with stunting, underweight and low BMI-for-age (as a measure of wasting) as outcomes in children aged one to 18 years were also investigated.

Methods

Study registration and ethics

These analyses were conducted as part of a cluster randomised controlled trial (RCT) in Manufahi District, Timor-Leste (Australian and New Zealand Clinical Trials Registry ACTRN12614000680662) [19]. The study protocol was approved by the University of Queensland 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 Human Research Ethics Committee. Participant informed consent processes involved explaining study purpose and methods, and obtaining signed

consent from all adults and parents or guardians of children under 18 years of age, as described previously [19].

Study setting, design and collection of data

This study was conducted as one of a series of baseline analyses for the "WASH for Worms" RCT, which aims to determine the extent of a reduction in burden of STH by integrating mass chemotherapy and community-based WASH programmes [19]. Rural communities in Manufahi District were selected for the study according to RCT-related inclusion and exclusion criteria (including being identified by the Timorese government as high-priority communities for WASH interventions) [19]. The RCT commenced in May 2012, with baseline surveys conducted in 18 communities until October 2013. Identically-collected data from six communities in Manufahi District were added – these communities were enrolled at the same time as the RCT communities but were not randomly allocated to each trial arm. Specifically, one cluster was excluded due to unsuitability of water source, one control cluster was excluded due to risk of contamination from a nearby intervention community, one was excluded due to unwillingness to wait two years for the WASH intervention, one was excluded due to small size, and one was excluded due to unwillingness to participate in building the water infrastructure [19]. Full details of study area and design [19], questionnaires and parasitological diagnostic approaches [4, 18] are provided elsewhere. Briefly, Manufahi District is comprised of rural Timorese villages with subsistence-based livelihoods. Community consultations and consent elicitation were conducted before questionnaire administration. Children aged less than 12 months and pregnant women in the first trimester of pregnancy were excluded because they could not

receive albendazole. Questionnaires were used to record details of water, sanitation and hygiene (WASH) practices, household and individual socioeconomic characteristics [4].

Measurement of anaemia status and anthropometry

Anaemia status was measured for all ages with haemoglobin concentration assessed by finger-prick blood test using a portable haemoglobinometer (HemoCue, Ängelholm, Sweden). Haemoglobin concentrations can be used to assess anaemia (being inadequate intakes and reserves of host iron and protein [20]). Data on haemoglobin were linked to household GPS coordinates and adjusted by -2 grams per litre for elevation of 1000 metres above sea level in accordance with World Health Organization (WHO) recommendations [21]; data from four communities, and part of a fifth community, were adjusted in this way; no communities had an elevation above 1500 metres. Haemoglobin was initially classified based on WHO definitions of anaemia severity (Table 1 [21]); however due to small numbers was re-categorised as a binary variable (anaemic/non-anaemic); which was used as the primary outcome.

[Please insert Table 1 here]

For children aged two to <18 years, weight was measured to the nearest 0.1kg using electronic scales (CAMRY, ED-301), and height was measured to the nearest 0.1cm using a portable stadiometer (Wedderburn, WSHRP). Children aged one to two years had length measured supine with a measuring mat (Wedderburn, SE210), and weight measured by taring (i.e. with the child held by an adult, and the adult's weight subsequently deducted).

Height-for-age (HAZ) and BMI-for-age (BMIZ, i.e. weight over height^2-for-age) z-scores were calculated for children aged 1<18 years. Weight-for-age (WAZ) z-scores were calculated for children aged 1<10 years, standardised to the international 2006 reference population using the software WHO Anthro and Anthroplus, for children up to five and aged five and over, respectively [22, 23]. These scores are expressed as differences from the reference median and are calculated based on sex and date of birth of each individual. Age in days was used for z-score calculations. Children of uncertain birthdate were assigned a mid-year birthdate (15th June) and records followed up with parents subsequently; 1038 children's records (95.4%) had completed birthdates. WAZ is only calculated up to ten years of age, because it is considered inadequate for monitoring growth beyond this age [24]; BMIZ complements HAZ in the assessment of thinness (low BMIZ) [24], and was used instead of weight-for-height (which is calculated for under-fives only) to assess wasting. Each of these continuous outcomes was categorised, with individuals classified as moderately stunted, underweight or wasted if HAZ, WAZ and BMIZ respectively were more than two standard deviations below the reference median, and severely stunted, underweight or wasted, respectively, if the z-scores were more than three standard deviations below the reference median [25].

Assessment of STH infection

Single stool samples were collected and fixed in 5% potassium dichromate. These samples were transported to QIMR Berghofer Medical Research Institute, Brisbane, Australia, for multiplex qPCR for presence and intensity of STH and protozoal infection using a method previously described [18] For purposes of this analysis, qPCR cycle threshold (Ct) values,

representing the amplification cycle where the signal exceeded background, was interpolated as a measure of the parasite DNA load in the stool sample [26], using a validated internal control. Ct-values were expressed on a $\log_{10}$ scale of the linear equation with fluorescence (i.e. $\log(b_0 + b_1x)$), where the slope (b_1) and the y-intercept (b_0) are provided from the PCR output and x is the Ct-value. Lower values therefore denote heavier intensity infection. In these assays a Ct-value of 31 for *Ascaris* (reported at genus level because the PCR assay was not species-specific for *Ascaris*), and a Ct-value of 35 for *N. americanus*, were set as the limits for detection of infection [18]. All assays showed Ct-values for the internal control within the expected range.

Data analysis

Data were analysed in STATA 14.0 (Stata Corporation, College Station, Texas). A wealth quintile was constructed using principal components analysis of variables assessing ownership of household assets (including animals, transport and appliances), house floor type, reported income and presence of electricity [4], according to established methods [27].

For faecal specimens, two runs were taken for each PCR assay. The arithmetic mean of the two (untransformed) Ct-values was taken to create a single measure per person. In this study, Ct-values were distinctly bimodal, particularly for *Ascaris* spp. (Figure 1). Statistically, bimodal explanatory variables present challenges for interpretation of model coefficients, even if the residuals are Normally distributed, as there is known poor fit to linear, quadratic or cubic equations [28]. Bimodality in explanatory variables is often

ignored. However, it was important to investigate in such samples because cut-points were being assigned to generate categories of intensity of infection. The distribution of Ct was examined by sex, age group, socioeconomic quintile, and STH co-infection, to investigate whether the modes varied with these variables. However, the distribution, and modes, appeared consistent across all groups. For ease of interpretation and for comparison with other studies, untransformed Ct-values were categorised in these analyses. Receiver-operating characteristic curves (ROCs) were used to assign initial cut-points for Ct-values, using a generated morbidity score (see Appendix 1). However, a very weak relationship between intensity of infection for either *N. americanus* or *Ascaris* and the morbidity score was observed (see below). This led to extremely poor predictive capacity using ROCs, so this technique was not ultimately used. Full detail of this is reported in Appendix 1, as statistical assignment of categories to infection intensities represent an important contribution that may be useful in assigning qPCR data to intensity of infection categories elsewhere.

[Please insert Figure 1 here]

An algorithm to assign intensity of infection based on approximations of epg was used for this analysis, with intensity classes being based on those endorsed by the WHO to represent high, moderate and low-intensity infections [29]. This algorithm was generated from seeding experiments as previously described [18] and was based on the linear relationship between the $\log_{10}$ of epg and Ct-value [18]. For *N. americanus* the equation was $epg=10^{(-0.43Ct + 14.88)}$, and for *Ascaris*, $epg=10^{(-0.275Ct + 9.622)}$ [18]. Because WHO endorsed

categorisations of epg intensity are based on the Kato-Katz diagnostic technique [29], a recovery factor of 0.2 was applied to current epg classes of infection intensity (based on a 20% recovery rate determined for faecal flotation of *Ascaris* eggs (R. Traub, unpublished data). This recovery factor was used in the absence of a recovery factor being known for Kato-Katz and the known poor accuracy of this technique in diagnosing STH infections [30]) (Table 2).

[Please insert Table 2 here]

Using classes of infection intensity for epg based on international standards [29], the Ct cut-point that correlated with heavy-intensity infection of (≥4,000*5=20,000) epg was selected for *N. americanus*, and the Ct cut-point that correlated with heavy-intensity infection of (≥50 000*5=250,000) epg was selected for *Ascaris*. The final intensity of infection variables for both STH were therefore categorised according to heavy-intensity, moderate- to low-intensity (hereafter called "moderate-intensity"), and no infection, whereby moderate-intensity infection was all Ct between the heavy-infection cut-point and the detectable Ct limits of 31 for *Ascaris*, and 35 for *N. americanus*. Sensitivity analyses were then undertaken, comparing these cut-points to a model that applied a cut-point of 10% heavy-intensity infection (based on the percentage of endemic populations deemed by the WHO as likely to suffer morbidity from heavy infections [29]), a model of 15% heavy-intensity infection, a model based on quintiles of infection intensity, and also comparing results from applying cut-points that have been reported elsewhere [31-33] to these data. The intensity cut-points selected using the WHO endorsed thresholds as estimated by the

algorithm performed comparably to these other cut-points on sensitivity analyses based on parameter estimates and Akaike's Information Criteria, and thus were used in all further analyses.

Chi-squared tests were conducted to compare prevalence of morbidity by age, sex and socioeconomic quintile; these variables were retained as core variables in all multivariable models. The associations between intensity of infection and morbidity by age group and sex were also explored. Mixed-effects logistic regression (for binary-coded anaemia as outcome) and mixed-effects multinomial regression within a generalised structural equation model framework (separately for outcomes of stunting, wasting and underweight) were undertaken to account for correlation among outcomes at the household and village level.

Initially univariable analyses were undertaken with each STH species, *G. duodenalis*, age group (categorical), sex as a binary variable, and socioeconomic quintile (categorical) included as explanatory variables for each of the morbidity outcomes. Although there was a high prevalence of *N. americanus* in this population [4], there was no multicollinearity with other STH species. Variables with $P<0.2$ from univariable analyses were added stepwise into a base model which included age group, sex, socioeconomic quintile, the categorical intensity of infection explanatory variables for *N. americanus* and *Ascaris* (described above), and binary *Ancylostoma* infection, until the most parsimonious adjusted model for each outcome was achieved. Variables were retained in final models if $P<0.1$ on the Wald test.

For models of stunting, underweight and wasting, anaemia prevalence was additionally included as a core variable because of its importance as a potential confounder. There were insufficient observations to investigate anaemia risk factors in children under five or within other childhood age groups. Anaemia was therefore modelled separately for children (aged 1<18 years) and adults, given the difference in prevalence and potential risk factors for these two groups. There were additionally insufficient observations to investigate stunting, wasting or underweight by age groups in regression analyses. Prevalence of both STH and morbidity vary with age and sex, and both the literature [34] and our earlier analyses [4] indicated the potential for moderation of the STH-morbidity relationship by age and sex. Given this, and observed differences in relationships between classes of *N. americanus* infection intensity and stunting across age and sex (identified in cross-tabulations), interactions between sex and *N. americanus* intensity of infection, and age group and *N. americanus* intensity of infection were investigated. Interactions were investigated by generating models with and without the interaction term and comparing these using the likelihood ratio test, with a requirement for $P<0.1$ for likelihood ratio tests, for interaction inclusion in the model. Using these criteria, no interactions were required in adjusted models for anaemia. A sex by *N. americanus* intensity of infection interaction was retained in the adjusted child stunting model, and an age group by *N. americanus* intensity of infection interaction was retained in the adjusted child underweight model. For the multinomial child anthropometry models, sensitivity analyses using binary-coded prevalence of outcome (of main effects only, i.e. no interaction terms) were undertaken to investigate the impact of increasing power. Additionally, post-hoc calculations were performed to determine the power to detect effects within each outcome, adjusting for

correlations within households and villages. These calculations indicated 80% power, with a 5% significance level, to detect odds ratios of 3.3 or more for anaemia outcomes, relative risks of 1.4 to 1.7 for stunting and underweight outcomes (depending on level of severity), and (reflecting lower numbers) relative risks of 2.1 to 9.5 for wasting as an outcome (according to level of severity).

Results

Prevalence of morbidity

Respondents from villages who provided both a stool and finger-prick blood sample were included in analyses of haemoglobin (2038 participants). Only 307 people (15% of the population) suffered from any anaemia, with the majority of these (n=222; 11%) being only mildly anaemic, and only three children being severely anaemic (Table 3). Anaemia was most prevalent in younger ages and generally decreased with increasing age ($P<0.0001$) (Figure 2). The observed zero prevalence of anaemia in females aged 65 years and over is of interest, although participant numbers in older age groups were generally low. Adult women of reproductive age (aged 18<45 years) had higher anaemia prevalence than men of the same age (18% compared to 7.9%, $P<0.0001$). The overall prevalence of *N. americanus* and *Ascaris* were 1346 (61%), and 536 (24%) participants, respectively [4]. The prevalence of *Ascaris* was highest amongst children of preschool age, whereas *N. americanus* was most prevalent in adults (Table 3) [4].

[Please insert Table 3 here]

[Please insert Figure 2 here]

Children 1<18 years old who provided stool and had height and/or weight measured were included in analyses of z-scores (Table 3). Extremely high levels of stunting (n=592. 60%), underweight (n=382, 60%), and wasting (n=189, 20%) were found, with 245 children (25%) severely stunted, 129 children (20%) severely underweight, and 44 children (4.5%) severely wasted (Table 3). This morbidity is reflected in the mean z-scores for each measure, all of which are well below zero. Stunting, underweight and wasting varied by age group with, generally, greater proportions of older children (≥10 years) experiencing severe morbidity compared to younger age groups. The prevalence of stunting was significantly higher among poorer households compared to wealthier households (P<0.0001) and males compared to females (P<0.0001), but the overall association between stunting and age was non-significant (P=0.117). Exploratory analyses indicated some unexpected, but not statistically significant, trends. A greater proportion of uninfected males were severely stunted than *N. americanus*-infected males (Table 4). This trend did not exist for females, who instead showed greater proportions of uninfected having normal (i.e. non-stunted) growth. Similarly, children aged one to five years with *N. americanus* infection were generally less severely stunted; a trend that reversed in the oldest age group (where a lower proportion of severe stunting was seen in uninfected children (Table 4). These complex and varying underlying relationships confirmed our decision to investigate interaction terms in our regression models.

[Please insert Table 4 here]

Prevalence and severity of being underweight was moderately higher for males (P=0.004), and generally increased by age (P=0.002), but not socioeconomic quintile (P=0.088). Prevalence and severity of wasting increased by age (P<0.0001), but did not differ by sex (P=0.651) or socioeconomic quintile (P=0.666).

Assignment of DNA intensity cut-points

From PCR output, there were no Ct-values above 35, indicating good reproducibility of the assays. There were very weak relationships between STH and all morbidity outcomes, which hampered statistical assignment of cut-points using ROC curves. Table 2 shows the final selected cut-points. Using our cut-points, 1155 (52%) people were categorised as having heavy-intensity *N. americanus* infection, and 191 (8.6%) with moderate-intensity infection (Table 5). For *Ascaris*, 220 (9.9%) people had heavy-intensity, and 318 (14%) people moderate-intensity infection. Amongst infected people, *N. americanus* mean infection intensity was Ct21.5 (95% CI: 21.3-21.8), and for *Ascaris* spp. 19.5 (95% CI: 19.2-19.9) (Table 5). Intensity of infection changed over age, with most heavy-intensity *Ascaris* infection occurring in young children. Heavy-intensity *N. americanus* infections were more evenly distributed across age groups, including older age groups.

[Please insert Table 5 here]

Factors associated with anaemia

Neither *Ascaris* nor *N. americanus* infection intensity was significantly associated with anaemia in the multivariable models, although *Ascaris* moderate-intensity infection was

marginally non-significant as a risk factor for adults (adjusted odds ratio (AOR) 1.6; 95% confidence intervals (CI) 0.93-2.7; Table 6). Heavy *N. americanus* infection in children had a protective association with anaemia. However, this was not a significant factor in adjusted models (AOR 0.69; 95%CI 0.41-1.1), potentially indicating the confounding influence of other core model factors. Increasing age was a highly significant, strongly protective factor in univariable and adjusted models for children (in children aged 6-11 years AOR 0.39, 95%CI 0.24-0.61; and in children aged 12 to 17 years AOR 0.19, 95%CI 0,09-0.38). There was no sex difference in odds of anaemia in children (AOR 0.97, 95%CI 0.64-1.5). Children in the poorest socioeconomic quintile had twice the odds of anaemia relative to those in the wealthiest quintile (AOR 2.1, 95%CI 1.0-4.3). For adults, age was non-significant (AOR 1.2, 95%CI 0.76-2.0), and neither sex nor socioeconomic status were associated with anaemia, with no evidence of an overall trend in odds with decreasing socioeconomic quintile.

[Please insert Table 6 here]

Factors associated with stunting

No level of either *N. americanus* or *Ascaris* infection intensity was associated with stunting of any severity in this population (Table 7). However, whilst not statistically significant, heavy-intensity *Ascaris* infection was associated with higher relative risks for both moderate and severe stunting; these relative risks were of reasonable size (moderate stunting adjusted relative risk (ARR) 1.6, 95% CI 0.87-2.9; severe stunting ARR 2.0, 95% CI 0.87-4.4). Sensitivity analysis of stunting prevalence showed that heavy-intensity

Ascaris infection was marginally non-significantly associated with greater odds of stunting compared to uninfected children (adjusted odds ratio (AOR) 1.8, 95% CI 0.98-3.4, *P*=0.057; results not shown). In the multinomial stunting model (Table 7), *Ancylostoma* infection was associated with moderate and severe stunting. However, the effect was in the opposite direction to anticipated: children with *Ancylostoma* infection were significantly less likely to have moderate or severe stunting than uninfected children.

[Please insert Table 7 here]

Due to inclusion of a sex by *N. americanus* interaction term, we report results of the association between *N. americanus* and stunting separately for males and females. Females with *N. americanus* infection of any intensity had no significant association with stunting of any severity. Being male and having heavy-intensity *N. americanus* infection (relative to being male and having no *N. americanus* infection) was associated with significantly reduced risk of severe stunting. It is important to note, however, that the main effect of *N. americanus* infection intensity showed that there was no association with stunting in females (the reference category). The main effect for sex indicates that, in those with no *N. americanus* infection, being male was highly significantly associated with almost three times the risk of moderate stunting (ARR 2.8, 95%CI 1.7-4.7), and almost seven times the risk of severe stunting (ARR 6.8, 95%CI 3.4-13.7). Relative to children aged one to five, children aged 12 to 17 had almost twice the risk of moderate stunting (ARR 1.7, 95%CI 0.98, 2.8), and almost three times the risk of severe stunting (ARR 2.7, 95%CI 1.3-5.4), although no significant associations were seen in children aged six to 11 years. Children in

the poorest socioeconomic quintile had twice the risk of moderate stunting (ARR 2.3, 95%CI 1.1-4.6), and five times the risk of severe stunting (ARR 5.1, 95%CI 1.8-14.7), compared to children in the wealthiest socioeconomic quintile. Anaemia was not a risk factor for stunting.

Factors associated with being underweight

The association between underweight and *N. americanus* infection is reported separately by age group because of the age group by *N. americanus* interaction term. In those aged one to five, *N. americanus* infection of any severity was not associated with being underweight. Whilst this same association was evident for children aged six to ten with *N. americanus* infection, the association with underweight was only marginally non-significant for those having moderate *N. americanus* infection intensity. Compared to children aged one to five years, being aged six to ten years was significantly associated with three times the risk of being severely underweight (ARR 3.3, 95%CI 1.5-7.0), but no increased risk of being moderately underweight (ARR 1.2, 95%CI 0.63-2.2), in *N. americanus*-uninfected children. There was no association between intensity of *Ascaris* infection and being underweight in this population (Table 8). *Ancylostoma* infection was close to significance for being moderately underweight (ARR 2.8, 95%CI 0.94-8.1), but not for being severely underweight (ARR 0.86, 95%CI 0.18-4.2). The presence of *G. duodenalis* infection, or of anaemia, were also not significantly associated with being underweight. Being male was highly significantly associated with three times greater risk of being severely underweight compared to normal growth children (ARR 3.1, 95%CI 1.7-5.6), but this association was not evident for moderate levels of underweight (ARR 1.3, 95%CI 0.81-2.0). Household

socioeconomic quintile was not associated with being underweight in these analyses, although the association with poorest socioeconomic quintile was only marginally not significant for being moderately underweight (ARR 2.5, 95%CI 1.0-6.1). With binary-coded underweight as outcome, a sensitivity analysis confirmed the consistency of these results with the multinomial results.

[Please insert Table 8 here]

Factors associated with wasting

Although no intestinal parasites were associated with wasting in adjusted analyses, observation numbers in this model were very low for assessment of some categories (Table 9). Additionally, presence of anaemia was not associated with wasting. Relative to being aged one to five years, being aged six to 11 years was associated with highly significant, threefold increased risk for being either moderately (ARR 2.9, 95%CI 1.6-5.1) or severely wasted (ARR 3.2, 95%CI 1.2-8.9). Strikingly, this trend worsened amongst children aged 12 to 17 years, with four times the risk of moderate wasting (ARR 4.3, 95%CI 2.2-8.4), and seven times the risk of severe wasting (ARR 7.1, 95%CI 2.3-21.8), seen in these children relative to the youngest age group. There was no association between sex or socioeconomic status and categories of wasting. Given low numbers with this multinomial wasting outcome, a sensitivity analysis was conducted using wasting as a binary-coded outcome; results were consistent with the multinomial analyses, with age group the sole significant factor.

[Please insert Table 9 here]

Discussion

In this first investigation of STH associations with community haemoglobin and child development indices in Manufahi District, Timor-Leste, a generally lower prevalence of anaemia than results reported previously [17] was observed, with 11% prevalence in children aged 1<5 years, and 18% prevalence in reproductive-aged women (18<45 years of age). For women, anaemia whilst moderately lower than reported previously [17], was significantly more frequent than in males of the same age, confirming the serious disease burden in this population group. This likely reflects the well-reported impact of pregnancies and menstruation on iron stores [35]. Anaemia in mothers is itself a risk factor for stunting or wasting in offspring. Iron-folic acid supplementation for pregnant women has been implemented by the Ministry of Health across all districts of Timor-Leste since 2003, with 61% of pregnant women reporting taking supplements in 2009-10 [16]. Strategies to increase uptake would be very beneficial.

Despite the difference in STH prevalence, being female was not demonstrated to be a risk factor for anaemia in adults. Interestingly, neither *N. americanus* nor *Ascaris* were significant risk factors, this despite the well-recognised association between hookworm infection and blood loss. Whilst *N. americanus* is implicated in blood loss, it causes measurably less blood loss than *Ancylostoma duodenale* [10]. Blood loss due to parasite infection needs to be greater than nutritional reserves and required intake for anaemia to develop [36]. In this population on the prevalence of the more pathogenic hookworm

species *Ancylostoma duodenale* as very low, representing a possible explanationfor the weak association between STH (particularly hookworm) and anaemia. Similar negligible associations have been identified in *N. americanus*-endemic populations elsewhere [36]. Socioeconomic status and age were not important risk factors for anaemia in adults, but were important, highly significant, risk factors for children. Different risk factor associations between children and adults point to the need to conduct further age-stratified analyses, ideally with children aged less than five analysed separately due to the higher prevalence of anaemia in this age group; observation numbers limited further age-stratification in our analysis. Anaemia can be caused by multiple concurrent factors including inadequate dietary iron, and it is inherently difficult to control for all of these in epidemiological studies. The lack of other identified risk factors in our models would suggest that additional unmeasured factors may be influencing these results. Of note, the prevalence of malaria had dramatically declined in Timor-Leste prior to the commencement of this study [37], indicating that this is not a likely confounding factor.

In the study area, an extremely high prevalence of stunting, underweight and wasting in children were observed compared to the international reference population, with considerable proportions of severe stunting, underweight and wasting. These are higher than national estimates, possibly reflecting the rurality of the study communities. Our reported prevalence, whilst being at a district, not national level (and therefore perhaps more susceptible to small geographic area fluctuations), are amongst some of the highest reported rates in the world [38]. This is despite relatively low community prevalence of anaemia. Proportions of child wasting in particular are well above the 15% level of severity

classified as critical [39]. Wasting represents rapid and severe malnutrition such as starvation, although it can also be the result of chronic unfavourable conditions [39]. With strong links between wasting and child mortality [39] this level is considered to be a public health emergency [40] that requires immediate response. However, as a cautionary note, the application of the 2006 WHO international reference standards to the Timorese population has not been assessed, and there is a possibility of this population being of a smaller stature than the international standards, leading to overstated morbidity. Further investigations into applicability of these thresholds within Timor-Leste are required.

