International Journal for Parasitology 49 (2019) 389–396

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Risk factors for infection with soil-transmitted helminths during an integrated community level water, sanitation, and hygiene and deworming intervention in Timor-Leste

Susana Vaz Nery[a,1,4,*], Naomi E. Clarke[a,1,4], Alice Richardson[a], Rebecca Traub[b], James S. McCarthy[c,d], Darren J. Gray[a], Andrew J. Vallely[e], Gail M. Williams[f], Ross M. Andrews[a,g], Suzy J. Campbell[a,2], Archie C.A. Clements[a,3]

[a] *Research School of Population Health, Australian National University, Canberra ACT 0200, Australia*
[b] *Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Parkville, VIC 3052, Australia*
[c] *Clinical Tropical Medicine Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, QLD 4006, Australia*
[d] *School of Medicine, University of Queensland, Brisbane, QLD 4006, Australia*
[e] *Kirby Institute, University of New South Wales, Sydney, NSW 2052, Australia*
[f] *School of Public Health, University of Queensland, Brisbane, QLD 4006, Australia*
[g] *Menzies School of Health Research, Charles Darwin University, Darwin, NT 0811, Australia*

ARTICLE INFO

Article history:
Received 8 August 2018
Received in revised form 10 December 2018
Accepted 12 December 2018
Available online 22 February 2019

Keywords:
Soil-transmitted helminths
Water
Sanitation and hygiene
Risk factors

ABSTRACT

Water, sanitation and hygiene interventions have been advocated as important complements to deworming programs to improve soil-transmitted helminth control. Evidence for the impact of water, sanitation and hygiene on soil-transmitted helminth infections is mixed, and based mainly on cross-sectional studies. In this study, we assessed associations between individual- and household-level water, sanitation and hygiene variables and soil-transmitted helminth infections, using data collected during the 2 year follow-up study period of the WASH for WORMS randomised controlled trial in Timor-Leste. Data were collected across four surveys, conducted at 6 monthly intervals in 23 communities. We analysed water, sanitation and hygiene and sociodemographic variables as risk factors for infection with *Necator americanus*, *Ascaris* spp., and undifferentiated soil-transmitted helminth infection, using generalised linear mixed models to account for clustering at community, household and participant levels. Water, sanitation and hygiene risk factors were examined both concurrently and with a 6 month lag period that coincided with the most recent deworming. The analysis included 2333 participants. Factors associated with *N. americanus* infection included age group, male sex (adjusted odds ratio (aOR) 3.1, 95% confidence interval (CI) 2.4–4.2), working as a farmer (aOR 1.7, 95% CI 1.2–2.4), and completing secondary school or higher (aOR 0.29, 95% CI 0.16–0.53). Risk factors for *Ascaris* spp. infection included age group, living in a dwelling with more than six people (aOR 1.6, 95% CI 1.1–2.3), having a tube well or borehole as the household water source (aOR 3.7, 95% CI 1.3–10.8), and using a latrine shared between households 6 months previously (aOR 2.3, 95% CI 1.2–4.3). Handwashing before eating was protective against infection with any soil-transmitted helminth (aOR 0.79, 95% CI 0.65–0.95). In the context of regular deworming, few water, sanitation and hygiene-related factors were associated with soil-transmitted helminth infections. Future research examining the role of water, sanitation and hygiene in soil-transmitted helminth transmission is required, particularly in low transmission settings after cessation of deworming. Identifying improved indicators for measuring water, sanitation and hygiene behaviours is also a key priority.

* Corresponding author at: Kirby Institute, University of New South Wales, Sydney NSW 2052, Australia.
E-mail address: snery@kirby.unsw.edu.au (S. Vaz Nery).

[1] These authors contributed equally to this work.
[2] Present address: Evidence Action: Deworm the World Initiative, Washington, DC 20005, USA.
[3] Present address: Faculty of Health Sciences, Curtin University, Bentley, WA 6102, Australia.
[4] Present address: Kirby Institute, University of New South Wales, Sydney, NSW 2052, Australia.

https://doi.org/10.1016/j.ijpara.2018.12.006
0020-7519/

1. Introduction

Ascaris lumbricoides, hookworm (*Necator americanus, Ancylostoma duodenale* and *Anyclostoma ceylanicum*) and *Trichuris trichiura* are the most common soil transmitted helminths (STH), and together constitute the most prevalent neglected tropical disease (NTD) worldwide (Hotez et al., 2014). STH transmission occurs through the faecal-oral route after a period of development in soil, or through direct penetration of the skin by hookworm larvae (Bethony et al., 2006). STH are therefore common in poor communities that lack access to safe water, improved sanitation and appropriate hygiene behaviour (Brooker et al., 2006).