Stunting is often associated with poor nutrient availability *in utero* and the neonatal period from maternal breast milk, exacerbated by continuing poor nutrient supply during the period of introduction of solids [41]. It represents a period of chronic malnutrition during the most rapid growth period of life, leading to long-term and often permanent failure to attain linear growth. Wasting and stunting share direct and underlying causal factors, but it is not yet well understood how much wasting contributes to stunting and *vice versa* [42]. There are ongoing nutritional initiatives in Manufahi District including provision of food at schools. However, given the prevalence of wasting, stunting and underweight, there is an urgent need to further investigate nutrition in this community and enhance strategies to ensure that children are receiving adequate nutrition. The greater ARRs for severe stunting and wasting in older compared to younger children may reflect more food security for young children following the end of Indonesian occupation of Timor-Leste.

After controlling for socioeconomic status, sex, anaemia prevalence and age, there were

few STH associations with child development indices, and most of the associations found fell short of the 5% significance threshold. Of importance were the sex by *N. americanus* interaction for stunting, and the age group by *N. americanus* interaction for underweight: the interaction terms highlight complex interrelationships occurring in the population, and main effects of *N. americanus* intensity of infection were not significantly associated with morbidity. It should not be interpreted from this analysis that *N. americanus* infection is associated with reduced risk of either stunting or wasting. The other significant helminth-associated finding was for *Ancylostoma* association with moderate and severe stunting, although trends were not in the expected direction, with *Ancylostoma* infection associated with reduced severe stunting risk (Table 7). The prevalence of *Ancylostoma* in this population was low, at 4.7%, leading to low numbers in regression models, and inability to categorise *Ancylostoma* into classes of infection intensity. Further, it was due to observed differences in the relationship between sex, age, *N. americanus* infection intensity and stunting that interactions were investigated. It could be that this underlying complex relationship also affected *Ancylostoma*. This finding of a 'protective' effect therefore needs to be interpreted cautiously.

The most significant risk factors for stunting and underweight were, generally, being male, and older child age groups, with additionally for stunting, being in the poorest socioeconomic quintile. Anaemia was not a risk factor. Underweight as a measure of child development is more variable than stunting, being influenced by both height and weight [39]. This may explain why poverty emerged as a risk factor for stunting but not being underweight in this population. Poverty could be a major contributing factor to chronic

nutrient deprivation at critical rapid growth stages in this community. High stunting rates are usually indicative of poor socioeconomic conditions [39]; these rates may represent a legacy of conflict in this country. No risk factors were found for wasting with the exception of a greatly increased risk with older child age group for both moderate and severe wasting. These results, coupled with high STH prevalence, point to unmeasured risk factors in our study population, likely including nutritional risk factors, but also possibly genetic, behavioural and environmental risk factors.

Given high STH prevalence, the lack of other STH associations with morbidity in our models is surprising. However, this could be linked to the low level of anaemia and its negligible associations with child development outcomes in this population, given the most likely causal pathway between STH and morbidity is the role of hookworms contributing to blood loss and anaemia, with impacts on child development being more indirect. STH-morbidity associations have not been consistently found in studies of many different designs, contributing to a picture of complexity in understanding STH impacts on morbidity in populations that are often suffering multiple potentially interacting insults associated with poverty and deprivation (reviewed in [11]).

An alternative reason for the lack of STH associations with morbidity in our analysis may be that *N. americanus* morbidity may have been inadvertently overemphasised in studies that do not differentiate the hookworm genera (e.g. *Necator* and *Ancylostoma*). It could be that the burden of hookworm stems predominantly from *Ancylostoma* infection, and not *N. americanus*. This has been raised previously [10, 43]. Further epidemiological studies

investigating differential morbidity, using advanced diagnostic methods, are required.

Studies reporting morbidity associations with STH show varied results (reviewed in [11]). The lack of consistent associations between STH and morbidity outcomes, particularly child-related z-scores, is problematic for quantifying disease burden. Early estimates of the global burden of disease from STH had widely varying ranges, primarily based on the interpretation of morbidity in school-aged children [44]. Of particular importance is that, historically, it was the perceived importance of disease in school-aged children that became a crucial factor in international advocacy and development of mass school-based STH control programmes [44]. However a Cochrane systematic review has concluded that there was "substantial evidence that deworming does not improve average nutritional status or haemoglobin" [45], although this conclusion is disputed due to there being insufficient evidence in existence to confirm or refute the findings, despite research efforts [11]. Lack of evidence likely reflects the underlying heterogeneity of STH in different populations, alongside numerous potentially influencing factors such as community nutrition, poverty and comorbidities. This highlights the importance of continuing detailed investigations into morbidity associations, in an attempt to meet evidence shortfalls. New technologies such as qPCR will potentially play a very important role in generating this evidence.

Population-based studies [27, 31-33, 46] are increasingly using PCR as a diagnostic tool for STH. However, using infection intensity has not been adequately undertaken to date. Of Ct cut-points that have been assigned, only one prior investigation was found that specified how cut-points were allocated; this study also used dilution experiments from gene

fragments for each species [31]. PCR data have therefore not yet been sufficiently validated using a wide range of infection intensities across populations, by contrast to categories of epg which are now widely used for microscopy-derived measures of infection intensity despite their not very well acknowledged methodological weaknesses [43]. In the absence of diagnostic 'gold standards' [30], and the increasing use of copro-diagnostic technologies, validating infection intensity using qPCR measures will become an increasingly important requirement. This analysis provides the first assignment of Ct cut-points based on an algorithm correlating infection intensity as measured by Ct value to an epg equivalent using the same faecal specimens. Major statistical inaccuracies can arise from setting data-driven cut-points [28], and this analysis should be seen as exploratory. Ideally further investigations of Ct-epg correlations are required. Further, population-based, and/or mathematical modelling studies are required to derive Ct cut-points that can be applied across populations.

Limitations and strengths

A limitation of this analysis is that there were insufficient observations to investigate anaemia risk factors in children under five, which was highly desired, or age-related differences for child development indices. Power calculations also indicated that there was power to detect risk factors for anaemia of odds ratios above 3.3, and for child morbidity relative risks of generally above 1.4. The prevalence was generally higher in young children; these are the most formative and highest-velocity growth years. Future research should investigate differential associations by age.

A further limitation of the study is that it was not possible to formally adjust for malaria prevalence. However, one of the largest recorded reductions in malaria incidence has recently been reported for Timor-Leste, with a 97% decrease in reported malaria incidence over 2006-2012 [37] to a very low level. This provides evidence for a low underlying incidence of malaria in these communities and hence this is believed to be a minor limitation.

A major strength of this study is that it provides the first quantitative assessment of the role of STH on measures of morbidity in Timor-Leste, using advanced parasitological and epidemiological methods. This provides an important epidemiological evidence base to inform policy and programmatic planning. In particular, differentiation of *N. americanus* and *Ancylostoma* coupled with the low morbidity association overall, has enabled us to hypothesise that *N. americanus* morbidity effects have possibly been overstated in settings where less sensitive STH diagnostic tools have been used. This is a very important research priority to investigate further. Additionally, in conducting a parasitological survey, the need to investigate nutritional epidemiology in this district has been identified. Such investigations should commence as a priority.

Conclusion

This report provides the first assessment of STH associations with haemoglobin, stunting, wasting and underweight anthropometric measures in Timor-Leste. In this high-prevalence setting, only weak associations between STH of any species and developmental measures were found. Despite this, high prevalence of stunting, underweight and wasting in children

illustrates the urgent need for investigating food quality and quantity in this community and providing nutritional enhancement, for example via micro- and macro-nutrient supplements. Additionally, regardless of the lack of association found between intensity of STH infection and morbidity in this study population, the high prevalence of STH provides a strong justification for introducing integrated STH control strategies. Deworming will reduce STH infections, but further nutritional interventions are going to be required to improve health.

List of abbreviations

AOR	Adjusted odds ratio
ARR	Adjusted relative risk
AUC	Area under ROC curve
BMI	Body mass index
BMIZ	BMI-for-age
CI	Confidence interval
Ct	Cycle threshold
DNA	Deoxyribonucleic acid
Epg	Eggs per gram of faeces
HAZ	Height-for-age
OR	Odds ratio
qPCR	Quantitative polymerase chain reaction
RCT	Randomised controlled trial
ROC	Receiver-operating characteristic curve

RR	Relative risk
SD	Standard deviation
STH	Soil-transmitted helminth
WASH	Water, sanitation and hygiene
WAZ	Weight-for-age
WHO	World Health Organization

Declarations

Ethics approval and consent to participate

These analyses were conducted as part of a cluster randomised controlled trial (RCT) in Manufahi District, Timor-Leste (Australian and New Zealand Clinical Trials Registry ACTRN12614000680662). The study protocol was approved by the University of Queensland 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 Human Research Ethics Committee. Participant informed consent processes involved explaining study purpose and methods, and obtaining signed consent from all adults and parents or guardians of children under 18 years of age.

Consent for publication

Not applicable.

Availability of data and material

The data for the WASH for Worms trial will be made freely and publicly available at the completion of the trial and analysis of trial outcomes. This is anticipated at the end of 2016.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SJC and ACAC designed the analysis. SVN was responsible for data collection and entry. SVN and SJC conducted data cleaning and management. SL analysed qPCR specimens. SJC conducted data analysis, with statistical advice from CADE and ACAC. All authors have contributed suggestions to the analysis and played a role in interpreting findings. SJC wrote the manuscript, and all authors have contributed to writing and approved the final manuscript.

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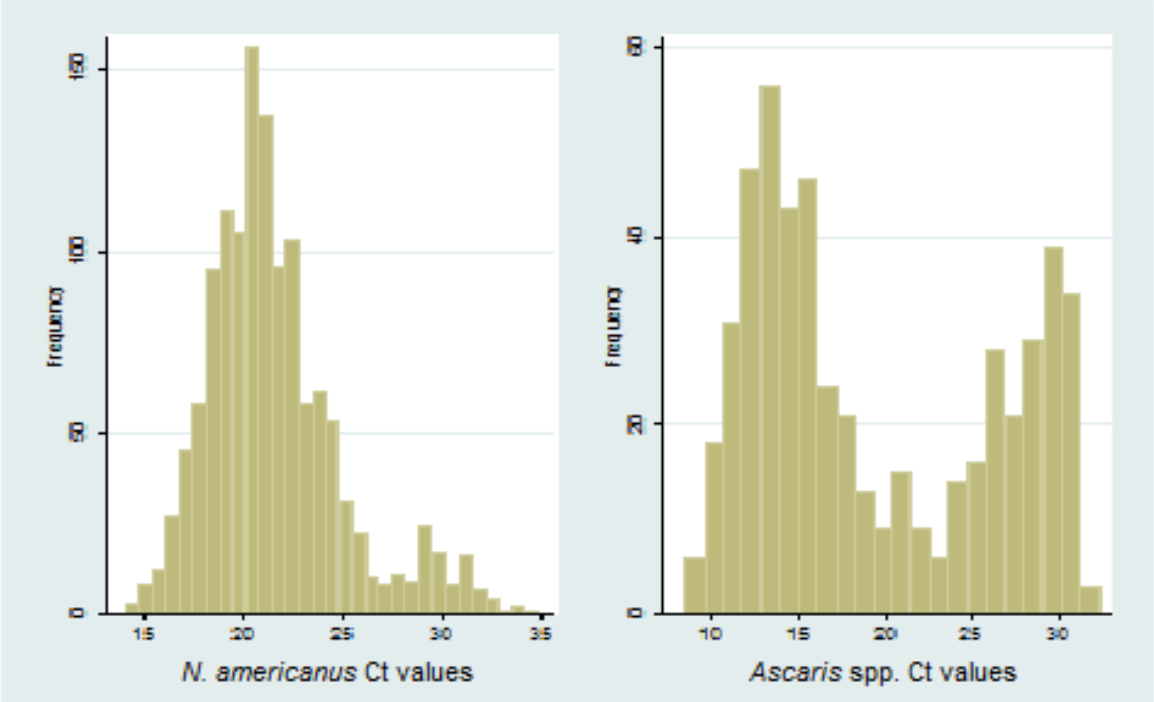
Table 1 Definitions of anaemia used in this study

	No anaemia	Mild anaemia	Moderate anaemia	Severe anaemia
Children <5 years	≥110g/L*	100-109g/L	70-99g/L	<70g/L
Children 5-11 years	≥115g/L	110-114g/L	80-109g/L	<80g/L
Children 12-14 years	≥120g/L	110-119g/L	80-109g/L	<80g/L
Non-pregnant women (≥15 years)	≥120g/L	110-119g/L	80-109g/L	<80g/L
Pregnant women	≥110g/L	100-109g/L	70-99g/L	<70g/L
Men (≥15 years)	≥130g/L	110-129g/L	80-109g/L	<80g/L

*Measure is grams per litre. Source (22)

Figure 1 *Necator americanus* and *Ascaris* spp. intensity of infection (Ct-value) distributions



Note: Uninfected people are excluded from these figures for purposes of scale.

Table 2 *Ascaris* spp. and *Necator americanus* intensity of infection cycle threshold (Ct) cut-points between heavy and moderate morbidity

Soil-transmitted helminth	Eggs per gram of faeces (EPG) class	EPG class with recovery factor applied*	Corresponding Ct-value
N. americanus	≥4 000	20 000	24.6
Ascaris spp.	≥50 000	250 000	15.4

Notes: "Ct", cycle threshold from PCR; DNA intensity from exponentiated Ct-values; epg, eggs per gram of faeces; epg intensity classes follow WHO definitions (28); recovery factor of 0.2 applied to epg intensity class based on recovery factor determined from faecal flotation (R. Traub, unpublished data).

Table 3 Baseline characteristics of study participants (*N*=2038)

Baseline community characteristics	**All ages (*N*=2038)** n (%)	**Children; aged 1<18 years (*N*=1018)** n (%)	**Adults; aged ≥18 years (*N*=1020)** n (%)
Mean haemoglobin (g/L, (SD))	131 (16)*	126 (13)*	136 (16)*
Non-anaemic (n, (%))	1 731 (85)	872 (86)	859 (84)
Mildly anaemic (n, (%))	222 (11)	95 (9.3)	127 (13)
Moderately/severely anaemic (n, (%))[#]	86 (4.2)	51 (5.0)	34 (3.3)
Ascaris spp. prevalence (n, (%))	536 (24)	302 (30)	215 (19)
N. americanus prevalence (n, (%))	1 346 (61)	522 (51)	785 (70)
Z-score characteristics	**Children aged 1<18 years for HAZ (*N*=983)**		
Mean HAZ (SD)	-2.25 (1.22)*		
Not stunted (n, (%))	391 (40)		
Moderately stunted (n, (%))	347 (35)		
Severely stunted (n, (%))	245 (25)		
	Children aged 1<10 years for WAZ (*N*=639)		
Mean WAZ (SD)	-2.19 (1.03)*		
Not underweight (n, (%))	257 (40)		
Moderately underweight (n, (%))	253 (40)		
Severely underweight (n, (%))	129 (20)		
	Children aged 1<18 years for BMIZ (*N*=985)		
Mean BMIZ (SD)	-1.19 (1.03)*		
Not wasted (n, (%))	796 (81)		
Moderately wasted (n, (%))	145 (15)		
Severely wasted (n, (%))	44 (4.5)		

Notes: * denotes mean and standard deviation presented instead of n and %. g/L, grams per litre; SD, standard deviation; HAZ, height-for-age z-score; WAZ, weight-for-age z-score; BMIZ, BMI-for-age z-score. WAZ (as indicator of underweight) only calculated for individuals 12 months to 10 years of age. HAZ (stunting) and BMI (wasting) calculated for individuals 12 months to <18 years of age. [#]Moderate and severe anaemia categories combined to maintain participant confidentiality.

Figure 2 Anaemia distribution by sex and age group (*N*=2000)

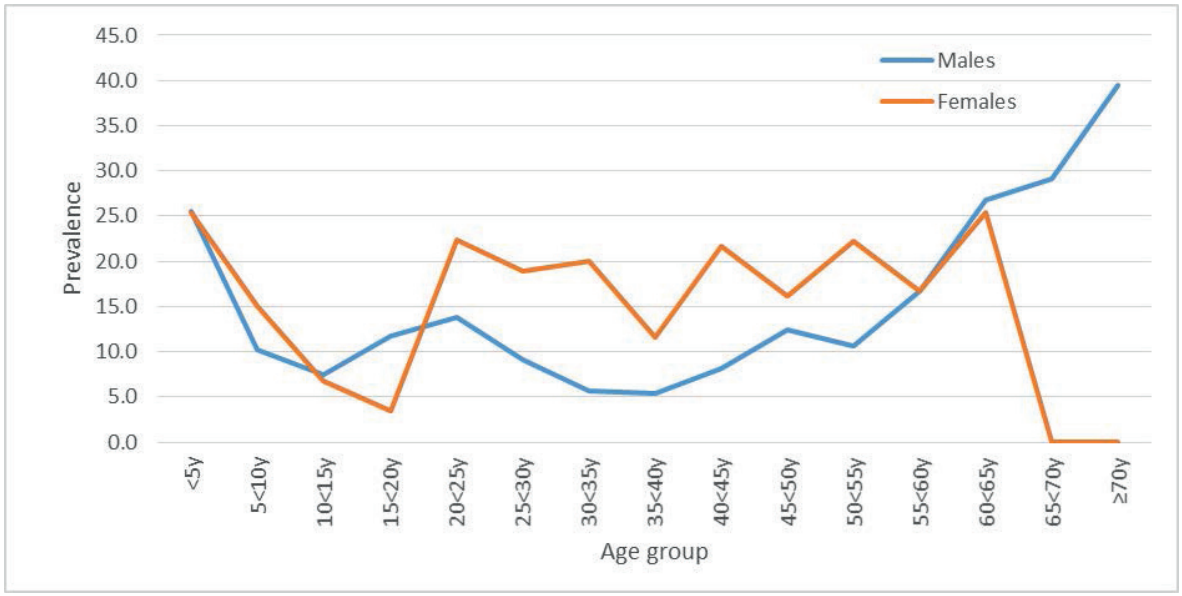


Table 4 Baseline characteristics of stunted children (*N*=592)

Baseline community characteristics	Not stunted (n, (%))	Moderately stunted (n, (%))	Severely stunted (n, (%))
Males			
High *N. americanus* infection intensity	92 (36)	96 (37)	70 (27)
Moderate *N. americanus* infection intensity	10 (31)	10 (31)	12 (38)
No *N. americanus* infection	57 (27)	80 (38)	76 (36)
Total	159 (32)	186 (37)	158 (31)
Females			
High *N. americanus* infection intensity	80 (48)	54 (32)	35 (21)
Moderate *N. americanus* infection intensity	16 (39)	17 (41)	8 (20)
No *N. americanus* infection	136 (50)	90 (33)	44 (16)
Total	232 (48)	161 (34)	87 (18)
Age group 1 to 5 years			
High *N. americanus* infection intensity	38 (48)	27 (34)	14 (18)
Moderate *N. americanus* infection intensity	12 (43)	11 (39)	5 (18)
No *N. americanus* infection	89 (38)	79 (34)	65 (28)
Total	139 (41)	117 (34)	84 (25)
Age group 6 to 11 years			
High *N. americanus* infection intensity	94 (43)	79 (36)	47 (21)
Moderate *N. americanus* infection intensity	10 (35)	11 (38)	8 (28)
No *N. americanus* infection	78 (43)	62 (34)	41 (23)
Total	182 (42)	152 (35)	96 (22)
Age group 12 to 17 years			
High *N. americanus* infection intensity	40 (31)	44 (34)	44 (34)
Moderate *N. americanus* infection intensity	4 (25)	5 (31)	7 (44)
No *N. americanus* infection	26 (38)	29 (42)	14 (20)
Total	70 (33)	78 (37)	65 (31)

Table 5 *Necator americanus* and *Ascaris* spp. intensity of infection profile

STH	Infection intensity profile n (%)			Mean Ct (95% confidence intervals)
	Heavy intensity	Moderate to low intensity	No infection	
N. americanus	1,155 (52%)	191 (8.6%)	873 (39%)	21.5 (21.3-21.8)
Ascaris spp.	220 (9.9%)	310 (14%)	1,689 (76%)	19.5 (19.2-19.9)

Notes: Numbers in this table are for all those who provided a stool sample, and therefore do not match the numbers used in separate morbidity analyses.

Table 6 Odds ratios for anaemia, by *Necator americanus* and *Ascaris* spp. infection intensity, Manufahi District, Timor-Leste

	Children; aged 1<18 years (*N*=1018)					Adults; aged ≥18 years (*N*=1020)				
	Univariable		Multivariable			Univariable		Multivariable		
Parameter	**OR**	**95% CI**	**AOR**	**95% CI**	***P***	**OR**	**95% CI**	**AOR**	**95% CI**	***P***
Ascaris heavy-intensity	0.93	0.49, 1.8	0.91	0.46, 1.8	0.8820	1.7	0.84, 3.3	1.8	0.86, 3.6	0.1165
Ascaris moderate-intensity	0.73	0.39, 1.4	0.85	0.44, 1.6		1.7**	1.04, 2.9	1.6	0.93, 2.7	
N. americanus heavy-intensity	0.52***	0.33, 0.83	0.69	0.41, 1.1	0.1606	0.92	0.62, 1.4	0.86	0.56, 1.3	0.7280
N. americanus moderate-intensity	0.42#	0.18, 1.0	0.50#	0.21, 1.2		0.99	0.52, 1.9	1.0	0.52, 1.9	
Ancylostoma infection	1.5#	0.58, 3.9	1.5#	0.58, 4.1	0.394	0.93#	0.42, 2.1	0.68#	0.27, 1.7	0.413
G. duodenalis infection	0.92	0.56, 1.5				0.42#	0.16, 1.1			
Male sex	1.1	0.70, 1.6	0.97	0.64, 1.5	0.894	0.93	0.66, 1.3	0.98	0.68, 1.4	0.918
Age group 6 to 11 years	0.35***	0.22, 0.54	0.39	0.24, 0.61	<0.0001					
Age group 12 to 17 years	0.20***	0.11, 0.38	0.19	0.09, 0.38	<0.0001					
Age group 65+ years						1.2	0.74, 1.9	1.2	0.76, 2.0	0.392
Socioeconomic quintile 4	1.0	0.49, 2.0	0.93	0.46, 1.9	0.0098	0.58	0.31, 1.1	0.58	0.31, 1.1	0.3718
Socioeconomic quintile 3	0.52	0.23, 1.2	0.49	0.22, 1.1		0.85	0.48, 1.5	0.83	0.46, 1.5	
Socioeconomic quintile 2	1.2	0.60, 2.4	1.1	0.55, 2.2		1.1	0.65, 2.0	1.1	0.62, 1.9	
Socioeconomic quintile 1 (poorest)	2.1**	1.0, 4.1	2.1	1.0, 4.3		0.97	0.54, 1.7	0.91	0.50, 1.7	

Notes: Logistic regression was used for investigating factors associated with anaemia. The outcome variable is bivariate anaemic/non-anaemic and associations therefore presented as odds ratios. *Ascaris* and *N. americanus* intensity infections defined according to following cut-points: *Ascaris*: heavy-intensity Ct≤15.4, moderate-intensity Ct>15.4<31, no infection Ct≥31; *N. americanus*: heavy-intensity Ct≤24.6, moderate-intensity Ct>24.6<35, no infection Ct≥35. OR, odds ratio; AOR, adjusted odds ratio; CI, confidence interval; *P*, Wald test. *** *P*<0.01, ** *P*<0.05 in univariable analysis, # less than 10 observations in subgroup; result should be interpreted cautiously. *Ascaris* (categorical infection intensity), *N. americanus* (categorical infection intensity), *Ancylostoma* infection prevalence (binary), age group, sex and socioeconomic quintile were included in all multivariable regression models as exposure outcomes and covariates. Reference categories: No *Ascaris* infection, no *N. americanus* infection, no *Ancylostoma* infection, no *Giardia* infection, age group 1-5 years (for child model), age group 18-64 years (for adult model), male sex, socioeconomic quintile 5 (wealthiest).

Table 7 Relative risk ratios for stunting, Manufahi District, Timor-Leste

	Moderate stunting n=347					Severe stunting n=245				
Parameter	**RR**	**95% CI**	**ARR**	**95% CI**	***P***	**RR**	**95% CI**	**ARR**	**95% CI**	***P***
Ascaris heavy-intensity	1.5	0.83, 2.7	1.6	0.87, 2.9	0.3007	1.8	0.79, 4.0	2.0	0.87, 4.4	0.2002
Ascaris moderate-intensity	1.1	0.65, 1.8	1.0	0.61, 1.7		1.0	0.50, 2.0	0.93	0.46, 1.9	
N. americanus heavy-intensity	0.82	0.56, 1.2	0.82	0.47, 1.4	0.6140	0.73	0.43, 1.2	0.91	0.41, 2.0	0.8647
N. americanus moderate-intensity	0.86	0.42, 1.7	1.3	0.52, 3.1		0.67	0.27, 1.7	0.70	0.18, 2.7	
Male sex	2.0***	1.4, 2.8	2.8	1.7, 4.7	<0.0001	3.7***	2.3, 6.0	6.8	3.4, 13.7	<0.0001
N. americanus heavy-intensity infection in males			0.65	0.31, 1.4	0.254			0.31	0.11, 0.85	0.023
N. americanus moderate-intensity infection in males			0.43	0.10, 1.8	0.244			0.82	0.13, 5.3	0.834
Ancylostoma infection	0.26***#	0.11, 0.65	0.27#	0.11, 0.68	0.005	0.33**#	0.11, 0.99	0.37#	0.12, 1.1	0.078
Giardia infection	**1.5**	0.96, 2.3				1.1	0.61, 2.0			
Anaemia prevalence	1.2	0.68, 2.0	1.1	0.65, 2.0	0.660	1.7	0.86, 3.3	1.7	0.83, 3.4	0.147
Age group 6 to 11 years	0.92	0.63, 1.4	1.1	0.71, 1.6	0.709	0.72	0.43, 1.2	1.1	0.60, 1.9	0.862
Age group 12 to 17 years	1.4	0.86, 2.3	1.7	0.98, 2.8	0.058	1.7	0.87, 3.2	2.7	1.3, 5.4	0.006
Socioeconomic quintile 4	1.1	0.59, 2.1	1.2	0.63, 2.2	0.2415	0.91	0.34, 2.5	1.1	0.39, 2.9	0.0165
Socioeconomic quintile 3	1.4	0.69, 2.7	1.4	0.70, 2.8		1.2	0.41, 3.4	1.3	0.45, 3.8	
Socioeconomic quintile 2	1.3	0.65, 2.4	1.2	0.64, 2.3		1.5	0.55, 4.0	1.5	0.57, 4.2	
Socioeconomic quintile 1 (poorest)	2.3**	1.1, 4.6	2.3	1.1, 4.6		5.1***	1.8, 14.2	5.1	1.8, 14.7	

Notes: Normal growth (no stunting) is reference category, i.e., moderate and severe stunting need to be interpreted relative to this reference. *Ascaris* and *N. americanus* intensity infections defined according to following cut-points: *Ascaris*: heavy-intensity Ct≤15.4, moderate-intensity Ct>15.4<31, no infection Ct≥31; *N. americanus*: heavy-intensity Ct≤24.6, moderate-intensity Ct>24.6<35, no infection Ct≥35. RR, relative risk; ARR, adjusted relative risk; CI, confidence interval; *P*, Wald test. *** *P*<0.01, ** *P*<0.05 in univariable analysis, #10 observations or less in subgroup; result should be interpreted cautiously. RRs in bold had univariable *P*<0.2 and were entered in multivariable regression models; for correct interpretation of this table, if a variable was significant for moderate stunting but not severe stunting, it was still included, therefore on occasion moderate stunting adjusted RRs are significant when severe stunting adjusted RRs are not, and *vice versa*. *Ascaris* (categorical infection intensity), *N. americanus* (categorical infection intensity), *Ancylostoma* infection prevalence (binary), anaemia prevalence (binary), age group, sex and socioeconomic quintile were included in all multivariable regression models as exposure outcomes and covariates. A sex**N. americanus* interaction is included in the model. Reference categories: No *Ascaris* infection, no *N. americanus* infection, female sex, no *N. americanus* infection in males (multivariable only), no *Ancylostoma* infection, no *Giardia* infection, no anaemia, age group 1-5 years, socioeconomic quintile 5 (wealthiest).

Table 8 Relative risk ratios for being underweight, Manufahi District, Timor-Leste

	Moderate underweight, n=253					Severe underweight, n=129				
Parameter	**RR**	**95% CI**	**ARR**	**95% CI**	***P***	**RR**	**95% CI**	**ARR**	**95% CI**	***P***
Ascaris heavy-intensity	1.0	0.53, 2.1	0.85	0.41, 1.7	0.8925	1.6	0.72, 3.5	1.2	0.49, 2.8	0.9379
Ascaris moderate-intensity	1.1	0.60, 2.1	1.0	0.52, 1.9		1.5	0.73, 3.3	1.0	0.46, 2.3	
N. americanus heavy-intensity	1.0	0.64, 1.7	0.59	0.28, 1.2	0.3584	0.85	0.47, 1.5	0.66	0.25, 1.8	0.1519
N. americanus moderate-intensity	0.94	0.41, 2.2	0.76	0.26, 2.2		0.87#	0.31, 2.5	0.12#	0.01, 1.2	
Age group 6 to 10 years	1.4	0.91, 2.2	1.2	0.63, 2.2	0.593	2.8***	1.6, 4.8	3.3	1.5, 7.0	0.003
Age group 6 to 10 years and *N. americanus* heavy-intensity infection	1.4	0.80, 2.5	1.8	0.67, 4.8	0.251	1.7	0.81, 3.6	0.70	0.20, 2.4	0.574
Age group 6 to 10 years and *N. americanus* moderate-intensity infection	1.8	0.39, 8.2	2.1	0.30, 13.9	0.463	7.0	1.36, 35.8	15.6	0.91, 266.4	0.058
Ancylostoma infection	2.5	0.88, 6.8	2.8	0.94, 8.1	0.064	0.69#	0.15, 3.2	0.86#	0.18, 4.2	0.848
Giardia infection	0.97	0.58, 1.6				1.1	0.58, 2.1			
Anaemia prevalence	0.99	0.56, 1.8	0.98	0.54, 1.8	0.943	0.70	0.34, 1.5	0.85	0.39, 1.9	0.682
Male sex	1.3	0.85, 2.0	1.3	0.81, 2.0	0.304	2.4***	1.4, 4.2	3.1	1.7, 5.6	<0.0001
Socioeconomic quintile 4	1.4	0.66, 3.1	1.5	0.66, 3.3	0.2606	0.57	0.20, 1.6	0.66	0.23, 1.9	0.3095
Socioeconomic quintile 3	1.1	0.46, 2.4	1.1	0.48, 2.6		1.0	0.36, 2.8	1.1	0.37, 3.0	
Socioeconomic quintile 2	1.1	0.53, 2.5	1.2	0.54, 2.7		0.87	0.33, 2.3	0.96	0.36, 2.6	
Socioeconomic quintile 1 (poorest)	2.4	1.0, 5.6	2.5	1.0, 6.1		2.0	0.72, 5.8	2.2	0.76, 6.6	

Notes: Normal weight is reference category, i.e., moderate and severe underweight need to be interpreted relative to this reference. *Ascaris* and *N. americanus* intensity infections defined according to following cut-points: *Ascaris*: heavy-intensity Ct≤15.4, moderate-intensity Ct>15.4<31, no infection Ct≥31; *N. americanus*: heavy-intensity Ct≤24.6, moderate-intensity Ct>24.6<35, no infection Ct≥35. RR, relative risk; ARR, adjusted relative risk; CI, confidence interval; *P*, Wald test. *** *P*<0.01, ** *P*<0.05 in univariable analysis, #10 observations or less in subgroup; result should be interpreted cautiously. RRs in bold had univariable *P*<0.2 and were entered in multivariable regression models models; for correct interpretation of this table, if a variable was significant for moderate underweight but not severe underweight, it was still included, therefore on occasion moderate underweight adjusted RRs are significant when severe underweight adjusted RRs are not, and *vice versa*. *Ascaris* (categorical infection intensity), *N. americanus* (categorical infection intensity), *Ancylostoma* infection prevalence (binary), anaemia prevalence (binary), age group, sex and socioeconomic quintile were included in all multivariable regression models as exposure outcomes and covariates. Being underweight not measured in children aged 11 to 17 years. An age group**N. americanus* interaction is included in the model. Reference categories: No *Ascaris* infection, no *N. americanus* infection, age group 1-5 years, no *N. americanus* infection in age group 6 to 10 years (multivariable only), no *Ancylostoma* infection, no *Giardia* infection, no anaemia, female sex, socioeconomic quintile 5 (wealthiest).

Table 9 Relative risk ratios for wasting, Manufahi District, Timor-Leste

	Moderate wasting n=145					Severe wasting n=44				
Parameter	**RR**	**95% CI**	**ARR**	**95% CI**	***P***	**RR**	**95% CI**	**ARR**	**95% CI**	***P***
Ascaris heavy-intensity	0.83	0.42, 1.6	0.83	0.40, 1.7	0.4465	0.51#	0.13, 2.1	0.37#	0.07, 1.9	0.4942
Ascaris moderate-intensity	0.73	0.38, 1.4	0.64	0.32, 1.3		1.1#	0.41, 3.1	0.92#	0.32, 2.7	
N. americanus heavy-intensity	1.7**	1.1, 2.8	1.3	0.76, 2.1	0.5964	2.1**	0.91, 4.7	1.2	0.51, 3.0	0.8431
N. americanus moderate-intensity	0.87#	0.35, 2.1	0.91#	0.36, 2.3		1.5#	0.40, 5.6	1.4#	0.35, 5.4	
Giardia infection	0.79	0.47, 1.3				0.46#	0.17, 1.2			
Anaemia prevalence	0.63	0.32, 1.2	0.83	0.41, 1.7	0.614	0.66#	0.22, 2.0	1.2#	0.37, 3.8	0.777
Male sex	1.3	0.84, 1.9	1.2	0.79, 1.9	0.358	1.3	0.68, 2.7	1.3	0.61, 2.6	0.534
Age group 6 to 11 years	3.0***	1.7, 5.2	2.9	1.6, 5.1	<0.0001	3.5**	1.3, 9.3	3.2	1.2, 8.9	0.022
Age group 12 to 17 years	4.9***	2.6, 9.2	4.3	2.2, 8.4	<0.0001	7.8***	2.6, 22.7	7.1	2.3, 21.8	0.001
Socioeconomic quintile 4	0.80	0.40, 1.6	0.82	0.39, 1.7	0.9181	0.89#	0.27, 3.0	0.92#	0.27, 3.2	0.8840
Socioeconomic quintile 3	0.72	0.35, 1.5	0.77	0.35, 1.7		1.3#	0.36, 4.4	1.3#	0.35, 4.6	
Socioeconomic quintile 2	0.69	0.34, 1.4	0.79	0.37, 1.7		0.60#	0.16, 2.3	0.70#	0.18, 2.7	
Socioeconomic quintile 1 (poorest)	0.66	0.31, 1.4	0.68	0.30, 1.5		0.59#	0.15, 2.3	0.66#	0.16, 2.7	

Notes: Normal growth (no wasting) is reference category, i.e., moderate and severe wasting need to be interpreted relative to this reference. *Ascaris* and *N. americanus* intensity infections defined according to following cut-points: *Ascaris*: heavy-intensity Ct≤15.4, moderate-intensity Ct>15.4<31, no infection Ct≥31; *N. americanus*: heavy-intensity Ct≤24.6, moderate-intensity Ct>24.6<35, no infection Ct≥35. Relative risks (RR) adjusted for age[2]. ARR, adjusted relative risk; CI, confidence interval; *P*, Wald test. *** $P<0.01$, ** $P<0.05$ in univariable analysis, #10 observations or less in subgroup; result should be interpreted cautiously. RRs in bold had univariable $P<0.2$ and were entered in multivariable regression models; for correct interpretation of this table, if a variable was significant for moderate wasting but not severe wasting, it was still included, therefore on occasion moderate wasting adjusted RRs are significant when severe wasting adjusted RRs are not, and *vice versa*. *Ascaris* (categorical infection intensity), *N. americanus* (categorical infection intensity), *Ancylostoma* infection prevalence (binary), anaemia prevalence (binary), age group, sex and socioeconomic quintile were included in all multivariable regression models as exposure outcomes and covariates. Insufficient *Ancylostoma* spp. observations to investigate in this regression model. No interactions were significant in this model. Reference categories: No *Ascaris* infection, no *N. americanus* infection, no *Ancylostoma* infection, no *Giardia* infection, no anaemia, female sex, age group 1-5 years, socioeconomic quintile 5 (wealthiest).

Appendix 1 Use of receiver-operating characteristic curves to statistically assign intensity of infection cut-points

Receiver-operating characteristic (ROC) curves show the trade-off between sensitivity and specificity, using the area under the ROC curve (AUC). Sensitivity is the proportion of individuals with the outcome of interest who are correctly classified according to the Ct threshold categorisation. Specificity is the proportion of individuals without the outcome who are correctly classified, where presence or absence of the outcome is defined by a "gold standard" which is assumed to correctly classified individuals. An AUC of 0.5-0.7 is interpreted as having poor predictive accuracy; 0.7-0.9 reasonable predictive accuracy; and >0.9 very good predictive accuracy [47].

A morbidity score was developed based on the presence/absence of moderate and severe stunting, wasting and underweight in children aged 1<10 years, with the following assignment: moderate stunting = 1, moderate wasting = 1, moderate underweight = 1, severe stunting = 2, severe wasting = 2, severe underweight = 2. These values were summed to a maximum possible score per child of 6. These scores were developed for the subgroup of children who were at the correct age to have all measures (i.e. children aged 1<10 years), and for purposes of generating scores we assumed that the relationship with Ct was the same for each of the morbidity measures. The morbidity score was then categorised as two versions of a binary variable (scores 0-1, scores 2-3, scores 4-6; whereby the cut-point between 1 and 2 represented the cut-point between low and moderate morbidity, and the cut-point between 3 and 4 represented the cut-point between moderate and high

morbidity) for running ROCs.

ROC analyses initially compared severe versus non-severe morbidity, and then moderate versus low morbidity. These were calculated as nonparametric curves, whereby the points on the curve were generated by using each possible outcome of the Ct as a classification cut-point and computing the corresponding sensitivity and specificity. For each of the intensity of infection outcomes, the Ct-value associated with the least distance between the ROC curve and 'perfect' classification was selected (i.e. the point on the ROC curve that had the greatest optimisation with sensitivity and specificity). Due to poor predictive capacity (Table S1), the assignment of cut-points between classes of infection intensity was discontinued. This demonstrated the weak underlying relationship between STH and morbidity in this population, with no apparent signal. That is, the test measure had no discriminating ability and an outcome could just as accurately have been based on chance. ROC analysis is an important statistical tool that can be used to assign cut-points, however it relies on the existence of a relationship between predictor and outcome.

Table S1 ***Ascaris*** **spp. and** ***Necator americanus*** **intensity of infection cycle threshold (Ct) cut-points between heavy and moderate morbidity assigned from receiver-operating characteristic curves**

STH	Ct cut-point	Distance	AUC score
N. americanus	Ct23	0.70	0.48
Ascaris spp.	Ct17	0.66	0.50

Notes: "Ct", cycle threshold from PCR; distance is calculated as $d=\sqrt{[(1\text{-sensitivity})^2 + (1\text{-specificity})^2]}$, with Ct cut-point selection based on the point on the Receiver Operating Characteristic (ROC) curve with least distance to the point of 'perfect' classification; "AUC", area under the ROC.

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Chapter 6 Analysis of WASH and environmental risk factors for intensity of STH infection

6.1 Chapter context

As was noted in Chapter 5, intensity of STH infection is a better marker of transmission potential than prevalence. Investigation of risk factors for intensity of STH infection is rarely undertaken, and all prior such analyses have used microscopic-based epg measures, which are of lower diagnostic accuracy than qPCR. Additionally, few analyses in any setting have investigated combined WASH and environmental risk factors in association with STH in detail. This is important due to the extensive potential interrelatedness of environmental, social, behavioural and host factors in any given setting influencing STH survival and transmission. Epidemiological analyses need to be informed by the underlying social and biological contexts. Assessing the full spectrum of measurable risk factors associated with WASH and the environment is therefore of prime importance for development of policy and programmatic decisions. Risk factors need to be considered not only for their clinical and statistical significance, but more broadly in terms of what may represent modifiable pathways for STH transmission.

In this chapter, detailed WASH, environmental and demographic factors associated with intensity of STH infection from qPCR were investigated. This analysis uses categorical intensity of infection variables for *N. americanus* and *Ascaris* that were developed in Chapter 5, and advanced statistical modelling to adjust for multinomial intensity outcomes,

dependency of observations, effects of poverty, and confounding from other measured variables. As such, this analysis augments both of the previous analyses of the PhD, to provide a comprehensive assessment of risk factors for STH, thereby meeting objective 5 of the thesis. This analysis is under review with *PLoS Neglected Tropical Diseases*.

6.2 Research objective

To assess WASH and environmental risk factors for intensity of infection from *N. americanus* and *Ascaris* spp. at baseline.

Variable selection

This analysis used the WASH risk factors from the first analysis (Chapter 4), and the intensity of infection variables developed in the second analysis (Chapter 5). Additionally, it examined environmental variables, including: temperature, elevation, precipitation, slope, vegetation, soil pH, soil texture, and land cover indices, linked to the parasitological data using a Geographical Information System. Sources for these data are included in the manuscript. The PhD Candidate undertook all checks for collinearity, univariable and multivariable modelling for this analysis.

Participants were excluded if they had not provided a stool sample for parasitological assessment, and if they had not answered an individual questionnaire.

Model building is described in the manuscript. Due to a multinomial outcome variable, models were underpowered to detect an effect in age-stratified analyses (i.e. preschool-aged children, school-aged children, adults). For this reason, these models were not stratified by age. Instead, age group as a categorical variable was included in the core model.

For this analysis, the PhD Candidate was responsible for 90% of the conception, 90% of the analysis, and 90% of the interpretation and writing of the paper. Clements A was responsible for 10% of the conception. Wardell R was responsible for 100% of the sourcing and smoothing of environmental variables, and 10% of the analysis. Clements A, Nery S, Wardell R, D'Este C, Gray D, McCarthy J, Traub R, Andrews R, Llewellyn S, Vallely A and Williams G are collectively responsible for 10% of the interpretation and writing of the paper.

Water, sanitation and hygiene (WASH) and environmental risk factors for soil-transmitted helminth intensity of infection in Timor-Leste, using real time PCR

Suzy J Campbell[a*], Susana V Nery[a], Rebecca Wardell[a], Catherine A D'Este[a], Darren J Gray[a,b,c], James S McCarthy[c,d], Rebecca J Traub[e], Ross M Andrews[f], Stacey Llewellyn[d], Andrew J Vallely[g], Gail M Williams[c], Archie CA Clements[a]

A. Research School of Population Health, College of Medicine, Biology and Environment, The Australian National University, 62 Mills Rd, Acton, ACT 2601, Australia (suzanne.campbell@anu.edu.au; susana.nery@anu.edu.au; rebecca.wardell@anu.edu.au; catherine.deste@anu.edu.au; darren.gray@anu.edu.au; director.RSPH@anu.edu.au)
B. Molecular Parasitology Laboratory, QIMR Berghofer Medical Research Institute, 300 Herston Rd, Brisbane QLD 4006, Australia
C. School of Public Health, University of Queensland, Herston Rd, Brisbane QLD 4006, Australia (gail.williams@uq.edu.au)
D. Clinical Tropical Medicine Laboratory, QIMR Berghofer Medical Research Institute, 300 Herston Rd, Brisbane QLD 4006, Australia (james.mccarthy@qimrberghofer.edu.au; stacey.llewellyn@qimrberghofer.edu.au)
E. Faculty of Veterinary and Agricultural Science, The University of Melbourne, Parkville VIC 3010, Australia (rebecca.traub@unimelb.edu.au)
F. Menzies School of Health Research, Charles Darwin University, Ellengowan Dr, Casuarina NT 0810, Australia (ross.andrews@menzies.edu.au)
G. Kirby Institute, University of New South Wales, Wallace Wurth Building, High St, Kensington NSW 2052, Australia (avallely@kirby.unsw.edu.au)

*Corresponding Author: Suzy Campbell, Global Health Division, Research School of Population Health, The Australian National University, 62 Mills Rd, Acton, ACT 2601, Australia. Email: suzanne.campbell@anu.edu.au, Phone: (+61) 2 6125 0538, Fax: (+61) 2 6125 5608

Water, sanitation and hygiene (WASH) and environmental risk factors for soil-transmitted helminth intensity of infection in Timor-Leste, using real time PCR

Abstract

Background: No investigations have been undertaken of risk factors for intensity of soil-transmitted helminth (STH) infection in Timor-Leste. This study provides the first analysis of risk factors for intensity of STH infection, as determined by quantitative PCR (qPCR), examining a broad range of water, sanitation and hygiene (WASH) and environmental factors, among communities in Manufahi District, Timor-Leste.

Methods: A baseline cross-sectional survey of 18 communities was undertaken as part of a cluster randomised controlled trial, with additional identically-collected data from six other communities. qPCR was used to assess STH infection from stool samples, and questionnaires administered to collect WASH, demographic, and socioeconomic data. Environmental information was obtained from open-access sources and linked to infection outcomes. Mixed-effects multinomial logistic regression was undertaken to assess risk factors for intensity of *Necator americanus* and *Ascaris* infection.

Results: 2152 participants provided stool and questionnaire information for this analysis. In adjusted models incorporating WASH, demographic and environmental variables, environmental variables were generally associated with infection intensity for both *N. americanus* and *Ascaris* spp. Precipitation (in centimetres) was associated with increased risk of moderate-intensity (adjusted relative risk [ARR] 6.1; 95% confidence interval [CI] 1.9-19.3) and heavy-intensity (ARR 6.6; 95% CI 3.1-14.1) *N. americanus* infection, as was sandy-loam soil around households (moderate-intensity ARR 2.1; 95% CI 1.0-4.3; heavy-

intensity ARR 2.7; 95% CI 1.6-4.5; compared to no infection). For *Ascaris*, alkaline soil around the household was associated with reduced risk of moderate-intensity infection (ARR 0.21; 95% CI 0.09-0.51), and heavy-intensity infection (ARR 0.04; 95% CI 0.01-0.25). Few WASH risk factors were significant.

Conclusion: In this high-prevalence setting, strong risk associations with environmental factors indicate that anthelmintic treatment alone will be insufficient to interrupt STH transmission, as conditions are favourable for ongoing environmental transmission. Integrated STH control strategies should be explored as a priority.

Funding: Australian National Health and Medical Research Council (NHMRC) Partnership project in collaboration with WaterAid Australia.

Key words

Soil-transmitted; helminth; hookworm; *Necator americanus*; *Ascaris*; intensity; risk factor; PCR

Background

Surprisingly little evidence convincingly demonstrates the benefits of water, sanitation and hygiene (WASH) interventions on reducing soil-transmitted helminth (STH) infections (1,2). Yet it is widely believed that WASH improvements together with anthelmintics could break STH transmission cycles in settings in which anthelmintics alone are insufficient (3,4). There has been inadequate epidemiological investigation of the role of improved WASH in reducing the STH burden, but there is a growing need for evidence to enable more effective investment in WASH and integrated strategies for STH control.

Intensity of STH infection is important to assess in epidemiological analyses. STH are highly aggregated in humans, with a small number of people harbouring large numbers of helminths, and the majority harbouring few or none (5). As with prevalence, intensity of worm burden is marked within various groups of the community such as different age groups and gender 6). This well-described phenomenon is a key feature of this macroparasite relationship with the human host. For quantitative investigations it is therefore problematic to use solely prevalence of infection as an indicator of STH burden or transmission, because large changes in intensity may only be accompanied by small changes in prevalence (6). STH do not reproduce within the host; infection intensity depends on the time and extent of exposure (7). Where STH are endemic, maximum worm intensity usually occurs at ages five to ten for *Ascaris lumbricoides* and *Trichuris trichiura*, and in adolescence or early adulthood for hookworm (6). Whilst the reasons for this are unknown, it may be due to behavioural and social factors, nutritional status, genetic and immunological factors (5, 8-11). There is evidence that some individuals are predisposed to heavy or light STH infections (9,12). Intensity of *T. trichiura* infection reacquired by an individual after treatment has been found to be significantly correlated with the intensity of infection prior to treatment (13). Additionally, intensity of infection with STH has been identified as substantially greater when any of the species occurred in combination with one or more of the others (14), probably also due to exposure, genetic and immunological factors, which could then act in determining risk of associated morbidities. Despite this knowledge, there is much focus on the use of prevalence to measure STH infection endemicity.