The mainstay of STH control is mass treatment with deworming drugs (World Health Organization, 2017); these kill adult worms but must be given on a regular basis due to reinfection after treatment (Jia et al., 2012). In order to interrupt STH transmission and achieve lasting control, interventions that successfully separate humans from excreta may be required, particularly in high transmission areas (Anderson et al., 2015; Coffeng et al., 2015). Interventions aimed at improving water, sanitation and hygiene (WASH) may therefore represent an important component of integrated control efforts against STH (Freeman et al., 2013b; Campbell et al., 2014).

Interventional studies examining the impact of WASH on STH epidemiology have revealed mixed results. School-based health education programs have been shown to reduce STH incidence and/or intensity in children (Bieri et al., 2013; Gyorkos et al., 2013), and a household level handwashing and nail clipping intervention decreased intestinal parasitic infections, including STH (Mahmud et al., 2015). On the other hand, several studies where community level sanitation interventions were implemented failed to demonstrate an impact on STH infections, likely due to poor intervention uptake (Clasen et al., 2014; Patil et al., 2014).

A small number of studies have examined multi-component WASH interventions. In a pilot trial, a combined sanitation, hygiene and deworming intervention had no impact on STH reinfection rates compared with deworming alone, but higher hookworm egg reduction rates were observed in intervention communities (Hürlimann et al., 2018). A school-based integrated WASH and deworming program reduced reinfection with *A. lumbricoides*, but not other STH, compared with deworming alone (Freeman et al., 2013a). The WASH Benefits study in Kenya found that a combined WASH intervention significantly reduced infection with *A. lumbricoides* (but not other STH) compared with the control group (Pickering et al., Integrating water, sanitation, handwashing, and nutrition interventions to reduce child soil-transmitted helminth and *Giardia* infections: a cluster-randomized controlled trial in rural Kenya. BioRxiv 464917 preprint, https://doi.org/10.1101/464917). In that study, a water only intervention achieved similar reductions in *A. lumbricoides* prevalence, while no impact on prevalence was observed following sanitation only or hygiene only interventions.

In our WASH for WORMS randomised controlled trial (RCT), no additional impact on STH infections was identified as a result of an integrated community level deworming and WASH intervention, compared with the significant reductions achieved by regular deworming of the entire community (Nery et al., 2019). Failure to sustain the sanitation intervention may have been a factor, with 40% of participants in the intervention arm practising open defecation at the end of the trial. Additionally, gradual improvements in WASH conditions were observed in the control arm of the study (Nery et al., 2019).

RCTs provide essential high quality evidence regarding the impact of interventions; however, in practice, implementing WASH improvements is challenging, particularly within a trial context (Campbell et al., 2018). Examining the overall impact of an integrated WASH intervention on STH infections is important, but offers limited insights into the relative contributions of discrete WASH components and practices, which may vary considerably among study participants. Investigating relationships between STH infections and specific WASH-related variables may generate important findings for policymakers seeking to understand what types of tailored interventions might have an impact on STH, and may similarly inform the development of future intervention trials.

Meta-analyses of observational studies have shown reduced odds of STH infection associated with a number of WASH-related variables, including access to and use of sanitation facilities (Ziegelbauer et al., 2012; Strunz et al., 2014), access to improved water (Esrey et al., 1991; Strunz et al., 2014), and handwashing behaviours (Strunz et al., 2014), although these results are not consistent across all studies. A cross-sectional analysis of WASH-related risk factors for STH infection conducted at the WASH for WORMS study baseline detected few WASH variables associated with STH infection (Campbell et al., 2016). It was argued that universally poor WASH conditions may have made it difficult to detect any associations (Campbell et al., 2016).

Although most existing analyses of WASH risk factors for STH infections are cross-sectional in nature, WASH conditions at the time of most recent deworming are likely to be important predictors of infection, in addition to concurrent WASH conditions. This is because reinfection can occur soon after deworming (Jia et al., 2012), and it is known that infective stages of hookworm and *T. trichiura* can persist in soil for up to several months (Udonsi and Atata, 1987; Brooker et al., 2006) and *A. lumbricoides* for several years (Muller, 2002). Furthermore, the prepatent interval (time between initial infection occurring and development of egg-laying adult worms) is up to 12 weeks for *A. lumbricoides* and *T. trichiura* and 8 weeks for hookworm (Bethony et al., 2006).

In the present study, we analyse data from the WASH for WORMS study, using an observational design, to investigate associations between specific WASH-related factors and STH infections. The specific objectives are to investigate the associations between STH infections and: (a) concurrent WASH variables (cross-sectional analysis), and (b) WASH variables 6 months previously, at the time of most recent deworming (longitudinal analysis).

2. Materials and methods

2.1. Study setting and design

This study took place in remote communities in Manufahi municipality, Timor-Leste. Data were collected from residents of the 23 communities initially enrolled in the WASH for WORMS RCT (of which five were sequentially, rather than randomly, allocated to a study arm and were therefore not analysed in the main trial) (Nery et al., 2015).