The relationships between intensity of STH infection and risk factors have been inadequately explored, yet could provide useful information as to why intensities differ by host age, environment, and helminth species. Because a key feature of the STH life cycle is the soil-dwelling stage, STH survival, development and transmission potential all rely on a complex assortment of environmental, social, behavioural and host factors. Therefore, in addition to investigating associations between WASH and STH, community-based associations must be considered within their environmental context (15,16). Although more evidence is required, STH associations with WASH have been systematically appraised (2). Studies have additionally identified temperature, rainfall, soil porosity and pH, vegetation and elevation ranges as influencing *N. americanus* larval development and STH transmission (16,17). We have previously separately reported on WASH (18) and environmental (19) risk factors for STH prevalence in Manufahi District, Timor-Leste. Given exposure-related risks, and associations between heavy-intensity infection and morbidity, this analysis was conducted to investigate whether WASH- and environmental-related risk factors in this district may also be associated with infection intensity, using categories derived from quantitative PCR (qPCR), a highly sensitive and specific diagnostic technique (20).

By combining data on both WASH and environmental risk factors this analysis provides a more complete picture of risks and thereby augments the current knowledge of risk factors for STH in Timor-Leste. Knowledge of WASH risk factors will be used to inform control strategies in this country. Whilst many environmental risk factors may not be modifiable, the inclusion of these factors will enable targeting of control strategies to areas of greatest

need. This is one of very few extensive investigations of combined WASH and environmental risk factors for STH undertaken. It is additionally the first epidemiological analysis of risk factors undertaken using categorised intensity of STH infection from qPCR.

Methods

Ethical approval and consent

This analysis used baseline data from 18 communities in a cluster randomised controlled trial (RCT), supplemented with data from an additional six communities, in Manufahi District, Timor-Leste (Australian and New Zealand Clinical Trials Registry ACTRN12614000680662) (21). STH have recently been reported as endemic in this community, with prevalence of *N. americanus* of 60% and *Ascaris* spp. of 24%, as detected by qPCR (18).

The University of Queensland 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 Human Research Ethics Committee approved the study protocol. Participant informed consent processes included explaining the study purpose and methods, and obtaining signed consent from all adults and parents or guardians of children under 18 years (21). Children aged less than 12 months were excluded (21).

Study setting, design and collection of data

The RCT commenced in May 2012. Detail on the RCT design is provided in the trial protocol (21). A baseline survey of 18 communities involved in the RCT, and six additional

communities, was conducted between May 2012 and October 2013. All communities surveyed were rural, and agrarian occupations predominated. Manufahi District has terrain varying from flat coastal plains to relatively mountainous inland areas (with elevation exceeding 1100 metres in some communities). It is a tropical region, with very high average rainfall of 190cm (19) and a wet season extending for close to ten months of the year. The average annual temperature is 24.5°C (19).

A single stool sample per participant was collected and fixed in 5% potassium dichromate. Multiplex qPCR was used to analyse stool samples for the presence and intensity of STH infection. Details on the qPCR diagnostic method are provided elsewhere (20).

Village, household and individual level questionnaires encompassing a broad range of potential WASH and socioeconomic risk factors were administered by trained field workers (18,21). Interviewer observation of household and village latrines, their type and cleanliness was undertaken; all other questions were self-reported. Data were collated and entered into a Microsoft Access database and extracted to STATA 13.0 (Stata Corporation, College Station, Texas) for error checking.

Data analysis

Individual-level data were linked to questionnaire and parasitological outcomes and household GPS coordinates (18,21). Principal component analysis was used to create a wealth index, based on ownership of household assets (animals, transport and appliances), house floor type, reported income, and presence of electricity (18, 22). Using eigenvalues

above 1, four principal components were retained and used to produce a final wealth score which was categorised into quintiles of relative socioeconomic status (18).

Outcome variables were intensity of *N. americanus* and *Ascaris* infection, which were analysed separately. Intensity of infection was derived from qPCR DNA cycle threshold (Ct) values, and categorised into two groups: (i) heavy-intensity, and (ii) moderate- to light-intensity infection (hereafter called "moderate intensity") using algorithms generated from seeding experiments to correlate Ct-values to egg per gram of faeces (epg) equivalents. Full detail of this method is provided elsewhere (20,23). Exposure variables were WASH variables from study questionnaires, grouped into domains of related variables (e.g. household sanitation; household water supply; household hygiene; household socioeconomic status), and environmental variables that were sourced separately. Environmental variables were selected for analysis based on reported prior relationships with STH development (17), and availability via open-access sources. Temperature, precipitation, elevation, soil texture, soil pH, landcover and vegetation data were selected for analysis (Table 1) and processed using the geographical information system ArcMap 10.3 (ESRI, Redlands, CA) (19). Very few environmental analyses incorporate information on soil texture and soil pH; it has been possible to incorporate these variables due to soil surveys conducted in the study region between 1960 and 1965 (24); soil type was not considered to have changed dramatically since that time. A range of environmental variables related to the above factors was produced according to long-term average data, seasonal periods, and spatial resolution (19), with household as the data point, and a 1 km buffer applied (whereby the median raster value within a 1 km radius of the household was

used (19)). Quality checks and exploratory analyses were undertaken to determine the most suitable version of each variable for analysis. Separate assessment of spatial autocorrelation was undertaken using semivariograms of residuals from multivariable models of selected environmental variables, with household and village random effects (19); no additional autocorrelation was identified (19). The analysis of environmental covariates in this study was limited to risk factor investigation. Predictive risk maps for STH infection in Manufahi District are published separately (19).

[Please insert Table 1 here]

Variables were investigated for multicollinearity according to likely relationships determined from literature, using tetrachoric analysis and the STATA "collin" user written package, according to the type of variable. Temperature and elevation were collinear; each variable was analysed in separate univariable models and subsequent variable selection was based on lower Akaike's Information Criterion (AIC), indicating better predictive performance of the model. Chi-squared tests were conducted to compare intensity of infection by age, sex and socioeconomic quintile. Using categorised intensity of infection as the outcome, univariable and multivariable mixed effects multinomial regression was undertaken, with household and village random effects to account for dependence of observations. Regression analyses were undertaken for *N. americanus* and *Ascaris* spp. separately.

Regression models were not age-stratified due to insufficient numbers for some combinations of outcome and explanatory variables. Univariable regression was undertaken for each risk factor, with inclusion of variables in multivariable regression if they had $P<0.2$ on the Wald test in univariable analyses. All multivariable models included age group, sex, and socioeconomic quintile as covariates. Forward stepwise variable addition was used with variables retained if $P<0.1$ within, then across, domains of variables, until the most parsimonious adjusted model for each outcome was achieved. A categorised age variable, and a sex*age interaction term, were investigated, as the association between sex and the outcome was anticipated to vary by age group. Interactions were investigated by developing models without, then with, the interaction term and comparing these using the likelihood ratio test, with $P<0.1$ being the inclusion criterion for the interaction. Applying this criterion, the interaction term was retained in the final *N. americanus* model, but not the *Ascaris* model. A 5% significance level was used, however this analysis reports results of up to 10% significance, which is important for epidemiological interpretation. Analyses were conducted using generalised structural equation models in STATA 14.1 (Stata Corporation, College Station, Texas). Due to uncertainty regarding the linearity of the association of continuous environmental variables and the infection outcomes, quadratic terns were also investigated in all models; however as none of these quadratic terms were significant in the adjusted models, these results are not presented. Post-analysis power calculations indicated 80% power, with a 5% significance level, to detect relative risks of 1.2 to 1.8 for *N. americanus* infection intensity (depending on level of intensity), and, reflecting lower prevalence overall, relative risks of 2.7 to 3.9 for *Ascaris* infection intensity.

Results

From 24 communities, 2827 eligible people provided baseline survey data, of whom 2152 participants (1038 males, 1114 females) completed both an individual questionnaire and provided a stool sample and were included in this analysis (Table 2, (18)). Using our infection intensity cut-points, more than half (52%) of participants had heavy-intensity *N. americanus* infection; 10% had heavy-intensity *Ascaris* infection (Table 2). There was very low prevalence of water or sanitation infrastructure, and most households owned few assets. Most heavy-intensity *Ascaris* infection occurred in children (Figure 1). Heavy-intensity *N. americanus* infections were more spread across age groups (Figure 2). Heavy-intensity *Ascaris* infection varied significantly by socioeconomic quintile (P=0.012); *N. americanus* infection intensity did not (P=0.468).

[Please insert Table 2 here]

[Please insert Figure 1 here]

[Please insert Figure 2 here]

Factors associated with N. americanus *intensity of infection*

Environmental factors were associated with *N. americanus* infection (Table 3). Of particular note, precipitation, measured in centimetres, was significantly associated with a six-fold increased risk of moderate-intensity (adjusted risk ratio [ARR] 6.1, 95% confidence interval (CI) 1.9, 19.3), and seven-fold increased risk of heavy-intensity

infection (ARR 6.6, 95%CI 3.1, 14.1), compared to no infection. Sandy-loam soil around the house was associated with more than two-fold higher risk of moderate-intensity (ARR 2.1, 95%CI 1.0, 4.3), and heavy-intensity infection (ARR 2.7, 95%CI 1.6, 4.5), respectively, compared to other soil types. Increasing elevation above sea-level was associated with slightly reduced risk of heavy-intensity infection (ARR 0.90, 95%CI 0.83, 0.97), but was not associated with moderate-intensity infection. Increasing normalised difference vegetation index (NDVI) was associated with increased risk for heavy-intensity infection (ARR 1.1, 95%CI 1.0, 1.1). Soil acidity was not included in *N. americanus* regression models (P>0.2 on univariable analysis).

[Please insert Table 3 here]

Co-infection with *Ancylostoma* spp. was associated with four-fold higher risk of heavy-intensity *N. americanus* infection (ARR 4.1, 95%CI 2.1, 8.0). *G. duodenalis* was marginally non-significant for heavy-intensity infection (ARR 0.71, 95%CI 0.49, 1.0). Due to the sex by age interaction term results are reported separately for females and males within age groups. Relative to no *N. americanus* infection, a significant gradient of increased risk of heavy *N. americanus* infection intensity with increasing age group was evident for females (ARRs increasing from 3.2 to 9.6; see table 3), however this was less evident for moderate-intensity infection (with the exception of being aged 65 years or older having four-fold increased risk of infection; ARR 4.4, 95%CI 1.6, 11.9). For males, relative to no infection, being aged 18 to 64 years was significantly associated with more than three-fold increased risk of any intensity infection (moderate-intensity ARR 3.3, 95%CI

1.3, 8.7; heavy-intensity ARR 3.6, 95%CI 1.8, 7.3). Sex in participants aged one to five years (i.e. reference group) was not associated with intensity of infection. A gradient of generally increasing risk of moderate- and heavy-intensity infection was also evident with worsening socioeconomic quintile (being significant across most subgroups for heavy-intensity), with people in the poorest quintile having more than twice the risk of infection for both intensity levels (moderate-intensity ARR 2.0, 95%CI 1.1, 3.7; heavy-intensity ARR 2.2, 95%CI 1.3, 3.6).

Few associations were found between WASH variables and STH outcomes in adjusted analyses. Of note is that a shared piped water supply was associated with strongly reduced risk of heavy-intensity infection compared to an unprotected stream (ARR 0.32, 95%CI 0.12, 0.84), and use of surface water was associated with twice the risk of moderate-intensity infection compared to an unprotected stream (ARR 1.9, 95%CI 1.1, 3.2). Boiling household water was associated with half the risk of moderate-intensity *N. americanus* infection compared to not boiling water (ARR 0.52, 95%CI 0.34, 0.80). Having one preschool-aged child in the household was protective against heavy-intensity *N. americanus* infection (ARR 0.57, 95%CI 0.40, 0.82). For moderate-intensity infection having one preschool-aged child in the house was not significant (ARR 0.81, 95%CI 0.52, 1.3), but having more than one was associated with reduced risk (ARR 0.57, 95%CI 0.34, 0.94). People reporting three or more bowel motions during the previous 24 hours (indicating diarrhoea) was associated with reduced risk of heavy-intensity infection compared to people who reported less than three bowel motions (ARR 0.40, 95%CI 0.17, 0.96). People who reported having access to anthelmintic drugs and people who reported

actually taking deworming treatment within the previous 12 months, was not associated with risk of infection in adjusted models, despite these factors being highly significant in univariable analysis for heavy-intensity infection. Methods of post-defecation anal cleansing, and shoe wearing, all of which were highly significant in univariable analyses for heavy-intensity infection, did not emerge as risk factors in adjusted analyses.

Factors associated with intensity of* Ascaris *infection

Factors significantly associated with *Ascaris* infection were age, and environmental variables, particularly alkaline soil and elevation above sea level (Table 4). Alkaline soil was significantly associated with highly reduced risks of moderate-intensity (ARR 0.21, 95%CI 0.09, 0.51), and heavy-intensity *Ascaris* infection (ARR 0.04, 95%CI 0.01, 0.25, note low numbers) compared to acidic soils. Neutral pH soil showed no association with risk of infection. Increasing elevation was associated with *Ascaris* infection, with observations of a mild gradient of increasing risk with increasing infection intensity (moderate-intensity ARR 1.3, 95%CI 1.2, 1.4; heavy-intensity ARR 1.4, 95%CI 1.2, 1.7). Increasing NDVI was also associated with mildly increased risk of heavy-intensity infection (ARR 1.2, 95%CI 1.1, 1.4). No WASH variables emerged as risk factors for *Ascaris* infection. Increasing age was associated with reducing risk of both moderate and severe infection intensity on a gradient that was significant for many age groups (particularly for heavy-intensity infections). Sex and socioeconomic status were not risk factors for *Ascaris* infection intensity.

[Please insert Table 4 here]

Discussion

This analysis presented the first investigation of combined WASH, environmental and demographic factors for intensity of STH infection in Timor-Leste. Using PCR-derived intensity of infection categorisation, similar infection intensity profiles to previous epg-based profiles (25) were found for each of *Ascaris* and *N. americanus*, with the most intense *Ascaris* infections in children, declining intensity and prevalence in adulthood, and prevalence and intensity of *N. americanus* being high in both childhood and adulthood. For *N. americanus*, heavy-intensity infections occurred in older age groups, although at low proportions. Whilst current risk factor models were not separately analysed by age groups, these results are in agreement with previous findings of different age-specific risk factors for different STH species in the study area (18). This highlights the potential role of exposure-related risk factors, although other factors, such as acquisition of some level of immunity, may play a role (25).

It has previously been hypothesised that sex and age associations with STH are strongly related to exposure-associated behaviours (26). Females showed a highly significant, increasing gradient for risk of heavy *N. americanus* infection with increasing age. Although less significant, a gradient was also evident for moderate-intensity *N. americanus* infection. Whilst overall male sex in those aged one to five was not a significant risk factor for *N. americanus* infection intensity compared to females of this age group, there was again an observation of greater heavy-intensity infection in males aged 18 to 64 relative to males aged one to five. These observations suggest that there are additional age- and sex-related factors occurring. This may include age as an expected indicator of time-accumulation

given STH do not multiply in the host (14) and the longevity of *N. americanus* (27). Alternatively, there could be exposure behaviours in older adults (compared to children) that are important to identify as they may be amenable to modification. There could also be differences in host immunity, particularly at different ages. Increased animal and soil contact through agricultural activities represents a direct potential transmission pathway (particularly in males) that requires further exploration. Further investigation into the female-age group association with heavy-intensity infection need to be undertaken; this could reflect particular household-related practices undertaken by women but not men. Further activities, such as constructing daily activity diaries, would be valuable to enable further insights in this setting. Alternatively, the findings of different sex and age patterns may be indicative of other factors such as host genetics (26).

Mixed-effects multinomial models were used to investigate the statistical relationship between intensity of STH infection, and WASH and environmental risk factors, whilst accounting for heterogeneity within village and household random effects. The lack of autocorrelation identified in semivariograms after accounting for large-scale environmental trends indicates that environmental variables explained the majority of spatial correlation in the data (19). In our adjusted models incorporating WASH, demographic and environmental variables, environmental variables were generally associated with the greatest ARRs for infection intensity for both *N. americanus* and *Ascaris* spp. Precipitation was associated with increased risk of *N. americanus* infection of any intensity, but not *Ascaris* intensity. It is important to note that the precipitation variable included in these analyses was derived from 50-year averaged data from the driest month (19); it is not

reflecting seasonality, which could have had an impact on *N. americanus* survival rates in the soil (17). Seasonal fluctuations could affect transmission potential but are not considered likely to have a strong influence on infection patterns given the longevity of *N. americanus* in the human host (17).

High rainfall contributes to suitably moist conditions for eggs and larvae to survive in soil, including the propensity for *N. americanus* larvae to remain near the soil surface and thus be available for human infection (17), but for *Ascaris*, excess rainfall may have negative impacts, possibly because the eggs sink lower in the soil as rainfall drains away. Our analysis showed strong associations between sandy-loam soil and highly increased risks of *N. americanus* infection, yet conversely, no significance in adjusted models for *Ascaris* spp. Observational associations between hookworms and sandy soil have been reported since the early 1900s (reviewed in (17)). Significance of soil type and rainfall likely reflect an important difference in life cycles and transmission potential between these two STH. *N. americanus* survive in the external environment as motile ensheathed larvae, but *Ascaris* spp. are present as (non-motile) eggs. The interrelated features of large-particle "sandy" soil, which tends to be less dense, aids both larval motility and water draining during/after rainfall, being therefore more amenable to *N. americanus* larval survival (17,26) and subsequent transmission potential. *Ascaris* eggs, on the other hand, are more susceptible to extremes, being able to dessicate in dry soil and to retard development in extremely wet soils (28); this supports the lack of association between *Ascaris* spp., sandy soil and precipitation in this analysis. These factors, plus the shorter developmental time to infectivity in the soil of *N. americanus* compared to *Ascaris* (16), may contribute to the

considerably greater prevalence of *N. americanus*.

We have previously reported on a protective association observed between alkaline soil type and *Ascaris* infection (19); in this current analysis there was some evidence of a gradient of increasing infection intensity, although numbers were low and this finding therefore requires verification. Other studies use soil acidity data in spatial analyses (29,30), with one study reporting associations between acidic soil and increased infection risk ((30); although this study used categories of pH that were all considered acidic compared to our definitions which defined neutral soil type as pH 6.6 to 7.3 (31)). Generally, soil acidity information is still rarely collected, yet this is an important potential determinant that could vary with precipitation, and other ecological or land use factors. Further analysis of pH ranges in epidemiological studies will contribute to knowledge of the optimal conditions for survivability of these helminths.

Differences in motility and survivability also potentially explain the direct association between increased elevation and *Ascaris* intensity of infection, with downhill runoff and draining after rainfall potentially facilitating survivability (and hence transmission) of those *Ascaris* eggs that remain in soil at higher elevations (i.e. those that do not get washed away); it would be plausible that *Ascaris* eggs that are washed downhill may be washed into rivers and streams, or lie within saturated environments that are less conducive to development. For *N. americanus* this was an inverse relationship for heavy-intensity infection; the protective association seen from elevation may reflect lower temperatures at higher elevations (as temperature was not included in multivariable analyses due to its high

correlation with elevation). Negative correlations between hookworms and elevation, and, less consistently, positive correlations between *A. lumbricoides* and elevation, have previously been reported (reviewed in (17)).

Given high STH prevalence, poverty and poor existing WASH infrastructure (18), and the large quantity of risk factors investigated, few WASH risk factors have emerged in these analyses. Homogeneously poor access to improved WASH resources in study communities would limit our ability to find major associations (18), and is the most likely explanation for this. No significant WASH risk factors for *Ascaris* intensity of infection were found in adjusted models. For *N. americanus*, the protective association with boiling water against moderate-intensity infection is slightly surprising. There is evidence that *N. americanus* larvae can survive and remain infective for several days in water (decreasing with duration of water exposure) (32). Whilst there is negligible published evidence for *N. americanus* infection via ingestion, this finding points to faecal contamination of drinking water sources as a possible exposure pathway. Water supply effects were also not significantly associated with intensity of *N. americanus* infection in the expected direction, with different levels of risk between surface water and an unprotected spring; both of which are unimproved water sources (33). This could possibly be due to location of communities downhill from springs (thus positioned for gravity-fed flow), whilst communities may be generally uphill from surface water. There may additionally be a greater tendency for people to remove footwear when going to surface water, compared to (potentially smaller) springs. Alternatively, this may reflect heavy *N. americanus* contamination in the vicinity of particular water sources in the study area. Self-reporting error or a misunderstanding of water source definitions

used in our study are also possible explanations. The protective effect of shared piped water, but not other 'improved' sources such as tubewells, is of interest and may reflect a heightened level of hygiene awareness in situations where multiple households use the same source, or alternatively, high correlation between some other variable and this one (although confounding and collinearity were investigated). The general lack of WASH associations, particularly with levels of sanitation, is similar to results that we have reported previously for prevalence (18); however it was not previously clear whether this was because prevalence models were age-stratified, which could have affected power to detect effects. Lack of WASH risk factors therefore most likely reflects homogeneously poor access to WASH infrastructure, with flow-on impacts on amenable hygiene behaviours, in these communities, or a true lack of association with STH in this district. Alternatively, with a multinomial outcome, analyses could have adequate power to detect only moderate-large associations (see limitations).

Prevalence of *N. americanus* has previously been reported to be significantly associated with low socioeconomic status in this study area (18). As has previously been identified socioeconomic status in this community reflects relative poverty that was still measurable within a general setting of poverty (18) and it is interesting that, for *N. americanus*, slightly higher estimates of association were seen for socioeconomic strata in heavy-intensity relative to moderate-intensity infection. This highlights an advantage of investigating socioeconomic status in defined districts on high-resolution (i.e. village-level) scale as opposed to national scale; it has been previously reported that between- and within-village heterogeneity may limit the usefulness of socioeconomic proxies in aggregated large-scale

analyses (28). The greater level of detail from this multinomial model provides additional insight into the *N. americanus*-poverty relationship. An interesting protective effect was the presence in the household of preschool-aged children; possibly this reflects adoption of hygienic behaviours when there are young children to protect them from disease exposures. The finding of reduced risk of heavy infection in people who reported three or more bowel motions is not surprising given that diarrhoea causes dilutive effects on quantities of helminths (34). Our finding that recent deworming was not significantly associated with infection in adjusted models may be due to self-report error, with possible confusion about medications received.

The risks associated with environmental variables have important implications for STH control. The high rainfall, mountainous, tropical environment combined with high levels of poverty, poor WASH infrastructure and behaviour, and the longevity of STH eggs and larvae survival in soil (16), provides a fertile environment for STH transmission in this district. This is a challenge for helminth control because environmental variables themselves are not modifiable. Despite this, awareness of high-risk factors can influence other activities, primarily hygiene- and sanitation-associated behaviours to manage environmental risks. This provides a strong justification for investment in WASH activities irrespective of their individual statistical significance in risk factor analyses, as this is an exposure-reduction pathway that can potentially be manipulated. As current evidence for hygiene behaviours on STH control is sparse (reviewed in (1,2)), further research on the hygiene behaviours that could have greatest impact in this scenario needs to be undertaken as a priority.

This analysis is an important contribution to an ongoing RCT that will assess the benefits of augmented albendazole with WASH for STH control in Manufahi District, Timor-Leste (21). As well as a detailed understanding of baseline WASH infrastructure and behaviours upon which to benchmark trial-related improvements in WASH, the knowledge of environmental factors is an essential prerequisite for effective targeting of interventions.

Limitations and strengths

This is an observational analysis and, as such, cause and effect cannot be determined. As has been noted previously (18) much of the WASH data collected involved self-report of infrastructure and behaviours. Presence, type and cleanliness of household and village latrines were verified by interviewer observation. Self-reporting is a frequently-encountered drawback of measuring WASH characteristics. Further, extensive heterogeneity in assessing WASH behaviours on STH outcomes makes assessment of WASH characteristics challenging (15,35). An important research priority is to develop specific WASH measurement guidelines for STH control. Power calculations indicated power to detect low associations for *N. americanus* and moderate associations for *Ascaris* infection intensity in multinomial models.

There are particular strengths to this study. This is one of very few epidemiological investigations of risk factors for STH infection intensity; this is particularly important to assess for environmental factors, given the links to STH transmission dynamics and correlations with morbidity. In this paper a community-based risk analysis is presented that combines high-resolution environmental, WASH and demographic variables in adjusted

models. Advanced statistical techniques have been used to adjust for multinomial intensity outcomes, dependency of observations, effects of poverty, and confounding from other measured variables. As with all analyses, there is the possibility of residual confounding from unmeasured factors. However this provides the most comprehensive assessment of STH risk factors that we have identified in any setting.

A further strength is the use of PCR; a highly sensitive and specific technique (20) that is increasingly used for STH diagnosis. PCR-derived intensity of infection categorisation is a recent development, and requires further validation in different epidemiological settings (23). Notwithstanding the need for further refinement of cut-points, different risk factors for moderate and heavy-intensity STH infections were found in this study area, with some evidence of a scale of increasing risk for factors such as soil type. This contributes useful, and highly relevant, information on risk factors within these communities. Use of infection intensity to determine risk factor associations requires more investigation. In particular, use of prevalence alone could mask significant intensity-related associations. This may mean that key evidence for WASH benefits may be overlooked in epidemiological studies that use prevalence of infection as the outcome. The possibility that WASH significance may be underreported in this way has been inadequately explored.

Conclusion

With intensity of STH infection as the outcome, a comprehensive risk analysis of environmental, WASH and demographic variables is presented for communities in Manufahi District, Timor-Leste. Strong risk associations with environmental variables were

identified. However, generally few associations with WASH risk factors were evident. This raises the importance of accurate measurement of WASH, and the need for clear guidelines on measuring WASH epidemiological research. This result also has important implications for STH control activities. Even in the absence of WASH significance, WASH infrastructure and behavioural-related activities are the only identified mechanism that could reduce or prevent transmission in an environment of high STH transmission potential. In this setting, anthelmintic treatment alone will not interrupt STH transmission; this provides a strong justification for application of integrated STH control strategies in this district.

List of abbreviations

AIC	Akaike's Information Criterion
ARR	Adjusted relative risk
CI	Confidence interval
Ct	Cycle threshold
DNA	Deoxyribonucleic acid
NDVI	Normalised difference vegetation index
qPCR	Quantitative polymerase chain reaction
RCT	Randomised controlled trial
RR	Relative risk
STH	Soil-transmitted helminth
WASH	Water, sanitation and hygiene

Declarations

Ethics approval and consent to participate

These analyses were conducted as part of a cluster randomised controlled trial (RCT) in Manufahi District, Timor-Leste (Australian and New Zealand Clinical Trials Registry ACTRN12614000680662). The study protocol was approved by the University of Queensland 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 Human Research Ethics Committee. Participant informed consent processes involved explaining study purpose and methods, and obtaining signed consent from all adults and parents or guardians of children under 18 years of age.

Consent for publication

Not applicable.

Availability of data and materials

The data for the WASH for Worms trial will be made freely and publicly available at the completion of the trial and analysis of trial outcomes. This is anticipated at the end of 2016.

Competing interests

The authors have declared that no conflicts of interest exist.

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Authors' contributions

SJC and ACAC designed the analysis. SVN was responsible for data collection and entry. SVN and SJC conducted data cleaning and management. SL analysed qPCR specimens. RW sourced and processed environmental variables. SJC conducted data analysis and drafted the manuscript. All authors have contributed suggestions to the analysis and interpretation of findings, and have approved the final manuscript.

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Table 1 Environmental variables selected for analyses

Variable	Source	Temporal resolution	Spatial resolution
Temperature (°C)[*] Mean temperature in coldest quarter (June-August) – *Ascaris* model Temperature range (maximum temperature in the hottest month - minimum temperature in coldest month) – *N. americanus* model	WorldClim†	Monthly average ambient temperature from 1950-2000	1000m
Precipitation (cm)[*] Mean precipitation in wettest quarter (December-February) – *Ascaris* model Precipitation in driest month (September) – *N. americanus* model	WorldClim†	Monthly average precipitation from 1950-2000	1000m
Slope (º)	ASTER on Terra satellite‡	GDEM, 2001	30m
Elevation per 100m[*]	ASTER on Terra satellite‡	GDEM, 2001	30m
Vegetation Average normalised difference vegetation index (NDVI)[*]	MODIS Terra satellite[#]	01/01/2012-31/01/2013	250m
Soil pH[*] pH in 3 categories: Acidic (pH 5.5-6.5), neutral (pH 6.6-7.4), alkaline (pH 7.3-8.4)[§]	O Solos De Timor survey[$]	1960's	N/A
Soil texture[*] Soil texture in binary: sandy-loam soil compared to other soil types (clay, clay-loam, sandy-clay, variable)[¤]	O Solos De Timor survey[$]	1960's	N/A
Landcover Landcover in binary: woody savanna compared to other landcover types (cropland/natural vegetation, evergreen forest, savanna)[¤] – *Ascaris* model Landcover in binary: woody savanna and evergreen forest compared to other landcover types (cropland/natural vegetation, savanna)[¤] – *N. americanus* model	MODIS Terra and Aqua satellites^	2012	500m

Notes: Environmental variables from (19). †WorldClim Version 1.4 (release 3), ‡ASTER Global Digital Elevation Model Version 2, [#]MOD13Q1 Version 5, [$]O Solos De Timor data available from Seeds of Life Timor, ^MCD12Q1 Version 5.1, [¤]Determined for Wash for Worms site (19). [§]Classified according to United States Department of Agriculture classification system (19,31). [*]Variables used a 1km buffer being the median value in 1km radius of household. ASTER, Advanced Spaceborne Thermal Emission and Reflection Radiometer; GDEM, global digital elevation model. Environmental variable selection for *Ascaris* and *N. americanus* models based on Akaike's Information Criterion.

Table 2 Selected baseline characteristics of study participants (*N*=2152)*

Characteristic	n (%)
N. americanus heavy-intensity infection	1,117 (52)
N. americanus moderate- to light-intensity infection	182 (8.5)
Ascaris spp. heavy-intensity infection	217 (10)
Ascaris spp. moderate- to light-intensity infection	311 (15)
Ancylostoma spp. prevalence	102 (4.7)
No STH infection	665 (31)
G. duodenalis prevalence	268 (13)
Male sex	1,038 (48)
Improved household water source	106 (18)
Uses unhygienic toilet	1727 (80)
Uses soap/ash to wash hands	1625 (76)
Always wears shoes when toileting	1361 (63)
Never attended school	451 (44)
Not finished primary school	207 (20)
Completed primary but not secondary school	245 (24)
Completed secondary school or higher	118 (12)
Reported taking anthelmintic in previous 12 months	97 (4.5)
Acidic soil (pH 5.5-6.5)	686 (32)
Neutral soil (pH 6.5-7.3)	986 (46)
Alkaline soil (pH 7.3-8.4)	480 (22)
Sandy-loam soil type	611 (28)
Woody savanna (*Ascaris* model)	661 (31)
Woody savanna and evergreen forest (*N. americanus* model)	739 (34)
	Median (interquartile range)
Mean temperature (°C) in coldest quarter (June-August) (*Ascaris* model)	23 (20.5, 24.7)
Temperature range (°C; maximum temperature in the hottest month - minimum temperature in coldest month) (*N. americanus* model)	11.4 (11.2, 11.5)
Mean precipitation (cm) in wettest quarter (December-February) (*Ascaris* model)	29.1 (23.1, 35.3)
Mean precipitation (cm) in driest month (September) (*N. americanus* model)	1.7 (1.4, 2.0)
Slope (º)	14.1 (3.5, 18.3)
Elevation per 100m*	4.2 (1.3, 7.4)
NDVI (average)	75.1 (70.4, 77.7)

Notes: *Baseline WASH and environmental risk factors for this population have previously been reported (18, 19). Parasitological outcomes determined by PCR, types of household latrines observed by interviewer, remaining WASH data are self-reported. Household water source reported at household (*N*=594) not individual level. Education level asked of adults only (*N*=1090). "Improved" household water source as defined by WHO/ UNICEF Joint Monitoring Programme (JMP) for Water Supply and Sanitation to include piped water into dwelling or yard, public tap or standpipe, tubewell or borehole, protected dug well, protected spring (33). "Unhygienic toilet" defined as any people who did not use a hygienic toilet (this included people who used a mixture of hygienic and non-hygienic toilets; hygienic toilets defined as use of a

house/school/village/neighbour toilet and nothing else). “Always wearing shoes” was contrasted to sometimes/never wearing shoes.

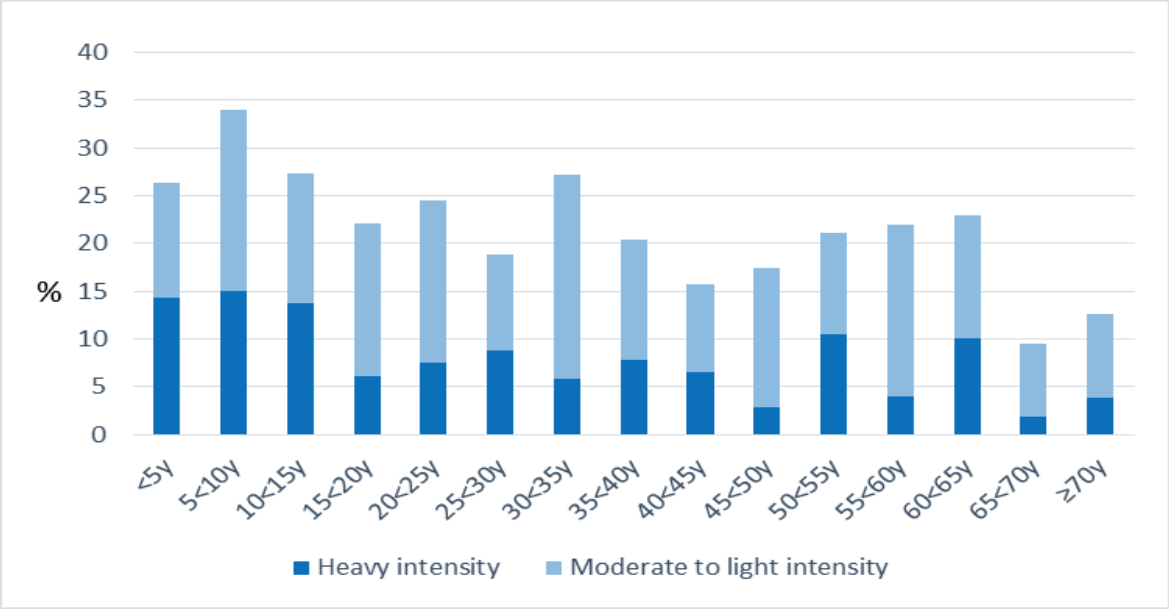

Figure 1 **Intensity of *Ascaris* infection by age group**

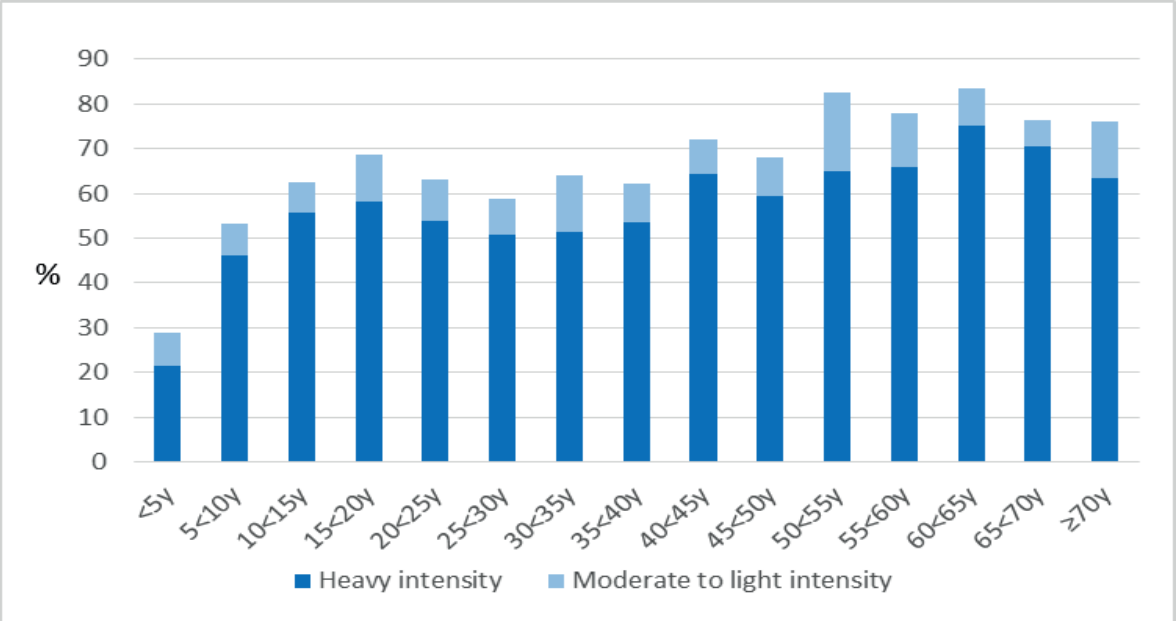

Figure 2 **Intensity of *N. americanus* infection by age group**

Table 3 Relative risk ratios for intensity of *N. americanus* infection, Manufahi District, Timor-Leste

	N. americanus moderate-intensity *N*=191					*N. americanus* heavy-intensity *N*=1155				
Parameter	**RR**	**95% CI**	**ARR**	**95% CI**	***P***	**RR**	**95% CI**	**ARR**	**95% CI**	***P***
Domain: General										
Age in years	**1.0*****	1.0, 1.0	N/A			**1.0*****	1.0, 1.0	N/A		
Age group 6 to 11 years°	**1.5**	0.88, 2.7	1.2	0.55, 2.8	0.621	**4.6*****	3.1, 6.6	3.2	1.8, 5.8	<0.0001
Age group 12 to 17 years°	**2.2****	1.1, 4.2	1.6	0.63, 4.2	0.311	**6.8*****	4.3, 10.6	4.8	2.5, 9.3	<0.0001
Age group 18-64 years°	**3.3*****	2.1, 5.3	1.6	0.80, 3.3	0.174	**8.1*****	5.7, 11.4	4.7	2.8, 7.9	<0.0001
Age group 65+ years°	**4.8*****	2.3, 10.0	4.4	1.6, 11.9	0.004	**15.9*****	9.3, 27.0	9.6	4.4, 20.7	<0.0001
Male sex: age group 6 to 11 years			1.1	0.34, 3.5	0.891			2.0	0.92, 4.4	0.081
Male sex: age group 12 to 17 years			1.6	0.41, 6.0	0.517			2.3	0.89, 5.7	0.087
Male sex: age group 18-64 years			3.3	1.3, 8.7	0.015			3.6	1.8, 7.3	<0.0001
Male sex: age group 65+ years			0.53	0.11, 2.5	0.419			1.7	0.60, 4.8	0.318
Male sex‡	**1.5****	1.1, 2.1	0.95	0.43, 2.1	0.901	**2.7*****	2.1, 3.3	1.3	0.73, 2.5	0.351
Domain: Individual hygiene										
Sometimes/never wears shoes inside house	**0.74**	0.52, 1.1				**0.76****	0.59, 0.98			
Sometimes/never wears shoes outside house	0.86	0.60, 1.2				**0.69*****	0.53, 0.88			
Sometimes/never wears shoes when toileting	**0.70**	0.48, 1.0				**0.64*****	0.49, 0.83			
Domain: Individual sanitation										
Uses unhygienic toilet	0.90	0.57, 1.4				1.0	0.70, 1.4			
Household has toilet	1.0	0.67, 1.6				0.85	0.60, 1.2			
Cleans self with water and hand only after toileting	**0.66**	0.41, 1.1				**0.42*****	0.29, 0.60			
Cleans self by other method after toileting	**0.70**	0.45, 1.1				**0.61*****	0.44, 0.84			
Domain: Household sanitation										
Household toilet: Pit latrine without slab	0.77	0.35, 1.7				**1.7**	0.89, 3.1			
Household toilet: Other toilet type	0.87	0.13, 5.9				0.76	0.13, 4.4			
No household toilet/no answer	0.84#	0.47, 1.5				1.5#	0.92, 2.6			
Toilet observed to be dirty	0.77	0.34, 1.7				**1.8****	0.94, 3.5			
Household rubbish disposed of by burning only	0.99	0.64, 1.5				1.0	0.72, 1.5			
Household rubbish disposed of by other method	1.0	0.64, 1.6				0.93	0.65, 1.3			
Domain: Household water supply										
Main water supply: piped water to dwelling	#		#			#		#		
Main water supply: piped water to yard	**2.1**	0.83, 5.2	1.6	0.58, 4.3	0.373	1.0	0.46, 2.3	1.6	0.53, 2.7	0.718
Main water supply: piped water shared	0.18#	0.02, 1.6	0.14#	0.02, 1.3	0.083	0.49	0.16, 1.5	0.32	0.12, 0.84	0.021
Main water supply: tubewell/borehole	0.48#	0.13, 1.8	1.4#	0.42, 4.6	0.583	**0.29****	0.11, 0.81	0.72	0.34, 1.5	0.382
Main water supply: unprotected dug well	0.97#	0.11, 8.9	1.3#	0.13, 13.2	0.822	0.44#	0.07, 2.7	0.71#	0.10, 5.1	0.737
Main water supply: protected spring	0.68#	0.13, 3.6	0.61#	0.11, 3.5	0.583	1.2	0.42, 3.7	0.99	0.33, 3.0	0.982
Main water supply: surface water	**1.7**	0.99, 2.7	1.9	1.1, 3.2	0.020	**1.2**	0.79, 1.8	1.3	0.85, 1.9	0.264
Main water supply: in household compound	0.91	0.50, 1.6				0.78	0.47, 1.3			
Distance to main water supply: 15 min or more	1.1	0.75, 1.7				**1.3**	0.93, 1.8			
Main water supply not running at least 1 week/month	0.78#	0.34, 1.8				0.98	0.53, 1.8			
Main water supply not running at least 1 month/year	0.77	0.46, 1.3				**0.60****	0.39, 0.93			
Water stored in covered container only	1.2	0.78, 1.9				**1.4**	1.0, 2.0			
Household water is boiled	**0.60****	0.40, 0.90	0.52	0.34, 0.80	0.003	0.88	0.63, 1.2	0.73	0.52, 1.0	0.057

Domain: Household hygiene										
Household has a food garden	0.62	0.34, 1.2				1.3	0.81, 1.9			
Human faeces used on food garden	2.6	0.80, 8.3				1.8	0.61, 5.2			
Domain: Household socioeconomic										
1 person aged <5 years in household	**0.68****	0.45, 1.0	0.81	0.52, 1.3	0.338	**0.39*****	0.28, 0.53	0.57	0.40, 0.82	0.002
2 or more people aged <5 years in household	**0.43*****	0.27, 0.67	0.57	0.34, 0.94	0.028	**0.39*****	0.28, 0.55	0.78	0.53, 1.2	0.221
1 person aged >65 years in household	1.3	0.63, 2.5				1.0	0.58, 1.9			
2 or more people aged >65 years in household	**1.9****	1.1, 3.5				**1.7****	1.1, 2.9			
Socioeconomic quintile 4	1.9**	1.1, 3.4	1.4	0.75, 2.5	0.2340	1.8**	1.2, 2.9	1.8	1.2, 2.9	0.0213
Socioeconomic quintile 3	1.5	0.87, 2.7	1.2	0.64, 2.2		1.5	0.97, 2.4	1.8	1.1, 2.9	
Socioeconomic quintile 2	1.3	0.70, 2.2	1.7	0.91, 3.0		1.8**	1.1, 2.8	1.7	1.1, 2.8	
Socioeconomic quintile 1 (poorest)	1.3	0.70, 2.2	2.0	1.1, 3.7		1.6**	1.0, 2.5	2.2	1.3, 3.6	
Domain: Village										
Village toilet type: Pit latrine without slab	0.43#	0.06, 3.0				0.93	0.17, 5.2			
Village toilet type: Pit latrine with slab	0.23#	0.03, 2.2				1.1	0.18, 6.0			
Village rubbish disposed of by burning only	0.55	0.10, 3.1				0.66	0.13, 3.4			
Village rubbish disposed of by other method	0.66	0.31, 1.4				0.80	0.39, 1.6			
Domain: qPCR										
Ascaris spp. infection	1.2	0.81, 1.9				1.2	0.86, 1.6			
Ancylostoma spp. infection	1.4#	0.50, 4.0	1.0#	0.32, 3.2	0.989	**4.0*****	2.2, 7.5	4.1	2.1, 8.0	<0.0001
G. duodenalis infection	**0.61****	0.37, 1.0	0.86	0.50, 1.5	0.585	**0.44*****	0.31, 0.61	0.71	0.49, 1.0	0.076
Domain: Individual recent history										
Deworming treatment taken within last 12 months	0.74#	0.33, 1.7				**0.43*****	0.23, 0.81			
3 or more bowel motions in last 24 hours	0.74#	0.28, 2.0	0.82#	0.30, 2.4	0.711	**0.32****	0.15, 0.70	0.40	0.17, 0.96	0.041
Loose stools during last 24 hours	0.94#	0.36, 2.5				**0.35*****	0.16, 0.76			
Diarrhoea during last 2 weeks	0.90	0.48, 1.7				0.89	0.58, 1.4			
Access to anthelmintics	**0.37****	0.17, 0.80				**0.35*****	0.20, 0.60			
Domain: Environmental										
Sandy-loam soil	**2.0****	1.1, 3.7	2.1	1.0, 4.3	0.038	**2.4*****	1.4, 4.0	2.7	1.6, 4.5	<0.0001
Woody savanna & evergreen forest landcover	**2.1**	0.95, 4.4				**2.3****	1.2, 4.4			
Temperature (°C)	**0.41**	0.13, 1.4	N/A			**0.47**	0.16, 1.4	N/A		
Precipitation (cm)	**5.5*****	2.4, 12.8	6.1	1.9, 19.3	0.002	**5.6*****	3.2, 9.9	6.6	3.1, 14.1	<0.0001
Elevation (m)	**1.1**	0.98, 1.2	0.94	0.83, 1.1	0.268	**1.1**	0.98, 1.2	0.90	0.83, 0.97	0.007
Slope (°)	**1.1*****	1.0, 1.1				**1.1*****	1.0, 1.1			
NDVI (average per 0.01)	**1.1*****	1.0, 1.2	1.1	0.98, 1.7	0.146	**1.2*****	1.1, 1.2	1.1	1.0, 1.1	0.023

Notes: No *N. americanus* infection is reference category, i.e., moderate- and heavy-intensity infection need to be interpreted relative to this reference. *N. americanus* intensity infection defined according to following PCR cycle threshold (Ct) cut-points: heavy-intensity Ct≤24.6, moderate-intensity Ct>24.6<35, no infection Ct≥35 (23). RR, relative risk; ARR, adjusted relative risk; CI, confidence interval; *P*, Wald test. *** $P<0.01$, ** $P<0.05$ in univariable analysis, # less than 10 observations in subgroup; result should be interpreted cautiously. RRs in bold had univariable $P<0.2$ and were entered in multivariable regression models; for correct interpretation of this table, if a variable was significant for moderate-intensity but not heavy-intensity, it was still included, therefore on occasion moderate-intensity adjusted RRs are significant when heavy-intensity adjusted RRs are not, and *vice versa*. Multivariable analysis did not include temperature due to collinearity with elevation; this is indicated as N/A=not applicable. Age (categorical), sex and socioeconomic quintile were included in all multivariable regression models as

covariates. Age (continuous) is indicated as NA=not applicable for multivariable models. Water supply variables follow JMP definitions (33), with the exception of "piped water" which was grouped due to low observation numbers. Definitions: "Used unhygienic toilet" any people who did not use a hygienic toilet (this included people who used a mixture of hygienic and non-hygienic toilets; hygienic toilets defined as use of a house/school/village/neighbour toilet and nothing else). "Other household toilet type" indicates hanging latrines (low observation numbers). "Household rubbish disposed of by other method" includes disposing it into a bin, a river, burying it or composting it. "Village rubbish disposed of by other method" includes burying it or disposing of it in the river. Reference categories: **General domain:** lowest age group in the stratum (age 1-5 years); female sex; female sex and age group 1 to 5 years. **Individual hygiene domain:** always wears shoes inside house; always wears shoes outside house; always wears shoes when toileting. **Individual sanitation domain:** uses hygienic toilet only; household has no toilet; cleans self with leaves only after toileting. **Household sanitation domain:** household toilet being a pit latrine with slab; household toilet observed (by interviewer) to be clean; household rubbish disposed of in bush only. **Household water supply domain:** main water supply being an unprotected spring; main water supply located separate from household compound; distance to main water supply less than 15 minutes; main water supply always running; household water stored in an uncovered container; household water is not boiled. **Household hygiene domain:** household does not own food garden; human faeces not used on food garden. **Household socioeconomic domain:** no people aged <5 years in household/no answer; no people aged ≥65 years in household/no answer; socioeconomic quintile 5 (wealthiest). **Village domain:** no village toilet; village rubbish disposed of in bushes only. **qPCR domain:** no *Ascaris* infection; no *Ancylostoma* infection; no *G. duodenalis* infection. **Individual recent history domain:** no deworming treatment taken within last 12 months; less than 3 bowel motions in last 24 hours; normal stools during last 24 hours; no diarrhoea in last two weeks; no access to anthelmintics. **Environmental domain:** acidic soil pH; other soil types (clay, clay-loam, sandy-clay, variable); other landcover types (cropland/natural vegetation, savanna).