The WASH for WORMS study methods are described in the published protocol (Nery et al., 2015). Briefly, this was a two-armed cluster RCT in which all communities received deworming treatment with albendazole (administered to all residents over 1 year of age, excluding pregnant women in the first trimester), every 6 months for 2 years. Additionally, in the intervention clusters, WaterAid Australia and their local partners implemented a WASH program, which consisted of: providing access to an improved water source; using a Community Led Total Sanitation-based approach to improve household sanitation; and promoting hand-washing with soap at critical times. Characteristics of the participating communities at the beginning of the study have been published elsewhere (Nery et al., 2015; Campbell et al., 2016).

2.2. Data collection

All community residents aged over 1 year were invited to participate in data collection surveys. These surveys were conducted at baseline and then at four follow-up data collection rounds that occurred at 6 monthly intervals for two years, immediately prior to each deworming round.

Faecal samples were provided by study participants and preserved in 5% (w/v) potassium dichromate. Samples were stored at room temperature and transported for further processing and molecular diagnosis at QIMR Berghofer Medical Research Institute (Brisbane, Australia). We used a multiplex real-time quantitative PCR (qPCR) technique to identify infections with *Ascaris* spp., *N. americanus, Ancylostoma* spp., and *Trichuris* spp. (Llewellyn et al., 2016).

Trained local fieldworkers interviewed participants and heads of households using a structured questionnaire. Information collected included demographic and socio-economic characteristics (age, sex, education, employment, income, and assets), clinical features (history of diarrhoea and deworming), and information related to WASH ownership and behaviour (presence and use of household latrines, defecation practices, availability of water, and hand-washing behaviours). As part of a thorough community census, age and sex were documented for all community members, including those who did not participate in the study. Most information about WASH access and behaviours was collected via self-report, or parental report for young children. Household water sources and the presence, features, and cleanliness of household latrines were directly observed by fieldworkers.

2.3. Statistical analysis

Participants for whom both parasitological and questionnaire data were available at one or more follow-up time points were included in the analysis. A household level wealth index was constructed using principal component analysis as described previously (Campbell et al., 2016), with minor modifications. Scores obtained on the wealth index were divided into quintiles to classify households according to relative poverty.

Due to low prevalence of *Ancylostoma* spp. and *Trichuris* spp., analyses were only performed for *Ascaris* spp. and *N. americanus*, as well as undifferentiated STH infection. We constructed generalised linear mixed models to examine associations between risk factors and infection. Models included random effects for community, household, and participant, to account for clustering at community and household levels, and measurements on the same individuals over time.

Data from all four follow-up time points were included in this analysis. We examined a wide range of WASH and socioeconomic variables as potential predictors; a full list is provided in the Supplementary Data S1. All WASH variables were examined both concurrently (i.e., at the same time point as STH diagnosis) and 6 months previously (i.e., at the previous study time point, when participants were most recently dewormed). Demographic and socioeconomic variables displayed no to minimal variability over the study period, and were examined concurrently only. The study time point was included as a covariate in all analyses to account for the number of deworming rounds that had been delivered. The study intervention was not included as a predictor, because this analysis focused on individual WASH access and behaviours, regardless of study assignment. The experimental findings of the WASH for WORMS RCT have been presented elsewhere (Nery et al., 2019).

To construct the generalised linear mixed models, we investigated multicollinearity between WASH variables using the "collin" command in Stata. Univariable regression models were run for each individual risk factor; these were run separately for concurrent and previous time point predictors. Variables were retained for further analysis if $P < 0.2$. Generalised linear mixed models were then constructed in a two step approach. Firstly, "within domain" multivariable models were constructed for each of 8 risk factor domains: demographic, individual hygiene, individual sanitation, school sanitation, individual socioeconomic, household sanitation, household water, and household socioeconomic variables. These "within domain" models included age, sex, study time point, and all variables retained from univariable analysis for that domain, including both concurrent and previous time point variables. Secondly, variables with $P < 0.1$ from the "within domain" models were retained in a full model and then removed iteratively until only age, sex, study time point, and variables significant at $P < 0.05$ remained in the final model. Analyses were conducted using Stata version 14.1 (College Station, TX, USA).

2.4. Ethics statement

Ethical approval was given by the Human Research Ethics Committees at The University of Queensland (2011000734), The Australian National University (2014/311), and the Timor-Leste Ministry of Health (2011/51). Written consent was provided by participants aged 18 years and older, and parents/guardians of those under 18 years of age. Written assent was provided by participants aged 12–17 years.

3. Results

3.1. Study participants

In total, 2333 individuals were included in this risk factor analysis. A summary of participation over time is shown in Supplementary Table S1. There was a higher proportion of females among participants (51.4%) compared with non-participants (42.6%, $P < 0.001$), mainly due to the fact that men were less likely to provide stool samples than women ($P < 0.001$). Participants ranged in age from 1 year to 94 years, and were slightly younger than non-participants (mean age 26.3 years versus 27.9 years; $P < 0.001$); see Table 1.