Interpretation notes: Because this table has an interaction term, parameterisation and correct interpretation in the adjusted model are as follows: º the age group main effect is the stated age group (e.g. six to 11 years) relative to age group one to five years in females because females are the reference group (i.e. demonstrating the relationship between age group and *N. americanus* infection intensity in females). Similarly, ǂ the male sex main effect is being male relative to being female in age group one to five years (reference group). Males in age groups six to 11 years and older is relative to males in age group one to five years (because males and older age groups are not the reference groups).

Table 4 Relative risk ratios for intensity of *Ascaris* spp. infection, Manufahi District, Timor-Leste

	Ascaris moderate-intensity *N*=310					*Ascaris* heavy-intensity *N*=220				
Parameter	**RR**	**95% CI**	**ARR**	**95% CI**	***P***	**RR**	**95% CI**	**ARR**	**95% CI**	***P***
Domain: General										
Age in years	**0.99*****	0.98, 1.0	N/A			**0.97*****	0.96, 0.98	N/A		
Age group 6 to 11 years	**1.9*****	1.2, 3.0	1.9	1.2, 3.1	0.005	**2.3*****	1.3, 4.1	2.4	1.3, 4.3	0.004
Age group 12 to 17 years	1.1	0.66, 2.0	1.1	0.63, 1.9	0.739	0.97	0.46, 2.0	0.91	0.43, 1.9	0.807
Age group 18-64 years	0.98	0.65, 1.5	0.95	0.62, 1.4	0.795	**0.58****	0.33, 1.0	0.56	0.32, 0.98	0.038
Age group 65+ years	**0.52**	0.26, 1.0	0.52	0.26, 1.0	0.059	0.19***#	0.06, 0.63	0.20	0.06, 0.66	0.008
Male sex	1.1	0.83, 1.5	1.1	0.83, 1.5	0.478	0.97	0.66, 1.4	0.88	0.59, 1.3	0.537
Domain: Individual hygiene										
Washes hands after defecation and at other times	0.83	0.54, 1.3				**0.46****	0.25, 0.84			
Washes hands at other times only (but not after defecation)	0.77	0.46, 1.3				**0.51**	0.24, 1.1			
Sometimes/never wears shoes outside house	0.95	0.70, 1.3				**1.7****	1.1, 2.6			
Sometimes/never wears shoes when toileting	**0.82**	0.59, 1.1				1.1	0.68, 1.7			
Domain: Individual sanitation										
Used unhygienic toilet	1.3	0.79, 2.0				**2.3**	0.91, 4.6			
Household has toilet	0.82	0.54, 1.2				**0.57**	0.27, 1.2			
Cleans self with water and hand only after toileting	0.85	0.53, 1.3				0.88	0.44, 1.8			
Cleans self by other method after toileting	0.92	0.62, 1.4				0.95	0.50, 1.8			
Village has public toilet	0.75	0.34, 1.7				0.19#	0.03, 1.1			
Domain: Household sanitation										
Household toilet: Pit latrine without slab	1.4	0.64, 2.9				0.72#	0.19, 2.8			
Household toilet: Other toilet type	0.54#	0.09, 3.2				0.39#	0.02, 6.9			
No household toilet/no answer	1.3	0.73, 2.4				1.5	0.56, 3.9			
Toilet observed to be clean	**0.44**	0.17, 1.1				1.0	0.30, 3.4			
Household rubbish disposed of by burning only	0.82	0.55, 1.3				1.2	0.61, 2.3			
Household rubbish disposed of by other method	0.78	0.52, 1.2				**0.38****	0.18, 0.77			
Domain: Household water supply										
Main water supply: piped water (to any point)	**1.8**	0.83, 3.8				1.4	0.40, 4.9			
Main water supply: tubewell/borehole	0.15#	0.01, 3.1				#				
Main water supply: protected spring	1.3#	0.37, 4.3				#				
Main water supply: surface water	1.4	0.83, 2.3				0.98	0.40, 2.4			
Main water supply located in household compound	**1.5**	0.85, 2.6				0.94	0.35, 2.5			
Main water supply not running at least 1 week/month	0.64	0.31, 1.3				0.73	0.21, 2.5			
Main water supply not running at least 1 month/year	0.79	0.48, 1.3				0.78	0.34, 1.8			
Household water is stored	0.61	0.18, 2.0				0.73	0.12, 4.6			
Secondary water source used	0.82	0.55, 1.2				0.70	0.36, 1.4			
Secondary water source is “improved”	0.86	0.30, 2.4				0.72#	0.14, 3.7			
Household water is boiled	0.87	0.58, 1.3				0.93	0.46, 1.9			
Domain: Household hygiene										
Household has a food garden	0.73	0.43, 1.2				0.76	0.31, 1.8			
Human faeces used on food garden	1.2	0.39, 3.4				1.7	0.28, 9.7			
Domain: Household socioeconomic										

1 person aged <5 years in household	**1.5****	1.0, 2.2				**1.5**	0.82, 2.9			
2 or more people aged <5 years in household	1.1	0.71, 1.6				1.5	0.76, 2.8			
1 person aged 5-17 years in household	0.82	0.48, 1.4				**0.37****	0.14, 0.95			
2 or more people aged 5-17 years in household	**1.4**	0.92, 2.1				1.6	0.84, 3.1			
1 person aged 65+ years in household	0.67	0.32, 1.4				0.49#	0.13, 1.8			
2 or more people aged 65+ years in household	0.85	0.48, 1.5				0.65	0.25, 1.7			
Socioeconomic quintile 4	0.76	0.43, 1.3	0.67	0.38, 1.2	0.6461	1.2	0.48, 3.2	0.76	0.26, 2.2	0.7453
Socioeconomic quintile 3	0.81	0.48, 1.4	0.96	0.56, 1.6		0.96	0.38, 2.5	1.0	0.38, 2.7	
Socioeconomic quintile 2	0.84	0.50, 1.4	0.91	0.54, 1.5		0.92	0.35, 2.4	1.2	0.45, 3.1	
Socioeconomic quintile 1 (poorest)	0.59	0.33, 1.1	0.82	0.48, 1.4		0.66	0.23, 1.9	1.5	0.58, 4.0	
Domain: Village										
Village rubbish disposed of by burning only	0.10#	<0.01, 2.6				0.05#	<0.00, 42.7			
Village rubbish disposed of by other method	0.42	0.11, 1.6				0.35	0.02, 5.7			
Domain: qPCR										
N. americanus infection	1.0	0.76, 1.4				**1.4**	0.88, 2.2			
Ancylostoma spp. infection	1.1	0.58, 2.2				**2.8****	1.3, 6.3			
G. duodenalis infection	1.2	0.77, 1.8				1.4	0.77, 2.4			
Domain: Environmental										
Soil pH: Alkaline	**0.09*****	0.03, 0.31	0.21	0.09, 0.51	0.001	0.01***#	<0.01, 0.09	0.04#	0.01, 0.25	0.001
Soil pH: Neutral	**0.54*****	0.30, 0.96	0.55	0.33, 0.91	0.019	**0.34****	0.12, 0.92	0.35	0.14, 0.84	0.019
Sandy-loam soil	**0.60**	0.33, 1.1				**0.24*****	0.09, 0.64			
Woody savanna landcover	**0.44****	0.23, 0.81				**0.28****	0.09, 0.85			
Slope (°)	**1.2*****	1.1, 1.3				**1.4*****	1.2, 1.5			
Temperature (°C)	**0.61*****	0.52, 0.71	N/A			**0.42*****	0.31, 0.57	N/A		
Precipitation (cm)	**1.3*****	1.2 1.4				**1.5*****	1.3, 1.7			
Elevation (m)	**1.4*****	1.3, 1.6	1.3	1.2, 1.4	<0.0001	**1.8*****	1.5, 2.2	1.4	1.2, 1.7	<0.0001
NDVI (average per 0.01)	**1.1****	1.0, 1.3	1.1	0.97, 1.1	0.201	**1.4*****	1.1, 1.7	1.2	1.1, 1.4	0.028

Notes: No *Ascaris* infection is reference category, i.e., moderate- and heavy-intensity infection need to be interpreted back to this reference. *Ascaris* intensity of infection defined according to following PCR cycle threshold (Ct) cut-points: heavy-intensity Ct≤15.4, moderate-intensity Ct>15.4<31, no infection Ct≥31 (23). RR, relative risk; ARR, adjusted relative risk; CI, confidence interval; *P*, Wald test. *** $P<0.01$, ** $P<0.05$ in univariable analysis, # less than 10 observations in subgroup; result should be interpreted cautiously. RRs in bold had univariable $P<0.2$ and were entered in multivariable regression models; for correct interpretation of this table, if a variable was significant for moderate-intensity but not heavy-intensity, it was still included, therefore on occasion moderate-intensity adjusted RRs are significant when heavy-intensity adjusted RRs are not, and *vice versa*. Multivariable analysis did not include temperature due to collinearity with elevation; this is indicated as N/A=not applicable. Age (categorical), sex and socioeconomic quintile were included in all multivariable regression models as covariates. Age (continuous) is indicated as NA=not applicable for multivariable models. No interaction term was required in *Ascaris* models. Water supply variables follow JMP definitions (33), with the exception of "piped water" which was grouped due to low observation numbers. Definitions: "Used unhygienic toilet" any people who did not use a hygienic toilet (this included people who used a mixture of hygienic and non-hygienic toilets; hygienic toilets defined as use of a house/school/village/neighbour toilet and nothing else). "Other household toilet type" indicates hanging latrines (low observation numbers). "Household rubbish disposed of by other method" includes disposing it into a bin, a river, burying it or composting it. "Village rubbish disposed of by other method" includes burying it or disposing of it in the river. Reference

categories: **General domain:** lowest age group in the stratum (age 1-5 years); female sex. **Individual hygiene domain:** uses soap/ash to wash hands; washes hands after defecation only; always wears shoes outside house; always wears shoes when toileting. **Individual sanitation domain:** uses hygienic toilet only; household has no toilet; cleans self with leaves only after toileting; village has no public toilet. **Household sanitation domain:** household toilet being a pit latrine with slab; household toilet observed (by interviewer) to be dirty; household rubbish disposed of in bush only. **Household water supply domain:** main water supply being an unprotected spring; main water supply located separate from household compound; main water supply always running; household water not stored; no secondary water source used; secondary water supply used was unimproved (according to JMP definitions (33)) or no answer provided; household water is not boiled. **Household hygiene domain:** household does not own food garden; human faeces not used on food garden. **Household socioeconomic domain:** no people aged <5 years in household/no answer; no people aged 5-17 years in household/no answer; no people aged ≥65 years in household/no answer; socioeconomic quintile 5 (wealthiest). **Village domain:** village rubbish disposed of in bushes only. **qPCR domain:** no *N. americanus* infection; no *Ancylostoma* infection; no *G. duodenalis* infection. **Environmental domain:** acidic soil pH; other soil types (clay, clay-loam, sandy-clay, variable); other landcover types (cropland/natural vegetation, evergreen forest, savanna).

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Figure legends

Figure 1 **Intensity of *Ascaris* infection by age group**

Figure 2 **Intensity of *N. americanus* infection by age group**

Chapter 7 Discussion and Conclusions

In this thesis the results of two reviews that demonstrate the major global importance of STH and strategies for their control, and three quantitative epidemiological analyses of STH in Timor-Leste, have been presented. The two reviews addressed objectives 1 and 2 of the thesis and the detailed epidemiological investigations addressed objectives 3-5. Finally, in this chapter, recommendations for STH control in Timor-Leste, and for the STH research agenda, are made, to meet the sixth and final objective of the thesis.

The findings and implications in this chapter progress from national to international level, to illustrate research priorities and bring them to a cohesive point. Therefore, the epidemiological analyses undertaken in Timor-Leste are discussed before the narrative reviews, which is the reverse order to presentation in the body of the thesis. Conclusions are made throughout the chapter and presented as a summary at the end.

7.1 Findings and implications of epidemiological analyses for Timor-Leste

In the first epidemiological analysis (Chapter 4), the prevalence of STH and risk factors associated with WASH in Manufahi District, Timor-Leste, were investigated. It was found that in this district there was a very high prevalence of *N. americanus* and *Ascaris* in particular, with lower levels of other STH and *G. duodenalis*. These infections were strongly associated with age, sex and (for *N. americanus*) socioeconomic status, none of

which are readily modifiable. Less evidence was found for WASH infrastructure and behavioural associations with STH. This may be due to low existing levels of measurable WASH factors in this pre-intervention setting. These results also could have been affected by a key judgement call, to age-stratify analyses into classes of preschool-aged, school-aged and adult age groups. In so doing, there was adequate power to detect only moderate associations. However, statistical models were generated for both *N. americanus* and *Ascaris* (results not reported) without any age stratification, for sensitivity analyses. These models had comparable significant WASH risk factors to the age-stratified models. Different STH age-prevalence profiles indicate that key important differences in risk factors were likely to be evident across different age groups, therefore the decision to age-stratify is justified. Additionally, certain risk factors could only be collected for population sub-groups, such as school attendance for school-aged children and employment type for adults. Age-stratifying enabled these risk factors to be investigated. Analysing baseline data from an RCT, which was designed and powered to detect different primary outcomes, could also have affected power to detect WASH associations in this cross-sectional analysis.

Regardless of lack of evidence for many WASH associations with STH at baseline, the first analysis has provided detailed STH prevalence information for a rural district of Timor-Leste, and identified inadequate WASH infrastructure and hygiene behaviour, which will directly inform the development and targeting of STH control programmes in this area. Of interest, as mentioned in Chapter 4, is that the very low level of *T. trichiura* in this setting means that there is a potential opportunity for single-dose albendazole to lead to

considerable rapid reduction of STH in this community. Although imperfectly, *G. duodenalis* is also affected by albendazole. Therefore, an STH control strategy could additionally have a positive impact on reducing the predominant intestinal protozoon identified in this community. Important recommendations for Timor-Leste from this first analysis are therefore to:

(i) Implement STH control programmes across Manufahi District as a priority, given the high STH prevalence and rural livelihoods of the villagers. This may require international liaison, for example with the WHO, to secure resources for control programmes. The programmes should include clear monitoring and evaluation criteria, including treatment coverage and regular analysis of prevalence and intensity data, to monitor impact of the control programme, particularly to assess the effectiveness of single-dose albendazole in these communities.

(ii) Undertake implementation research and planning to increase WASH infrastructure in this District; this is broader than STH control given the associations between WASH and improved socioeconomic development. As a priority component of this, planning should be informed by investigations (still under way as Aim 3 of the WASH for Worms trial) to understand barriers and enablers associated with acceptability and uptake of the WASH programme in Manufahi District (237). Implementation planning needs to ensure intersectoral collaboration, especially between District and national levels, and across health, education and non-government sectors, so as to achieve more effective and integrated STH control.

In the second analysis of STH associations with morbidity (Chapter 5), several significant findings emerged. That there was no major link between STH infection and the high level of morbidity that was prevalent in this study community was unexpected. This is, itself, very important to highlight. STH are subtle, confounders are difficult to measure, and to assume that clear morbidity associations can be found in all prevalent areas would be unlikely. There are numerous studies previously undertaken in which the link between morbidity and infection has not been demonstrated (reviewed in Chapter 2). Sometimes reporting a 'lack of association' is much harder than reporting a clear result but it is important to communicate this, as bias can be introduced if only positive findings are reported. It is highly likely that the low *Ancylostoma* spp. prevalence, coupled with low underlying anaemia (as the most direct morbidity association), is a large part of the reason for this lack of association.

The prevalence of stunting, wasting, and being underweight in children was among the highest rates of stunting or wasting reported anywhere in the world (252), and this requires urgent consideration. The impact of such high morbidity in children is likely to contribute to an entire generation of disadvantaged people, if strategies to address this cannot be developed (253). That we have been able to demonstrate the lack of strong association between STH and morbidity still contributes an important component of knowledge that highlights the importance of nutrition; this will aid in development and/or refinement of nutritional strategies to address these morbidities. Given the high prevalence of morbidity, a major conclusion of this thesis is that additional nutritional assessments be undertaken in

this community, with strategies developed to provide essential micro- and macro-nutrients to reduce these deficits.

However a cautionary note needs to be added, which is that, despite extensive effort in developing the 2006 WHO international reference standards for child growth (254), the application of these thresholds to the Timorese population has not been assessed. Asian people from many countries are of smaller stature than other populations and 'averaging' to an international reference point, whilst considerably more accurate than referring to a Western population, may still be overstating morbidity rates. Further investigations into thresholds are required, however it is accepted that the WHO 2006 international reference standards are currently the most accurate reference point. Recommendations for Timor-Leste arising from the second analysis are therefore to:

(i) Undertake, as a priority, investigations into the nutrition that children are receiving in these communities, and in particular to determine whether existing strategies, such as provision of school nutritional programmes, are reaching the most vulnerable children.

(ii) Advocate for the WHO to assess the applicability of the 2006 WHO international reference standards to Timorese children.

In the third analysis (Chapter 6) a detailed risk factor investigation of WASH and environmental variables associated with intensity of STH infection in Manufahi District was conducted. This is an interesting picture to complete, specifically to investigate the addition of environmental variables on measures of more modifiable risk such as WASH. With a multinomial intensity of infection outcome, it is believed that this is the first

investigation of its kind reported. Environmental variables were found to be associated with both *N. americanus* and *Ascaris* intensity of infection, with, generally, few WASH associations emerging. This contributes important knowledge for Timor-Leste, as the environment provides favourable conditions for ongoing STH development and transmission in soil. Therefore, although chemotherapy could rapidly reduce STH burden and as stated previously potentially only single-dose treatments may be required, ongoing transmission is almost assured unless WASH is integrated into any STH control strategy. The recommendation for Timor-Leste arising from this final analysis is therefore to secure long-term commitment to STH control, again if possible from the WHO, but also from the Ministries of Health and Education. The necessity of applying a long-term lens to control programming is evidenced by the favourable environmental conditions for STH transmission in Manufahi District, and the longevity of STH egg survival in the environment. For this reason, ideally, STH control should be applied to the full community, not delivered solely through school-based programmes as tends to be most standard in developing countries.

The findings from all of these analyses have specific importance for planning STH control and morbidity reduction strategies in Timor-Leste. Key results and recommendations from these analyses will be distributed to programme managers in the Timor-Leste Ministry of Health, the National Laboratories, the WHO Country Office, and other identified personnel who will require this information to inform policy and programmatic activities relevant for Manufahi District, and Timor-Leste more broadly. For the Ministry of Health and WHO personnel in particular, this information will provide very useful context for mass drug

administration, which commenced in a selected number of districts in late 2015. Additionally, the need to investigate nutritional strategies will be highlighted.

In addition to the applicability of these findings to Timor-Leste, there are specific results from these analyses which contribute to the broader epidemiological and parasitological research agendas. These are discussed in the following section.

7.2 Implications of the epidemiological analyses beyond Timor-Leste: growing the research agenda

Strengthening WASH for STH epidemiology

At present most evidence for the health impact of WASH is observational, often uses self-reporting for measurement, and suffers from a lack of clear guidelines in terms of the best measures to use in epidemiological studies. These key factors contribute directly to the evidence shortfall for demonstrating WASH impacts on STH, which, as reviewed in Chapter 3, would likely reduce international investment. The health impact of WASH activities are still often measured in terms of health outcomes such as diarrhoea, which is an understandable position for the WASH sector, which does not invest in controlling one single aetiological agent of disease. However, for STH, and other NTDs, this means that opportunities to elicit evidence are missed. For STH, WASH questionnaires need to cover the specific features related to STH exposure and biology. As STH life cycles are likely to be restricted only by human habitation, behaviour, and environmental factors influencing

egg and larval survival (66), particular consideration needs to be given to the interplay between direct STH life cycles and aspects of WASH that could reduce this, such as hygienic behaviour that reduces soil contact.

More epidemiological research on the impact of WASH on STH needs to be conducted. Over time, and with knowledge sharing, this will enable refinement of epidemiological questionnaires so that targeted characteristics can be investigated, rather than attempting to cover the quantity of factors investigated in Chapter 4. However, this analysis has been an important preliminary step to tease out aspects of WASH that are more or less readily measurable and provide meaningful information in developing country contexts for which there is no known baseline position. Another main conclusion of this thesis, which has been strongly raised elsewhere (255,256), is the urgent requirement for development of guidelines for measuring WASH in epidemiological research, including research on STH. Such guidelines need to be informed by research into the best measures to use, and as such an iterative process of guideline development will be required. Further, given generally insufficient epidemiological investigation of WASH, and the issues raised with regard to statistical power of studies in the previous section, it is also concluded that WASH risk factor investigations need to be adequately powered to detect meaningful WASH effects.

Further to the above, WASH characteristics have a potential for multiple overlapping relationships. For example, hygiene requires a degree of water and/or sanitation infrastructure and *vice versa* (Chapter 3). Therefore, as raised elsewhere, an important research agenda is to determine the best statistical approach to identify risk factors from an

interrelated suite of WASH predictors for STH prevalence modelling (257). Analyses could include statistical methods such as recursive partitioning (257), and random forest analysis, and, in future research, will be compared to results from the mixed-effects logistic regression analysis completed in Chapter 4.

Finally, comprehensive WASH measurement guidelines must include indicators that will require mixed-methods approaches, as highlighted in the review on STH control strategies (Chapter 3), so as to more thoroughly investigate amenable behavioural patterns that could reduce STH exposures. Observational research, including mixed-methods approaches, will not be able to demonstrate a causal link between WASH and STH. RCTs are essential, but due to their complexity and expense will be rare, and for many risk factors or exposure not ethically or logistically possible. There needs to be consideration by key international policy-makers of inclusion of appropriate observational research in decision-making processes.

Intensity of STH infection diagnostics

The second quantitative analysis makes an important research contribution to the application of PCR for STH diagnosis. There are major drawbacks with microscopy-based methods of STH diagnosis in human stool. Furthermore, given the greater accuracy of using intensity of infection for assessment of STH compared to prevalence, the application of highly sensitive and specific PCR to STH diagnosis needs to be extended beyond use for prevalence assessments only. This is extremely important, to maximise the potential of this

contemporary technology. Chapter 5 has provided the first attempt to assign classes of infection intensity to PCR data for use in epidemiological analyses. At this stage, this is hypothesis-generating, however in the third analysis, evidence of a scale of intensity impact was seen for some risk factors, as an indication of the potential usefulness of this approach.

A particularly interesting and unexpected finding is our observation of lack of morbidity associated with *N. americanus* in this endemic setting, facilitated by the identification of hookworm genera by qPCR. An important research priority which should be further investigated is to determine whether *N. americanus* infection (both prevalence and intensity of infection) contribute to morbidity, or whether the burden of disease attributed to hookworms stems predominantly from *Ancylostoma*. Implications of this, in terms of STH control programmes, should also be explored, for example the possibility of less frequent dosing in areas of low *A. duodenale* endemicity.

The statistical application of cut-points to Ct-values adds to knowledge of STH transmission and disease burden. Although there was no evidence of a relationship between morbidity and STH in our population to enable decisions to be made via the application of receiver-operating characteristic curves, the statistical method should receive attention in further consideration of PCR categorisation of infection intensity.