WASH characteristics in the study population over time are shown in Table 2. These data were collected following the implementation of the study WASH intervention in 12 of the 23 communities; as a result, overall WASH coverage among the study population was higher than that reported at baseline (Campbell et al., 2016).

At the first study follow-up, the prevalence of *N. americanus* was 33.6% (95% confidence interval (CI) 31.3–36.0), while *Ascaris* spp. prevalence was 17.9% (95% CI 16.1–19.9). As expected given regular deworming in both study arms, prevalence decreased by the end of the study, with final *N. americanus* prevalence of 14.6% (95% CI 12.8–16.5) and *Ascaris* spp. of 10.5% (95% CI 9.0–12.2); see Fig. 1 and Supplementary Table S2.

3.2. Factors associated with STH infections

Univariable analyses revealed a range of WASH, demographic and socioeconomic variables with $P < 0.2$ that were retained for initial (within domain) multivariable models. Supplementary Tables S3–S5 depict the results of univariable analyses of all risk factors examined.

Table 1
Characteristics of study participants.

Characteristic	n (%)
Individual characteristics (n = 2333)	
Age group	
1–5 years	459 (19.7)
6–11 years	488 (20.9)
12–17 years	238 (10.2)
18–64 years	954 (40.9)
65 + years	194 (8.3)
Male sex	1135 (48.7)
For children aged 6–17 (n = 726)	
Attends school	619 (85.3)
For adults aged 18+ (n = 1148)	
Education level	
Never went to school	535 (46.6)
Not finished primary school	197 (17.2)
Finished primary but not secondary	283 (24.7)
Finished secondary or higher	126 (11.0)
Employment	
Not employed outside the home	428 (37.3)
Employed as farmer	629 (55.8)
Employed, other occupation	84 (7.3)
Household characteristics (n = 565)	
At least one child under 5 years of age	257 (45.5)
More than 6 people sharing a dwelling	222 (39.3)

Participation was defined as providing both a questionnaire and stool sample for at least one follow-up time point. Data in this table are from the first time point at which participation was recorded.

3.2.1. Factors associated with N. americanus infection

Results of the final adjusted multivariable model for *N. americanus* are shown in Table 3. The odds of infection decreased with each follow-up, with lowest odds at the final follow-up (adjusted odds ratio (aOR) 0.15, 95% CI 0.11–0.20). All age groups were found to have significantly higher odds of infection compared with children aged 1–5 years, with highest odds among those aged 18–64 years (aOR 7.8, 95% CI 4.7–12.8) and 12–17 years (aOR 6.0, 95% CI 3.7–9.8). Males had significantly higher odds of infection than females (aOR 3.1, 95% CI 2.4–4.2). Adults who obtained at least primary school education had lower odds of infection compared with those who never went to school, with lowest odds among those who completed secondary school or higher (aOR 0.29, 95% CI 0.16–0.53). Farmers had higher odds of infection compared with those who did not work outside the home (aOR 1.7, 95% CI 1.2–2.4). The only WASH variable that was associated with *N. americanus* was having water available for personal cleaning after defecation, which was associated with marginally increased odds of infection (aOR 1.4, 95% CI 1.0–2.0).

3.2.2. Factors associated with Ascaris spp. infection

Results of the final adjusted multivariable model for *Ascaris* spp. are shown in Table 4. Similar to results for *N. americanus*, odds of infection decreased with each follow-up, with odds at the final follow-up nearly two-thirds lower than the first follow-up (aOR 0.37, 95% CI 0.24–0.55). Adults aged 18–64 (aOR 0.37, 95% CI 0.23–0.61) and 65+ years (aOR 0.37, 95% CI 0.19–0.71) had significantly lower odds of infection compared with children aged 1–5 years. Those living in a household with more than six people had higher odds of infection (aOR 1.7, 95% CI 1.2–2.4). Those who finished primary school but not secondary school were found to have higher odds of infection compared with those who never went to school (aOR 2.1, 95% CI 1.2–3.6). In terms of concurrent

Table 2
Water, sanitation and hygiene characteristics over time.

	Follow-up 1, n (%)	Follow-up 2, n (%)	Follow-up 3, n (%)	Follow-up 4, n (%)
Individual sanitation variables	*n = 1598*	*n = 1459*	*n = 1465*	*n = 1412*
Main place of defecation is toilet	768 (48.1)	723 (50.5)	724 (49.4)	813 (57.6)
Practises open defecation	852 (53.4)	802 (55.0)	806 (55.0)	675 (47.8)
Uses water to clean self after defecation	659 (41.2)	635 (43.5)	649 (44.3)	815 (57.7)
Household sanitation variables	*n = 482*	*n = 467*	*n = 442*	*n = 428*
Household has toilet	226 (46.9)	232 (49.7)	224 (50.7)	244 (57.0)
If yes, toilet has slab	102 (45.1)	141 (60.8)	119 (53.1)	125 (51.2)
If yes, toilet is pour-flush latrine	137 (60.6)	171 (73.7)	157 (70.1)	181 (74.2)
If yes, toilet is clean	67 (29.7)	66 (28.5)	42 (18.8)	66 (27.1)
Toilet shared with another dwelling	26 (5.4)	27 (5.8)	28 (6.3)	18 (4.2)
Household garbage disposed of in bush	295 (61.2)	292 (62.5)	296 (67.0)	283 (66.1)
Household garbage buried	54 (11.2)	47 (10.1)	19 (4.3)	21 (4.9)
Household garbage burnt	273 (56.6)	196 (42.0)	174 (39.4)	128 (29.9)
For households with children < 5 years old	*n = 175*	*n = 157*	*n = 161*	*n = 127*
Child waste disposed of hygienically[a]	38 (21.7)	14 (8.9)	24 (14.9)	7 (5.1)
Household water variables	*n = 482*	*n = 467*	*n = 442*	*n = 428*
Main water source				
Piped water	167 (34.7)	240 (51.4)	249 (56.3)	293 (68.5)
Protected spring	13 (2.7)	20 (4.3)	9 (2.0)	8 (1.9)
Tube well/borehole	27 (5.6)	61 (13.1)	30 (6.8)	41 (9.6)
Unprotected spring/dug well	208 (43.2)	59 (12.6)	80 (18.1)	50 (11.7)
Surface water	62 (12.9)	79 (16.9)	70 (15.8)	32 (7.5)
Distance to household water source more than 15 min	124 (25.7)	94 (20.1)	114 (25.8)	95 (22.2)
Water always available from main water source	373 (77.4)	405 (86.7)	270 (83.7)	368 (86.0)
Water stored in covered containers only	431 (89.4)	434 (92.9)	419 (94.8)	393 (91.8)
Household water treated	288 (59.8)	305 (65.3)	259 (58.6)	246 (57.5)
Individual hygiene variables	*n = 1598*	*n = 1459*	*n = 1465*	*n = 1412*
Washes hands using soap or ash	1060 (66.3)	1233 (84.5)	1306 (89.2)	1379 (97.7)
Washes hands before contact with food	745 (46.7)	580 (39.8)	612 (41.8)	709 (50.2)
Washes hands after contact with faeces	956 (59.9)	1127 (77.2)	1192 (81.4)	1243 (88.0)
Washes hands after contact with dirt	939 (58.8)	934 (64.0)	1093 (74.6)	1124 (79.6)
Always wears shoes indoors	493 (30.9)	516 (35.4)	590 (40.3)	694 (49.2)
Always wears shoes outdoors and while toileting	891 (55.8)	802 (55.0)	869 (59.3)	1001 (70.9)

[a] Hygienic disposal defined as disposing of a child's faeces in the household toilet or with household waste.

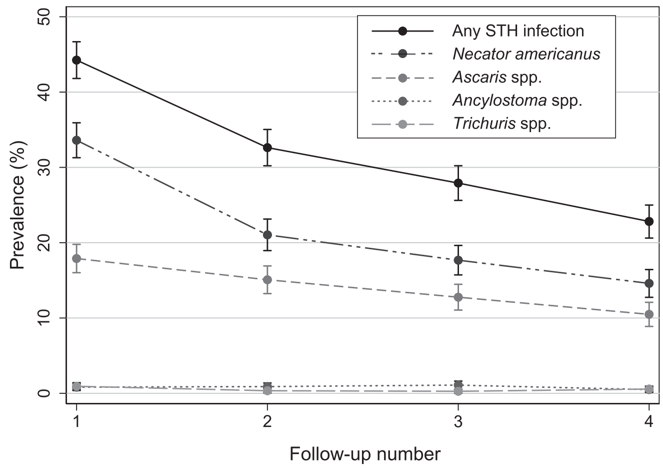

Fig. 1. Prevalence of soil-transmitted helminth infections in the study population over time.

Table 3
Results of final multivariable mixed effects logistic regression examining risk factors for *Necator americanus* infection (n = 2333).