Contemporary STH epidemiology

An extremely useful analysis which should be conducted in future research is the investigation of whether using prevalence of infection alone could mask significant intensity-related associations with WASH factors, and whether this could mean that key evidence for WASH benefits may be being overlooked in epidemiological studies that rely on prevalence of infection as the outcome. With both prevalence and infection intensity variables developed, this analysis could be undertaken for the "WASH for Worms" study population. However, ideally, this should also be done in populations where stronger WASH associations have been identified (likely to be areas with more heterogeneity in levels of WASH). This could provide important information as to the most appropriate method to assess WASH, so as to generate evidence of benefits raised previously in this chapter. To date, few investigations (255) have analysed WASH risk factors on both STH prevalence and STH infection intensity in the same population, and the possibility of underreporting of WASH significance based on prevalence has been inadequately explored.

The analysis presented in Chapter 6 brings together many common elements of the thesis: it measures STH infection intensity as a better measure of transmission and morbidity in epidemiological analyses, it combines environmental and WASH risk factors in adjusted models, it addresses issues of poverty, multicollinearity, and helminth clustering, all of which are known issues affecting spatial models of STH (66). This has required the use of contemporary biostatistical models and contributes to addressing identified gaps in the epidemiological research agenda. Finally, and crucially, this analysis indicates the likely

benefits of integrating STH control with WASH, even in situations where WASH characteristics themselves may not present as risk factors in adjusted analyses. WASH is the only exposure-reduction pathway that could potentially negate the impact of environmental conditions that favour STH survival. This situation raises the importance of accurate measurement of WASH, again a theme that has been consistently raised throughout the thesis. As well as promoting an integrated STH control agenda, this further raises an important issue which will be discussed in the last section of this chapter about using evidence to inform policy.

The PhD Candidate has additionally aimed to contribute to STH epidemiology by investigating the global STH context, as has been undertaken with the two narrative reviews. Key findings and implications of these reviews are presented below.

7.3 Findings and implications of narrative reviews

The need for an evidence debate: STH morbidity

The vast majority of STH burden is due to morbidity, not mortality. This creates an abundance of difficult issues for attributing disease burden, and requires epidemiologists to consistently refine evidence, and assess the strength of such, in burden investigations. Recent GBD estimates of STH are a pertinent example of this. Extensive effort was applied to develop advanced analytical models that use the best available evidence (1), to come up with these estimates (258). Yet the results for STH were lower than previous estimates for

several reasons. First, the improved availability and modelling of prevalence and morbidity data; second, reduced prevalence resulting from STH control programmes; and third, because evidence was insufficient to justify including some STH-associated morbidities into GBD calculations. From a quantitative epidemiological position, this approach is more than justifiable: it is a fundamental requirement for veracity. Despite the debate on GBD estimates, the reality is that major gaps remain in underlying evidence that limit estimation of total STH morbidity. This lack of evidence undoubtedly leads to an underestimated STH disease burden. Given the importance of GBD for health priority setting, strengthening morbidity evidence is of paramount importance and will require ongoing research contributions.

In the critical appraisal of STH morbidity (Chapter 2), it was identified that there is a recent paucity of quantifiable evidence of STH morbidity, when assessed by direct morbidity measures such as changes in height, weight, haemoglobin, and cognition. Reasons for this lack of recent evidence may be three-fold: first, the inherent difficulties in measuring morbidity attributable to subtle and heterogeneous parasites such as STH as indicated above; second, the possible focus on prevalence and intensity of infection as primary research outcomes which may have meant that studies are not designed to measure direct morbidity effects; and third, because there are ethical difficulties in conducting trials for sufficient time periods to assess anthelmintic delivery (replicating the programme context in which it is applied). Problems with the analysis of RCTs for systematic reviews were highlighted, as was the importance of continuing to assess direct measures of morbidity in epidemiological studies of STH.

There are many important considerations to be made for assessing evidence from STH morbidity. First, from an evidence-strengthening perspective, each of the STH should ideally be assessed as individual species, rather than as an amalgamation. Teasing out the relative contributions of each STH species may be required to strengthen morbidity evidence. However, this is a very purist evidence perspective: it would require use of advanced diagnostic techniques (for example PCR to differentiate hookworm species) which may not be feasible or affordable in many settings, and it negates the fact that STH usually occur in common with each other, and other neglected and infectious diseases. The polyparasitism impact is well-recognised by researchers (259), yet as raised above this is not measurable with current GBD methodologies.

The role of observational evidence for morbidity due to STH is important. Chapter 2 investigated evidence by key topic areas. However, many study designs to investigate morbidity or mortality evidence from STH are unlikely to be ethically acceptable; and, of those that are, most evidence generated will be observational, not experimental. This, then, begs the question, "will the impact of STH on morbidity outcomes ever be quantified?". It further highlights the critical need to consider observational evidence in assessing burden of disease due to STH. It will be incorrect to solely use RCTs in systematic reviews and meta-analytical techniques if the intention is to investigate morbidity outcomes.

Cochrane systematic reviews have already negated the inclusion of associations between STH infection and reduced cognition in GBD estimates (1). Numerous studies over decades have reported observational associations between STH and a range of morbidities.

Although it is acknowledged that such findings have not been consistently found across all studies, it seems incorrect to simply ignore these associations, when trying to follow an experimental agenda. In the face of the finding (Chapter 2) that heterogeneity affects the conclusions of the Cochrane systematic review (of experimental evidence), it is recommended that the approach of solely using RCTs in systematic reviews of STH morbidity be reconsidered. Further, the NTD sector needs to determine the role of all evidence for STH morbidity. As well as continuing to provide STH prevalence and intensity data, if the STH research community can provide quantifiable, attributable evidence of STH morbidities, even greater precision will be brought to future DALYs for STH. The rationale for large-scale deworming programmes is to reduce STH morbidity. Given this, and reinfection data seen from many deworming programmes in the absence of integrated interventions (such as investment in WASH), an important conclusion from the narrative review is that further investments in appropriately designed studies that are adequately powered to measure STH morbidity are required.

Evidence-based policy

The main question addressed by the narrative review on control strategies for STH (Chapter 3), "what control strategies will work for STH in the longer term?", is crucial. The NTD community recognises the importance of longer-term strategies such as WASH and multi-component integration. However, major international agencies, such as the WHO, will be slow to recommend investment in these in the absence of sound evidence. Ideally, this needs to include cost-effectiveness, which will be extremely challenging to demonstrate.

Even the Bill and Melinda Gates Foundation, which invests so heavily in NTDs (including chemotherapy for STH), does not currently list STH as a high-opportunity target in its NTD strategy; instead most funding is directed towards those NTDs that present the greatest opportunity for control, elimination, or eradication (260). This position is justified given current evidence shortfalls. In the international and resource-constrained era of NTD elimination and concurrent 'universal coverage' priorities which include the major disease investments of HIV/AIDS, malaria and tuberculosis, policy-makers will rarely invest in strategies of unclear benefit, to address heterogeneous STH impacts on morbidity. Quite simply, there are stronger investment justifications. In addition to more evidence for morbidity from STH, research must provide practical evidence for STH control strategies that work; otherwise there is a risk of losing impetus to address STH in the broader NTD environment.

Evidence-based policy?

The above section highlighted that policy-makers are unlikely to invest without evidence. Yet, investment without sufficient evidence is also what policy-makers are required to do. Evidence is rarely clear-cut, and more lateral approaches, such as investigation of integration, or investment in WASH based on its potential to break transmission pathways despite insufficient direct evidence, are required. This is the direction that major international agencies are moving in, although for WASH in particular it is a slow-turning wheel.

The WHO refers to integrated NTD control and elimination a “litmus test” for universal health coverage (4). The international health community now needs to determine what a truly integrated health agenda should encompass and, ideally, this needs to include guidelines for how integrated disease control can, and should, be done. This is a significant research opportunity: there are multiple existing, often localised, examples across the health sector where integration at a community level has worked (although evaluation of these tends to be sparse). More rigour now needs to be brought to this.

Setting a truly integrated primary health agenda is in direct alignment with macro-political strategies as set by the World Bank (261), the WHO (4) and other parties. With the current focus on intersectoral, transdisciplinary cooperation and learning, this is an unprecedented opportunity to drive impetus for entire health system redesign and strengthening activities. By necessity, this agenda is broader than NTDs, however is a major opportunity for experts to collectively share knowledge and in so doing propose critical requirements of integrated health care. A key recommendation from the narrative review on STH control strategies is that integration, as it is directly influenced by NTD control and elimination strategies, needs to be strengthened with inclusion of structural system enhancements delivered as primary health care. For NTDs, this requires consideration beyond chemotherapy treatment to be “multi-component integration”. However, it is clearly acknowledged that more evidence is required. Second, and vitally, integrated NTD control and elimination strategies need to be soundly evaluated, with results disseminated for within- and cross-program knowledge sharing.

7.4 Summary of recommendations

1. Information from all epidemiological analyses undertaken in Timor-Leste needs to be presented to the Ministry of Health, the WHO Country Office, and other programmatic managers, to inform the development and targeting of STH control programmes.
2. Nutritional assessments need to be undertaken in Manufahi District, and strategies developed to provide essential micro- and macro-nutrients to reduce these deficits.
3. The WHO should undertake investigations of the applicability of the WHO 2006 child growth standards to the Timor-Leste population.
4. There is an urgent need for WASH guidelines to be developed for measuring WASH in epidemiological research involving STH.
5. More WASH risk factor investigations need to be undertaken. Such studies need to be well-designed and adequately powered. More statistical investigations of WASH correlations need to be undertaken.
6. Intensity of infection categories for PCR need to be robustly developed for STH diagnostics.
7. Research should be undertaken to investigate whether *N. americanus* and *A. duodenale* have different morbidity impacts in individuals, and what the STH control program implications of this would be.
8. For STH morbidity, STH control (including WASH) and integration, more research needs to be undertaken to strengthen the evidence base as specifically highlighted throughout the chapter.

9. Observational research needs to be a component of the international health agenda for NTD decision-making.
10. Integrated NTD control needs to be broadened to "multi-component" integration.
11. NTD control and elimination strategies need to be evaluated and results disseminated.

7.5 Concluding remarks

The ill-defined relationship between STH infection and (often nonspecific) morbidity, duration of morbidity, lack of a diagnostic gold standard for measuring STH, and paucity of disability, disease and vital registration data from many areas of the world (129,262) will continue to frustrate the NTD community for years to come. However, major investment in STH control drives the requirement for increasingly detailed investigations and cutting-edge research, in attempts to find answers to questions on justifiable and cost-effective control and prevention approaches.

In addition to chemotherapy, breaking transmission cycles through direct intervention should be a key. This is not a new concept. However, generation of evidence is complex; STH are insidious. If research cannot consistently provide directly measurable benefits, it should not be interpreted that such benefits are unobtainable.

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Appendices

Appendix 1 PhD Candidate's work on WASH for Worms RCT

This appendix provides an overview of the PhD Candidate's work on questionnaire development and trial activities, and data cleaning and management conducted prior to undertaking the analyses of the thesis. Chapters 2-6 have detailed the analytical methods specific to each chapter.

Conducting an RCT involves an entire research team, and this work is ongoing. The trial context has been introduced in Chapter 1, and therefore this appendix does not cover all the RCT components, of which this thesis is only one part. It does not include aspects in which the PhD Candidate was not directly involved. The PhD Candidate's contributions to the team are highlighted. Further information on the RCT is provided in the trial protocol (237).

Questionnaire and pictorial development for the WASH for Worms trial

Many RCTs use cross-sectional questionnaires to elicit information on a range of social and demographic characteristics from study participants. Such questionnaires can then be repeated through the RCT to collect follow-up information, with the advantage that using the same questionnaire means the same characteristics are measured in the same way over

time. This requires, as much as possible, use of questions that ideally have been validated in surveys elsewhere, and also interviewer training to ensure that questions are asked in the identical manner (for example by asking the question exactly as it is written), to avoid interviewer bias (263) and variability in interpretation and responses. Careful questionnaire design is therefore crucial. Self-reporting of information is a further potential source of bias. Mechanisms to validate questions, such as eliciting correctness of response by asking a similar question two ways, or confirming with interviewer observation (as is appropriate) are important. Further, using the same questionnaire throughout the trial means that if any bias has been introduced it is systematic. Such surveys are an important means of measuring risk factors, such as the WASH risk factors in the "WASH for Worms" trial. Post-trial knowledge-sharing of validated questionnaires is also important for international research.

With expertise provided by trial investigators, and WASH experts from the University of Queensland and the International WaterCentre, the PhD Candidate developed the trial questionnaires during late 2011-early 2012. Questionnaire development was heavily informed by JMP definitions (189), the United States Agency for International Development (USAID)-supported Water and Sanitation Indicators Measurement Guide (264), WASH questionnaires from Pakistan (265), Cameroon (266) and Belize (267), and the PhD literature review on STH risk factors (Chapters 1,4). Appendix Table 1 provides a summary of the components of the questionnaires that were either directly validated or influenced from questions asked elsewhere. Whilst the majority of WASH questions addresses aspects of STH infection risk, additional questions considered to be specific to

STH were added, based on previous research. These included: wearing shoes, use of night-soil as fertiliser, animal ownership and type, reusing water on food gardens, household floor construction, number and ages of household members, agricultural occupation, and use of anthelmintics in previous 12 months.

Appendix Table 1: Sources of information for questionnaire development

Question	Questionnaire reference	Source
How hands are washed	Individual questionnaire B6	Billig et al., 1999 (264)
How hands are dried	Individual questionnaire B7	Billig et al., 1999 (264)
When hands are washed	Individual questionnaire B8	Billig et al., 1999 (264); Kumba Rural Council Cameroon (266); ACF, 2011 (265)
Main household food preparer	Individual questionnaire B12	Billig et al., 1999 (264)
Place used for defecation	Individual questionnaire C13, C19	Kumba Rural Council Cameroon (266); ACF, 2011 (265)
Use/ownership of household/school/village latrine	Individual questionnaire C14, C15, C17, C21, C22, C29, C31; household questionnaire B3; village questionnaire B3	Billig et al., 1999 (264); Kumba Rural Council Cameroon (266); Ministry of Education Belize, 2009 (267); WHO/UNICEF, 2010 (189)
Type of household/village latrine	Individual questionnaire C30; household questionnaire B4; village questionnaire B4	WHO/UNICEF, 2010 (189)
Interviewer-verified cleanliness of household/village latrine	Household questionnaire B5; village questionnaire B5	Billig et al., 1999 (264); Kumba Rural Council Cameroon (266); Ministry of Education Belize, 2009 (267); WHO/UNICEF, 2010 (189)
Reasons for non-use of household/school/village latrine	Individual questionnaire C16, C18, C23, C32	Billig et al., 1999 (264); Kumba Rural Council Cameroon (266)
Sharing of household/village latrine with neighbours	Household questionnaire B6; village questionnaire B6	Kumba Rural Council Cameroon (266); WHO/UNICEF, 2010 (189)
Public latrine availability	Individual questionnaire C20; village questionnaire B3	Kumba Rural Council Cameroon (266)
How respondent cleans self after defecation	Individual questionnaire C20	Ministry of Education Belize, 2009 (267)
How child faeces are disposed of	Household questionnaire B7	Billig et al., 1999 (264); Kumba Rural Council Cameroon (266)

Rubbish disposal in household and community	Household questionnaire B8; village questionnaire B7	Kumba Rural Council Cameroon (266); Ministry of Education Belize, 2009 (267)
Number and type of animals owned	Household questionnaire D30	ACF, 2011 (265)
Disposal of animal droppings	Household questionnaire C31	Kumba Rural Council Cameroon (266)
Type of household water source	Household questionnaire C9, C18	WHO/UNICEF, 2010 (189)
How household water is used	Household questionnaire C10, C19	Billig et al., 1999 (264); Kumba Rural Council Cameroon (266)
Distance and location of household water source	Household questionnaire C11, C12, C20	Billig et al., 1999 (264); Kumba Rural Council Cameroon (266); ACF, 2011 (265)
Water source non-availability	Household questionnaire C13, C14	Billig et al., 1999 (264); Ministry of Education Belize, 2009 (267)
How household water is stored	Household questionnaire C15	Kumba Rural Council Cameroon (266)
Containers for storing water	Household questionnaire C16	Kumba Rural Council Cameroon (266); Ministry of Education Belize, 2009 (267); ACF, 2011 (265)
How household water is treated	Household questionnaire C21, C23	Ministry of Education Belize, 2009 (267); ACF, 2011 (265)
Purposes for which household water is treated	Household questionnaire C22	Ministry of Education Belize, 2009 (267)
Highest level of education	Individual questionnaire D34	KTL, 2012 (268)
Current employment status	Individual questionnaire D35	KTL, 2012 (268)
Number and age of people per household	Household questionnaire D25	ACF, 2011 (265)
Type of house construction	Household questionnaire D33	Doku et al., 2010 (269)
Level of household income over last year	Household questionnaire D34	QUT, 2012 (270)
Type of assets owned by the household	Household questionnaire D35, D37	Pole & Ikeme, 1976 (271); Doku et al., 2010 (269)
Electricity at home	Household questionnaire D36	Doku et al., 2010 (269)

Questionnaires incorporated “skip rules” to ensure that only questions relevant of the respondent were asked. The benefit of conducting interviewer-led questionnaires was that no minimum level of respondent education was required (which in traditional villages could not have been assumed). As there is a risk that interviewers can introduce bias by asking leading questions or prompting when not indicated, questionnaires explicitly specified where prompting was, and was not, allowed.

Data relating to WASH-related risk factors were collected at village, household and individual level. To supplement questionnaires and minimise confusion, pictorial charts were developed to highlight different types of sanitation and water supply using JMP definitions (189). Final questionnaires included: an individual questionnaire answered by all participants with a parent or guardian answering for children under 12 years (Appendix 2), a household questionnaire completed by one adult per household (Appendix 3), and a village questionnaire completed by one village representative (Appendix 4). Accompanying these were the pictorial charts. Once documents were completed, the PhD Candidate liaised with a hired translator to facilitate their translation into the Timorese national language, Tetum. The PhD Candidate made a 90% contribution to the development of the questionnaires and pictorial charts.

Field work conducted by PhD Candidate

The PhD Candidate conducted additional preparatory work prior to RCT commencement including developing the Standard Operating Procedure (SOP) for conducting anthropomorphic measurements and participant interviews. The PhD Candidate assisted with diluting potassium dichromate for fixing stool specimens and shipping trial inventory to Timor-Leste.

After meetings between the Principal Investigator and senior Ministry of Health and WaterAid officials, the PhD Candidate commenced the baseline survey of the RCT with a Timorese employee at the first two villages in Timor-Leste in May 2012. This included meeting with Ministry of Health, National Laboratory, and district health staff to explain the trial, and recruit district health staff to assist with field work. Additionally, meetings were conducted with WaterAid staff to confirm village scheduling, and permission sought from village chiefs to conduct community consultations. These were then conducted, covering all aspects of the trial according to SOPs and village information sheets, with opportunities for villagers to ask questions.

Participants were then enrolled, consent forms completed, and the study commenced. Participants supplied a finger-prick blood sample for the purpose of measuring their haemoglobin concentration, and children aged one to 18 years had their height and weight recorded. Individuals provided a stool sample for parasitological analysis, and individual,

household, and village questionnaires were completed. Household global positioning system (GPS) coordinates were sited. The PhD Candidate's role involved coordination and checks that consent was being correctly obtained and recorded, quality checks of questionnaire completion and measurements conducted, and fixing stool specimens for parasitological analysis. Back-translations of the questionnaires into English were also checked with the Timorese trial employee. Whilst the PhD Candidate was involved in the field work for the first two villages, baseline measurements for the RCT continued from May 2012 to October 2013 under the management of the Trial Coordinator once she was recruited.

Upon return from Timor-Leste the PhD Candidate worked with the Data Manager to develop the trial database, and with the Trial Coordinator to check the performance of the questionnaires. A second round of field work was undertaken in 2013, to oversee the (by then established) field team conducting village baseline and follow-up surveying, administration of albendazole, and to conduct data double-entry (from Tetum to English).

Data management is an essential component of good research, and it is extremely important that data are clearly described, consistent across software, and managed by one person (i.e. that there is not more than one person creating or removing variables). After data were entered into Microsoft Access, the PhD Candidate has been responsible for the exporting and ongoing management of trial baseline data in STATA, and has compiled a database codebook listing all variables in the dataset, their range and meaning, including extensive notes on variables created or dropped throughout the analyses. At various times the PhD

Candidate has met with the Principal Investigator and other trial members to clarify variable creation and removal, and the reasons (e.g. collinearity), and ensure agreement regarding management of variables for analysis. The codebook is an essential ongoing data management tool for the RCT.

Data collection and cleaning for the WASH for Worms trial

Baseline data were compiled into three separate Microsoft Access databases, each containing the information for eight villages. Double data entry, by different personnel for first and second entry, was undertaken to minimise transcription errors. The PhD Candidate did second-entry of baseline data for 15 of the 24 villages, from Tetum paper-based questionnaire records into an English language database. An error script was then run by the Data Manager to check for errors between records. Extensive field work and quality assessment checks were also undertaken by the Trial Coordinator throughout the trial, to ensure accuracy and consistency of responses to questions and measurements.

The PhD Candidate played an extensive role in cleaning and management of the baseline trial data. Data cleaning is an essential component of epidemiological analysis, as accuracy of the underlying variables is required for any analyses to have meaning. Household participant register information and all questionnaire information was extracted from each of the three Access databases and imported into STATA. This information was then appended so that information on all 24 villages was collated, and saved as STATA datasets

separately for household participant register, village questionnaire, household questionnaire and individual questionnaire. Within each dataset, variables were labelled and recoded as required (e.g., converting string variables to numeric variables), and formatted dates to a STATA-recognisable form. “Other specify” answers were cleaned by determining overlap with existing variable categories, or by creating new variables or categories where more than ten of the same response were recorded.

Parasitological data consisted of STH and *G. duodenalis* Ct-values from qPCR diagnosis. These data were obtained as three separate Excel files and imported into STATA, where they were reshaped and saved as a STATA dataset. All datasets were saved with a unique individual identification variable to enable matching of data from the different datasets. The PhD Candidate then linked the five different study datasets of household participant register, individual questionnaire, household questionnaire, village questionnaire and parasitological outcome by individual unique identifier, so that each observation in the household participant register was linked with its corresponding village, household and individual questionnaire information and parasitological outcome. This process identified several records that did not link correctly due to incorrectly-matching unique identification numbers; these records were investigated for correctness and decisions made with the Trial Coordinator on a case-by-case basis, until all discrepant variables could be linked correctly.

After compilation of full baseline data, further data cleaning checks in STATA were conducted to investigate ‘nonsense’ answers to variables, missing information (such as age and sex), and incorrect adherence to questionnaire skips. Accuracy was also checked

amongst several key variables that contained 'quality checking' information across individual, household and village questionnaires, for example verification from the individual questionnaire cross-checked with the village questionnaire as to whether the village actually had a latrine. In liaison with the Trial Coordinator, discrepant records were then checked in turn in Timor-Leste.

Inclusion and exclusion criteria

The "WASH for Worms" RCT has specified inclusion and exclusion criteria. Residents must be living within the identified villages, and have completed a consent form, to be included. Children aged less than one year and women in their first trimester of pregnancy are excluded. These criteria were applied at study entry; however the age variable was checked to ensure that there were no children aged less than one year.

Data management and descriptive analyses

Risk factor variables were restructured because the questionnaire design allowed for multiple answers to be provided per question. Each possible answer was then coded as a separate binary variable in the database. Restructuring these according to frequency of responses into a single new categorical variable provided a more useful basis for analysis, as a single measure was then used per question. Variables were restructured according to the following protocol:

1. If a binary variable had no similarity to other variables and sufficient numbers (≥30 observations) it formed its own subgroup in the new categorical variable;
2. If a binary variable had low numbers (<30 observations) it was combined where possible with a similar subgroup to form a clinically meaningful category in the new categorical variable - if it did not make sense to do this, it was kept separate;
3. For variables which were similar to other variables, consideration was firstly given to whether it made sense to combine them or not and to investigate subgroups;
4. Variable recategorisation additionally tried to minimise numbers of subgroups with low cell numbers.

Variables were checked for low observation numbers (including in subgroups), with the following protocol applied:

1. If less than ten observations in a variable, there were insufficient observations for analysis therefore variables were either excluded from analyses or combined with other variables (if feasible);
2. Variables with between 10-30 observations were examined on a case-by-case basis, to determine whether they would be sufficient for regression analysis according to the number of observations that were related to the outcome;
3. Variables with low numbers of observations that could be merged with other variables were prioritised in tetrachoric investigation (see following paragraphs), e.g., household water treatment type could be merged with household water treatment (yes/no) to create a new categorical variable of: household water

treatment - no/boil/other/missing. This was to retain information that would otherwise be excluded from analyses.