Covariate	aOR	95% CI	*P* value
General variables			
Follow-up number[a]			
Follow-up 2	**0.34**	**0.27–0.44**	**<0.001**
Follow-up 3	**0.24**	**0.18–0.31**	**<0.001**
Follow-up 4	**0.15**	**0.11–0.20**	**<0.001**
Age group[a]			
6–11 years	**3.87**	**2.59–5.76**	**<0.001**
12–17 years	**6.03**	**3.71–9.78**	**<0.001**
18–64 years	**7.76**	**4.69–12.82**	**<0.001**
65 + years	**4.05**	**2.21–7.42**	**<0.001**
Male sex	**3.14**	**2.37–4.16**	**<0.001**
Individual socioeconomic variables			
Education level (adults age 18 + years only)[b]			
Didn't finish primary school	0.79	0.53–1.19	0.259
Finished primary but not secondary school	0.76	0.51–1.12	0.170
Finished secondary school or higher	**0.29**	**0.16–0.53**	**<0.001**
Employment (adults age 18 + years only)[c]			
Employed – farmer	**1.66**	**1.16–2.38**	**0.005**
Employed – other job	1.58	0.85–2.95	0.152
Household sanitation variables			
Water available to clean self after defecating	**1.43**	**1.00–2.03**	**0.050**
Random effects variance (95% CI)			
Community	1.01 (0.51–2.02)		
Household	1.65 (1.20–2.26)		
Participant	2.22 (1.66–2.97)		

aOR, adjusted odds ratio; CI, confidence interval.
Reference categories are as follows: [a]follow-up 1; [b]aged 1–5 years; [c]never went to school; [d]not employed outside the home.
Bold values indicate those reaching statistical significance, defined as $P < 0.05$.

Table 4
Results of final multivariable mixed effects logistic regression examining risk factors for *Ascaris* spp. infection (n = 2333).

Covariate	aOR	95% CI	*P* value
General variables			
Follow-up number[a]			
Follow-up 2	**0.61**	**0.44–0.87**	**0.001**
Follow-up 3	**0.35**	**0.24–0.51**	**<0.001**
Follow-up 4	**0.37**	**0.24–0.55**	**<0.001**
Age group[b]			
6–11 years	0.92	0.63–1.36	0.681
12–17 years	0.70	0.42–1.18	0.184
18–64 years	**0.37**	**0.23–0.61**	**<0.001**
65+ years	**0.37**	**0.19–0.71**	**0.003**
Male sex	1.07	0.82–1.41	0.608
Individual socioeconomic variables			
Education level (adults age 18 + only)[c]			
Didn't finish primary school	1.39	0.77–2.53	0.329
Finished primary but not secondary school	**2.07**	**1.18–3.63**	**0.011**
Finished secondary school or higher	1.81	0.81–4.03	0.197
Household socioeconomic variables			
More than 6 people living in dwelling	**1.70**	**1.18–2.43**	**0.004**
Household sanitation variables			
Household has shared toilet – six months previously	**2.29**	**1.22–4.32**	**0.010**
Household water variables			
Household main water source[d]			
Tube well/borehole	**3.69**	**1.26–10.78**	**0.017**
Unprotected spring/dug well	1.07	0.62–1.88	0.795
Protected spring	1.38	0.55–3.50	0.492
Surface water	1.46	0.61–3.48	0.396
Random effects variance (95% CI)			
Community	5.09 (2.64–9.84)		
Household	0.85 (0.51–1.42)		
Participant	0.58 (0.25–1.37)		

aOR, adjusted odds ratio; CI, confidence interval.
Reference categories are as follows: [a]follow-up 1; [b]aged 1–5 years; [c]never went to school; [d]household main water source is piped water.
Bold values indicate those reaching statistical significance, defined as $P < 0.05$.

WASH variables, using household water from tube wells or boreholes was associated with significantly higher odds of infection (aOR 3.7, 95% CI 1.3–10.8). In terms of WASH variables identified 6 months previously, higher odds of infection were observed among those who used a toilet shared between two or more households (aOR 2.3, 95% CI 1.2–4.3). No other WASH variables were significantly associated with *Ascaris* spp. infection.

3.2.3. Factors associated with undifferentiated STH infection

Results of the final adjusted multivariable model for undifferentiated STH infection are shown in Table 5. Mirroring the results for individual STH species, increasing follow-up time was associated with lower odds of infection. All age groups had higher odds of infection compared with children aged 1–5 years, while adults who finished secondary school or higher had lower odds of infection compared with those who never went to school (aOR 0.40, 95% CI 0.24–0.65). In terms of concurrent WASH variables, those using household water from tube wells or boreholes were again found to have higher odds of infection (aOR 2.5, 95% CI 1.3–4.8). Variables that were associated with lower odds of infection were having a water source more than 15 min walk from the household (aOR 0.74, 95% CI 0.59–0.94) and handwashing before eating (aOR 0.80, 95% CI 0.66–0.96). No other WASH variables were significantly associated with undifferentiated STH infection.

A summary of all significant predictors of infection with *N. americanus*, *Ascaris* spp., and undifferentiated STH infection is provided in Table 6.

Table 5
Results of final multivariable mixed effects logistic regression examining risk factors for undifferentiated soil-transmitted helminth infection (n = 2333).

Covariate	aOR	95% CI	*P* value
General variables			
Follow-up number[a]			
Follow-up 2	**0.44**	**0.35–0.55**	**<0.001**
Follow-up 3	**0.31**	**0.25–0.39**	**<0.001**
Follow-up 4	**0.21**	**0.76–0.28**	**<0.001**
Age group[b]			
6–11 years	**2.08**	**1.54–2.80**	**<0.001**
12–17 years	**2.72**	**1.86–3.98**	**<0.001**
18–64 years	**3.62**	**2.57–5.09**	**<0.001**
65+ years	**1.94**	**1.26–2.97**	**0.002**
Male sex	**2.34**	**1.90–2.87**	**<0.001**
Individual socioeconomic variables			
Education level (adults age 18 + only)[c]			
Didn't finish primary school	0.87	0.61–1.25	0.454
Finished primary but not secondary school	0.89	0.64–1.25	0.512
Finished secondary school or higher	**0.40**	**0.24–0.65**	**<0.001**
Individual hygiene variables			
Washes hands before eating	**0.80**	**0.66–0.96**	**0.020**
Household water variables			
Household main water source[d]			
Tube well/borehole	**2.54**	**1.34–4.84**	**0.004**
Unprotected spring/dug well	1.09	0.79–1.52	0.594
Protected spring	1.72	0.88–3.37	0.112
Surface water	1.20	0.75–1.93	0.447
Water source ≥ 15 min walk from household	**0.74**	**0.59–0.94**	**0.014**
Random effects variance (95% CI)			
Community	1.83 (0.97–3.49)		
Household	1.06 (0.78–1.44)		
Participant	1.37 (1.01–1.86)		

aOR, adjusted odds ratio; CI, confidence interval.
Reference categories are as follows: [a]follow-up 1; [b]age 1–5 years; [c]never went to school; [d]household main water source is piped water.
Bold values indicate those reaching statistical significance, defined as $P < 0.05$.

4. Discussion

This risk factor analysis was conducted to further investigate the role of WASH on STH infections in the context of the WASH for WORMS intervention trial. A novel component of our analysis was that we examined WASH-related risk factors both concurrently and 6 months prior, at the time of the previous deworming round. In a previous cross-sectional analysis conducted at the study baseline, few WASH variables were associated with STH infections (Campbell et al., 2016). The analysis presented here included data from four study follow-up time points over a 2 year period, with more diverse WASH conditions compared with the study baseline. Nonetheless, only a small number of WASH-related variables were found to be associated with STH infections in this analysis. The odds of infection with both *N. americanus* and *Ascaris* spp. decreased significantly with increasing follow-up time, reflecting the impact of regular, community-wide deworming.

Although water contamination is not thought to play a crucial role in the STH transmission cycle, meta-analysis has demonstrated that piped water is protective against multiple STH species (Strunz et al., 2014), and the recent WASH Benefits study in Kenya found that an intervention aimed at improving water quality was protective against *Ascaris* spp. (Pickering et al., BioRxiv 464917 preprint, cited earlier). Our results also provide some evidence that water source affects the risk of STH infection, lending support to suggestions that contaminated water may play a more important role in transmission than previously recognised (Pickering et al., BioRxiv 464,917 preprint, cited earlier). Using water from a tube well or borehole was associated with significantly higher odds of *Ascaris* spp. and undifferentiated STH infection, while those using a water source more than 15 min walk from the household had lower odds of undifferentiated STH infection. Tube wells and boreholes are considered protected water sources; however, in study communities, these were predominantly used when a community's usual water source was unavailable (i.e., in the dry season). Our results suggest that water contamination may have occurred at the source or during collection. Similarly, the protective effect of water sources located further from the household likely represents decreased faecal contamination.

In terms of sanitation, previous meta-analyses have found that sanitation access and use are protective against both *A. lumbricoides* (Ziegelbauer et al., 2012; Strunz et al., 2014) and hookworm (Ziegelbauer et al., 2012), although results are not consistent across all studies, and these findings have not been mirrored in community level sanitation intervention studies (Clasen et al., 2014; Patil et al., 2014). In our analysis, using a shared latrine (shared between two or more households) 6 months previously was associated with higher odds of *Ascaris* spp. infection, possibly due to increased faecal contamination. Surprisingly, having water available for personal cleaning after defecation was marginally associated with higher odds of *N. americanus* infection. This may

Table 6
Summary of factors associated with *Necator americanus*, *Ascaris* spp. and undifferentiated soil-transmitted helminth infection in final adjusted multivariable models.