Any two correlated independent variables may have multicollinearity (which may or not be severe). Severe multicollinearity can lead to inflated coefficients in regression analysis, and unreliable model interpretation. Variables that were related or that appeared to measure similar concepts were investigated for collinearity, to enable the best variable selection for regression models. Tetrachoric correlation analysis (for binary variables) and a separate STATA "collin" user written package (for other variables) were used.

Results of the "collin" test gave a Variance Inflation Factor (VIF); an indication of how much the standard error could be inflated by multicollinearity. Tolerance for a variable is 1 minus the R^2 that results from the regression of that variable on the other relevant variables. The corresponding VIF is 1/tolerance. If variables are completely uncorrelated, both the tolerance and VIF are 1. If a variable is very closely related to another variable(s), the tolerance nears zero, and the VIF becomes very large. A tolerance of 0.1 or less (equivalently VIF of 10 or greater) indicates collinearity (272). Tetrachoric analysis gives a correlation coefficient, with the following interpretation: coefficient between ±0-0.15 = low correlation; ±0.16-0.4 = modest correlation; ±0.41-0.7 = moderate correlation; ±0.7-0.99 = high correlation; ±1.00 = perfectly collinear (250).

The methods of assessing multicollinearity were undertaken within, and then across, domains. Where multicollinearity was identified, the protocol was to:

1. Merge variables where possible to avoid loss of individual variable information;
2. Drop variables that were collinear and retain one for analysis. This was done on the priority basis of: largest number of observations; numbers associated with outcome; and knowledge of the most important variable to retain - i.e. from knowledge of risk factors (these were explicitly noted when used as the basis for variable selection).

In addition to the above, the PhD Candidate investigated missing data, checked units of measurement, variable structure and distributions, explanatory associations with outcomes (chi-squared and Wilcoxon's rank sum tests), and conducted sensitivity analyses of variables. All descriptive epidemiological analyses have been conducted using STATA versions 13 and 14.

Development of socioeconomic quintile

STH are closely linked to poverty and as such, adjustment for socioeconomic status was an important component of all analyses. Principal component analysis (PCA) was used to develop a socioeconomic score from demographic variables used in the questionnaires. This score was then categorised into quintiles to enable consideration of relative socioeconomic status in all analyses.

PCA is a statistical technique for variable reduction. It is useful for reducing a quantity of similar variables (for example, individual socioeconomic variables) into one composite

variable for analysis. The technique describes a series of uncorrelated linear combinations of the variables that contain most of the variance (251) thereby allowing their identification and selection to build a composite. It is frequently used to generate a single measure of socioeconomic status from multiple socioeconomic questions derived from surveys, because accounting for individual variation in a composite index is more accurate than selecting one single variable for usage. Because the socioeconomic variable is derived from the study population, this measure of socioeconomic status is relative to that population only.

There are several assumptions for PCA. The variables considered for the development of the socioeconomic variable were any variables that could serve as indices of household wealth. However, variables were specifically excluded if it was considered important to adjust for them in regression analyses due to of anticipated risks, such as ownership of a household latrine. Further, any variables with close to 90% of responses in a single subgroup were also excluded, due to insufficient variation. Included variables were those that provided information on ownership of household assets (including animals, transport and appliances), house floor type, reported income and presence of electricity. These data were household-level variables and therefore PCA was conducted on household-level observations only. That is, because individual observations had been 1:many linked it was important not to replicate household-level observations across multiple-linked data, as it could potentially affect the variance explained.

PCA was used to develop weights for the above asset variables (273). Four principal components (PCs) with eigenvalues above one were retained, individually each contributing over 10.0% of variance explained (cumulatively 57.1% explained). Varimax rotation was used to produce orthogonal factors (i.e., uncorrelated factors, needed to create a composite index). The first PC explained 21.2% of the variance in included variables and gave greatest weight respectively to ownership of household appliances (0.60), bicycles and vehicles (0.56), and presence of household electricity (0.52). The second PC explained an additional 14.4% of variance and gave greatest weight to number of household horses (0.70) and cows (0.66) owned. The third PC explained an additional 10.9% of variance and gave greatest weight to number of household pigs (0.66), chickens (0.61) and dogs (0.42) owned. The fourth PC explained an additional 10.7% of variance and gave greatest weight to household floor type (0.79) and reported household income (-0.52).

The "factortest" STATA user written package was used to calculate tests of accuracy of the principal component analysis technique. This calculates Bartlett's test for sphericity and the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy (251). The Bartlett's test for sphericity tests the hypothesis that the associated correlation matrix is an identity matrix, indicating that variables are unrelated and therefore unsuitable for assessing intercorrelations. A Bartlett's test $P<0.01$ indicates usefulness of the PCA approach (274). The KMO score measures the adequacy of the PCA sampling by indicating the proportion of variance in the variables that might be caused by underlying factors. KMO takes values between zero and one, with small values indicating that overall the variables have too little in common to warrant PCA analysis (251). A KMO of ≥ 0.5 provides good justification of

the PCA model as there is high correlation in the dataset (251).

To build the composite socioeconomic index, each included variable was weighted according to the proportion of its variance explained by its associated principal component (i.e. the normalised squared loading) (274). Components were restricted to include only those variables that contributed 0.3 or more to the normalised squared loading, to reduce the impact of minor "noise" on the composite score. Each PC was then weighted according to its contribution to the proportion of the explained variance in the dataset (i.e. the normalised sum of squared loadings) (274), with these scorings summed for the four PCs into one resultant socioeconomic score. Finally, this score was categorised into quintiles, to classify each household according to relative poverty.

Appendix 2 Individual questionnaire

INDIVIDUAL QUESTIONNAIRE

A cluster randomised control trial (RCT) of the impact of a community-based hygiene and sanitation programme on infection with intestinal parasites following mass albendazole chemotherapy in Timor-Leste

PLEASE NOTE: ALL ELIGIBLE HOUSEHOLD MEMBERS ARE TO COMPLETE THIS INDIVIDUAL QUESTIONNAIRE (INCLUDING THE HOUSEHOLD QUESTIONNAIRE RESPONDENT).

Date : ☐☐ / ☐☐ / 2 0 ☐☐ Interviewer initials: ☐☐

Consent checklist

Has written consent been obtained? Y ☐ N ☐ *Only proceed if Yes*

A. Participant details

Village *Household* *Person*

Participant ID ☐☐☐ - ☐☐ - ☐☐

2. Name ☐

2.1 Gender M or F : ☐

ALL INDIVIDUALS AGED 12 YEARS AND OVER ARE ABLE TO COMPLETE THIS QUESTIONNAIRE FOR THEMSELVES IF THEY WISH TO, OTHERWISE THE MOTHER/CAREGIVER CAN DO IT. MOTHERS/CAREGIVERS ARE TO COMPLETE ONE COPY OF THE QUESTIONNAIRE FOR THEMSELVES, AND ONE COPY FOR EACH CHILD LESS THAN 12 YEARS OF AGE. WHERE A MOTHER IS COMPLETING A QUESTIONNAIRE FOR THEIR CHILD, QUESTIONS 3 TO 5 MUST BE COMPLETED.

3. Name ☐

Village *Household* *Person*

4. ID number ☐☐☐ - ☐☐ - ☐☐

5. Relationship with participant ☐

Clinical Information

Pregnancy

If female and aged 14-50 years…

C1. To your knowledge, are you pregnant? Y ☐ N ☐

Antiparasitic drugs

C4. To your knowledge, have you taken deworming medication in the last 12 months? Y ☐ N ☐

Participant's diarrhoea history

C5. Are you currently experiencing symptoms of diarrhoea? Y ☐ N ☐

C6. In the last 24 hours, how many bowel motions have you passed? ☐

C7. In the past 24 hours, has the consistency of your bowel motions been: (Tick ONLY ONE correct response)

- Normal ☐
- Loose and/or watery ☐
- Other ☐

C8. In the last two weeks have you had diarrhoea? Y ☐ N ☐

B. Personal hygiene data

6. Would you explain and show me what you do when you wash your hands? *Tick (√) all items mentioned or demonstrated WITHOUT reading out options:*

Uses water	
Uses soap or ash	
Washes both hands	
Rubs hands together at least three times	
Cleans under fingernails	
Other	
Don't know	
Refused	

Specify: ☐

7. Would you explain and show me what you do when you dry your hands after washing? *Tick (√) all items mentioned or demonstrated WITHOUT reading out options:*

Dries hands hygienically – by air drying or using a clean cloth	
Dries in clothes	
Other	
Don't know	
Refused	

Specify: ☐

8. When do you wash your hands? *Tick (√) all items mentioned WITHOUT reading out options:*

After defecation or urination	
After cleaning babies' bottoms/ disposal of children's faeces	
Before food preparation	
Before eating/ Before feeding children	
After contact with animals	
After contact with soil or dirt	
Other	
Don't know	
Refused	

Specify: ☐

For the following actions, please consider whether you always, sometimes, or never do them:
Tick (√) the correct box:

	Always	Sometimes	Never
9. When inside your home, do you wear shoes?	☐	☐	☐
10. When outside your home, do you wear shoes?	☐	☐	☐
11. Do you wear shoes when defecating/urinating?	☐	☐	☐

12. Are you the main food preparer for your household? Y ☐ N ☐

C. Sanitation data

13. Where is the main place you defecate? CHOOSE ONLY ONE OPTION

Household toilet	
Village toilet	
School toilet	
Neighbour toilet	
On the ground	
Other	
Don't know	
Refused	

Specify: _____

14. Does your house have a toilet? Y ☐ N ☐ *If 'N', skip to question 20*

15. Do you use your household toilet? Y ☐ N ☐ Refused ☐

If 'Y', and female with 14 years or more, skip to question 17

If 'Y', and male any age or female under 14, skip to question 19

16. If no, why is this? *Tick all items mentioned or demonstrated WITHOUT reading out options:*

Toilet is dirty	
Toilet is broken	
Toilet pit is full/overflowing	
Toilet is not suitable for children	
Toilet is in an unsafe location	
Other	
Don't know	
Refused	

Specify: _____

GO TO QUESTION 20

For women and girls 14 years or more:

17. Do you use the household toilet when menstruating? Y ☐ N ☐ Refused ☐

If 'Y', skip to question 19

18. If no, why not? Choose only one option

Not allowed/not appropriate	
Other	
Don't know	
Refused	

Specify: ☐

19. Besides your household toilet, where else do you usually defecate? *Tick all items mentioned or demonstrated WITHOUT reading out options:*

Nowhere else	
Village toilet	
School toilet	
Neighbour toilet	
On the ground	
Other	
Don't know	
Refused	

Specify: ☐

20. How do you clean yourself after DEFECATING? *Tick all items mentioned or demonstrated WITHOUT reading out options:*

Use toilet paper	
Use newspaper	
Use leaves or sticks	
Use water and hand	
Other	
Don't know	
Refused	

Specify: ☐

21. Does your village have a public toilet? Y ☐ N ☐

If 'N" skip to question 24 if aged 6 to 17 OR question 33 if aged otherwise

22. If yes, do you use the village toilet? Y ☐ N ☐ Refused ☐

If 'Y' skip to question 24

23. If no, why is this? *Tick all items mentioned or demonstrated WITHOUT reading out options:*

Must pay a fee to use the toilet		
Women and girls are not permitted to use it when menstruating		
Toilet is dirty		
Toilet is broken		
Toilet pit is full/overflowing		
Toilet is not suitable for children		
Toilet is in an unsafe location		
Other		Specify:
Don't know		
Refused		

If aged 6-17 years…

24. Do you go to school? Y ☐ N ☐ *If 'N" skip to question 33*

25. Is the school in the same
Tick if the same, cross if different:

Bairo?	
Aldeia?	
Suco?	
Sub-district?	
District?	

For the following actions, please consider whether you always, sometimes, or never do them:
Tick the correct box:

	Always	Sometimes	Never
26. When inside your classroom, do you wear shoes?	☐	☐	☐
27. When outside your classroom, do you wear shoes?	☐	☐	☐
28. Do you wear shoes when defecating/urinating at school?	☐	☐	☐

29. Does your school have a toilet? Y ☐ N ☐ *If 'N" skip to question 33*

30. If yes, what type of toilet? *Show respondent the picture sheet of different toilet types. Observe school toilet if possible.*

Flush toilet				
Pit latrine	With slab		Flush to pit	
	Without slab		Flush elsewhere	
			No water	
Composting toilet				
Bucket				
Hanging toilet or latrine				
Other		Specify:		
Don't know				
Refused				

31. Do you use this toilet? Y ☐ N ☐ Refused ☐ *If 'Y', skip to question 33*

32. If no, why is this? *Tick all items mentioned*

Girls are not permitted to use it OR it is not suitable for both genders	
Toilet is dirty	
Toilet is broken	
Toilet pit is full/overflowing	
Toilet is not safe for children	
Other	Specify:
Don't know	
Refused	

---

33. Do you have access to treatments for deworming? *Tick all items mentioned:*

No, not at all		
Yes, in my village		
Yes, in another village		Village name:
Yes, at school		School name:
Yes, at health centre/outreach health team (CISCA)		Health unit:
Yes, other		Specify:
Don't know		
Refused		

D. Socio-economic characteristics – Complete ONLY IF 18 years and over

34. What is your highest level of education? (tick only one option)

Never went to school	
Not finished primary school (6 years)	
Completed primary school	
Not finished pre-secundario (9 years)	
Completed pre-secundario (9 years)	
Not finished secundario (12 years)	
Completed secundario (12 years)	
Completed professional training	
Not finished university	
Completed university	
Don't know	
Refused	

35. What is your current employment status?

Employed/has job		Specify job:	Farmer				
Doing housework			Fisherman				
Student			Animal Keeper				
Retired			Clerk/administration				
Long-term disabled			Health worker				
Unemployed			Selling at market				
Don't know			Ironsmith				
Refused			Other		Specify:		
			Refused				

This completes the questionnaire. We are grateful for your participation - thank you.

Appendix 3 Household questionnaire

HOUSEHOLD QUESTIONNAIRE

A cluster randomised control trial (RCT) of the impact of a community-based hygiene and sanitation programme on infection with intestinal parasites following mass albendazole chemotherapy in Timor-Leste

PLEASE NOTE: ONE HOUSEHOLD MEMBER SHOULD COMPLETE THIS HOUSEHOLD QUESTIONNAIRE. WHEREVER POSSIBLE THIS SHOULD BE THE MOTHER OF THE FAMILY. ALL HOUSEHOLD MEMBERS ARE TO COMPLETE THE INDIVIDUAL QUESTIONNAIRE.

Date : ☐☐ / ☐☐ / 2 0 ☐☐ Interviewer initials: ☐☐

Consent checklist

Has written consent been obtained? Y ☐ N ☐ *Only proceed if both Q are Yes*

A. Participant details

1. ID number *Village* ☐☐☐ - *Household* ☐☐ - *Person* ☐☐

2. Name ☐

B. Sanitation data

3. Does your house have a toilet? Y ☐ N ☐ *If 'N', skip to question 7*

4. Can you please show me your household toilet? *Tick off (v) toilet type, using picture sheet of different toilet types as a guide:*

Flush toilet				
Pit latrine	With slab		Flush to pit	
	Without slab		Flush elsewhere	
			No water	
Composting toilet				
Bucket				
Hanging toilet or latrine				
Other		Specify: ☐		
Don't know				
Refused				

5. *Tick off (V) all relevant observations WITHOUT reading out question:*

Toilet is clean (no urine, faeces, flies)	
Water or other personal cleaning materials are evident	
There is a hole cover	
Urine on seat	
Faeces on seat	
Urine on floor/walls	
Faeces on floor/walls	
Odour	
Flies present	
Other	
Don't know	
Refused	

Specify: ☐

6. Is this toilet shared with another household? Y ☐ N ☐ Refused ☐

If a baby or toddler in the house (CHILDREN BETWEEN 1 AND 3 YEARS OF AGE):

7. How do you dispose of the child's faeces? *Tick all items mentioned or demonstrated WITHOUT reading out options:*

In household waste	
In the household toilet	
In the bushes	
In the garden	
In the river	
Other	
Don't know	
Refused	

Specify: ☐

---

8. Can you please show me where you dispose of household waste? *Tick all items mentioned or demonstrated WITHOUT reading out options. Use prompts:*

Communal bin	
In the bushes	
In the river	
Dig/bury it	
Burn it	
Compost it	
Recycle it	
Other	
Don't know	
Refused	

Specify: ☐

Specify: ☐

C. Water supply data

9. What is the main source of water for your household? *CHOOSE ONLY ONE OPTION with a tick (√). Show respondent the picture sheet of different water sources. Observe the source if possible:*

Piped water into dwelling	
Piped water to yard/plot - this house only	
Piped water shared with other houses (to yard/plot or to communal place)	
Tubewell or borehole or protected dug well	
Unprotected dug well	
Protected spring	
Unprotected spring	
Rainwater	
Cart with small tank/drum or tanker-truck	
Surface water	
Bottled water	
Other	
Don't know	
Refused	

Specify: ____

10. Do you use the main water source (as indicated in the previous question) for: *Read all options and tick all items mentioned:*

Drinking?	
Cooking or Dishwashing?	
Hand/body washing?	
Laundry?	
House cleaning?	
Other	
Don't know	
Refused	

Specify: ____

11. Where is this main water source? *CHOOSE ONLY ONE OPTION with a tick (√):*

In your household compound	
Elsewhere in your village/bairro	
In a neighbouring village/bairro	
Other	
Don't know	
Refused	

Specify: ____

12. Approximately how far is the main water source from the house (in time)? *CHOOSE ONLY ONE OPTION with a tick (√)*

Less than 15 min	
15 min – 1 hour	
1 – 3 hours	
More than 3 hours	

13. Is water always available from this main source? Y ☐ N ☐ *If 'Y', skip to question 15*

14. If no, how often would you say that water is not available? *CHOOSE ONLY ONE OPTION with a tick (√):*

At least once per day	
One or two days per week	
More than 2 days per week	
More than one week per month	
More than one month per year (eg dry season)	
Other	
Don't know	
Refused	

Specify:

15. Do you store water from this main source in the household? Y ☐ N ☐ *If 'N', skip to question 17*

16. If yes, what type of container do you use to store water? *Tick all items mentioned or demonstrated:*

Jerry-can		Covered: Y		N		
Balde/basin (plastic)		Covered: Y		N		
Ceramic pot		Covered: Y		N		
Other		Covered: Y		N		Specify:
Don't know						
Refused						

17. Is there an alternative source of water that you also use for your household? Y ☐ N ☐

If 'N', skip to question 21

18. If yes, what is this alternative source of water? *CHOOSE ONLY ONE OPTION with a tick (√):*

Show respondent the picture sheet of different water sources. Observe the source if possible:

Piped water into dwelling	
Piped water to yard/plot - this house only	
Piped water shared with other houses (to yard/plot or to communal place)	
Tubewell or borehole or Protected dug well	
Unprotected dug well	
Protected spring	
Unprotected spring	
Rainwater	
Cart with small tank/drum or Tanker-truck	
Surface water	
Bottled water	
Other	
Don't know	
Refused	

Specify:

19. Is an alternative water source the water that you use for: *Read all options and tick (√) all items mentioned:*

Drinking?	
Cooking or Dishwashing?	
Hand/body washing?	
Laundry?	
House cleaning?	
Other	
Don't know	
Refused	

Specify: _____

20. Where is this alternative water source? *CHOOSE ONLY ONE OPTION with a tick (√):*

In your household compound	
Elsewhere in your village/bairro	
In a neighbouring village/bairro	
Other	
Don't know	
Refused	

Specify: _____

---

21. Do you treat (or boil) any of your household water? Y ☐ N ☐ *If 'N', skip to question 24*

22. If yes, which purposes would you treat the water for? *Read all options and tick (√) all items mentioned:*

Drinking?	
Cooking or Dishwashing?	
Hand/body washing?	
Laundry?	
House cleaning?	
Other	
Don't know	
Refused	

Specify: _____

23. What do you treat it with?

Household bleach	
Boil	
Filter	
Other	
Don't know	
Refused	

Specify: _____

---

24. Where do you tip out used water?

On a food garden		
On the ground next to the house		
On the ground away from the house (at least 5 metres)		
In the bushes		
In a lake or stream		
Other		Specify:
Don't know		
Refused		

D. Risk factors

25. How many people of the following age groups live in your household?

<5 years	
5-17 years	
18-65 years	
>65 years	
Total	

26. Do you have a food garden? Y ☐ N ☐

27. What water do you use on the garden? *Tick as appropriate:*

Rain	
Reused/waste water	
Same as main household water source	
Same as alternative household water source	
Other	
Don't know	
Refused	

28. Do you use human faeces in your garden as a fertilizer? Y ☐ N ☐

If 'N', skip to question 30

29. If yes, do you: *Tick as appropriate:*

Put it on the garden straight away		
Dry it out first, then put it on the garden		
Other		Specify:
Don't know		
Refused		

30. Does your household keep any of the following animals?

Dogs?	Y		N		If yes, how many?	
Pigs?	Y		N		If yes, how many?	
Chickens?	Y				If yes, how many?	
Other animals?	Y		N		If yes, please specify which animals and how many:	
Don't know						
Refused						

31. If yes, where do you dispose of animal faeces? *Tick as appropriate:*

Just leave them where they are		
On a food garden		
On a non-food garden		
On the ground next to the house		
On the ground away from the house (at least 5 metres)		
In the bushes		
In a lake or stream		
Other		Specify:
Don't know		
Refused		

E. Household characteristics

32. What type of floor does your house have?

Earth		
Cement/tile		
Wood		
Other		Specify:
Don't know		
Refused		

33. What type of walls does your house have? *Note to interviewer: this question is for the main external walls of the dwelling.*

Wood		
Weatherboard		
Brick		
Straw/bamboo		
Tin sheets		
Bebak (palm leaf)		
Other		Specify:
Don't know		
Refused		

34. How much income did your household receive over the last year?

Less than USD $365	
USD $365-730	
USD $730-1460	
More than USD $1460	
Don't know	
Refused	

35. Does your household own any of the following types of transport?

A bicycle	Y		N		
A motor vehicle	Y		N		
Other	Y		Specify:		
Don't know					
Refused					

36. Do you have electricity/solar panel at home? Y ☐ N ☐

37. If yes, does your household have any of the following home appliances that work? *Tick as appropriate:*

Television	Y		N	
Refrigerator	Y		N	
Radio set	Y		N	
Computer	Y		N	
Don't know				
Refused				

This completes the questionnaire. We are grateful for your participation - thank you.

Appendix 4 Village questionnaire

VILLAGE/BAIRRO QUESTIONNAIRE

A cluster randomised control trial (RCT) of the impact of a community-based hygiene and sanitation programme on infection with intestinal parasites following mass albendazole chemotherapy in Timor-Leste

Date : ☐☐ / ☐☐ / 2 0 ☐☐ Interviewer initials: ☐☐

Village/Bairro name: ☐

Consent checklist

Has written consent been obtained? Y ☐ N ☐ *Only proceed if Yes*

A. Participant details

2. ID number *Village* ☐☐☐ - *Household* ☐☐ - *Person* ☐☐

2. Name ☐

B. Sanitation data

3. Does your village/bairro have a public toilet? Y ☐ N ☐ Refused ☐

If 'N' skip to Question 7

4. If yes, can you please show me your village/bairro toilet? *Tick off (√) toilet type, using picture sheet of different toilet types as a guide:*

Flush toilet				
Pit latrine	With slab		Flush to pit	
	Without slab		Flush elsewhere	
			No water	
Composting toilet				
Bucket				
Hanging toilet or latrine				
Other				
Don't know				
Refused				

Specify: ☐

5. *Tick off (V) all observations WITHOUT reading out question:*

Toilet is clean (no urine, faeces, flies)	
Water or other personal cleaning materials are evident	
There is a hole cover	
Urine on seat	
Faeces on seat	
Urine on floor/walls	
Faeces on floor/walls	
Odour	
Flies present	
Other	
Don't know	
Refused	

Specify:

6. Does anyone from another village/bairro use this toilet? Y ☐ N ☐ Which village?

7. Can you please show me where village/bairro garbage is disposed? *Tick off (V) all items mentioned or demonstrated WITHOUT reading out options. Use prompts:*

Communal bin	
In the bushes	
In the river	
Dig/bury it	
Burn it	
Compost it	
Recycle it	
Other	
Don't know	
Refused	

Specify:

Specify:

This completes the questionnaire. We are grateful for your participation - thank you.