Variable domain	*N. americanus*	*Ascaris* spp.	Undifferentiated STH infection
General variables	*Study follow-up number* Age groups 6–11, 12–17, 18–64 and 65 + years[a] Male sex	*Study follow-up number* *Age groups 18–64 and 65 + years*[a]	*Study follow-up number* Age groups 6–11, 12–17, 18–64 and 65 + years[a] Male sex
Individual socioeconomic variables	*Did not finish primary school*[b] Employed as farmer[c]	Finished primary but not secondary school[b]	*Did not finish primary school*[b]
Household socioeconomic variables	–	More than 6 people living in household	
Household sanitation variables	Water available to clean self after defecating	Household has shared toilet	
Household water variables	–	Tube well/borehole as main water source[d]	Tube well/borehole as main water source[d] *Water source ≥ 15 minutes' walk from household*
Individual hygiene variables	–	–	*Washes hands before contact with food*

Italics indicates that variable is associated with decreased odds of infection.
Reference categories are as follows: [a]aged 1–5 years; [b]never went to school; [c]not employed outside the home; [d]household main water source piped water.

represent a chance finding, or may relate to skin contact with water containing infective filariform hookworm larvae (that can penetrate the skin) while washing after defecation. No other sanitation-related variables emerged as significant predictors in our analysis. The lack of association between most sanitation variables and STH infections is likely due to persistent open defecation by a proportion of individuals throughout the study period. Ongoing open defecation within the community allows environmental contamination and transmission to be maintained, such that even those who regularly use a latrine can still be exposed to contaminated soil and become reinfected with STH.

A protective effect of handwashing behaviour on STH has been previously identified in both observational (Strunz et al., 2014) and intervention studies (Mahmud et al., 2015). Our analysis identified lower odds of any STH infection in individuals who reported handwashing before eating, but this protective effect was not seen for individual STH species, and other handwashing variables were not significant. Given our reliance on self-reported hygiene behaviours, this may reflect a degree of measurement error. Previous work has shown that participants may exaggerate responses that reflect more desirable WASH practices (Manun'Ebo et al., 1997), and indeed, hygiene behaviours such as handwashing with soap and handwashing after defecation were reported by more than 75% of participants in our study at the baseline (Nery et al., 2019). A more reliable method of measuring handwashing behaviours may detect a more consistent protective effect against STH. However, structured observations can also lack validity (Ram et al., 2010). Identifying accurate, unbiased methods for measuring WASH behaviour remains a significant challenge.

Demographic and socioeconomic factors were strongly associated with STH infections. Consistent with known age- and sex-related patterns of STH infection (Brooker et al., 2004; Bethony et al., 2006), *N. americanus* infections were highest among males and adults, while *Ascaris* spp. infections were higher among children. Aligned with findings at the study baseline (Campbell et al., 2016), adults with higher levels of education had lower odds of *N. americanus* infection, reflecting that STH disproportionately affect the most disadvantaged people (Holland et al., 1988). Individuals living in a dwelling with more than six people had increased odds of *Ascaris* spp. infection, implicating a role of overcrowding in environmental contamination and transmission. Similar findings have been described previously (Holland et al., 1988; Scolari et al., 2000). Importantly, those working as farmers had increased odds of *N. americanus* infection. This finding can be explained by occupational exposure to contaminated soil (Brooker et al., 2004), and suggests that an educational component tailored specifically to adults working in high risk occupations could be of benefit in community level WASH interventions.

A limitation of this observational analysis is the likelihood of residual confounding. Although we included a wide range of WASH and sociodemographic variables in our analysis, it is likely that other, unmeasured factors contributed to the risk of STH infections. A further limitation is that most sanitation and hygiene variables were self-reported by participants, and in the case of young children, by their parents. As discussed above, self-reported data are prone to bias. This potential source of measurement error decreases the reliability of detected associations (or the lack thereof) between WASH and STH. Finally, it is possible that the highly sensitive diagnostic technique used to detect STH infections may have led to weakening of associations between WASH and STH, due to the detection of very light-intensity infections that would be missed by more conventional microscopy-based approaches (Campbell et al., 2016).

In summary, this risk factor analysis found that only a small number of WASH variables were associated with STH infections, with regular deworming, socioeconomic variables, and demographic factors representing the major predictors of infection. Compared with a cross-sectional analysis conducted at the study baseline (Campbell et al., 2016), the current analysis identified more WASH associations in the context of more diverse WASH conditions. However, many WASH variables were not associated with STH infection, and identified WASH-related risk and protective factors were not consistent across STH species. These findings align with the overall study results of the WASH for WORMS trial, in which no additional impact of the community-based WASH intervention on STH infections was seen, compared with deworming alone (Nery et al., 2019).

STH life cycles rely on individuals being exposed to a faecally-contaminated environment; thus, a link between WASH and STH transmission is undisputable. In practice, however, generating evidence for the impact of WASH interventions, access and practices on STH infections remains challenging. Contributing factors include the complexity of implementing WASH interventions in low income settings, the challenge of achieving sustained behavioural change, and the difficulties in accurately measuring WASH behaviours (Campbell et al., 2018).

Despite these challenges, further research should be undertaken to elucidate the impact of WASH on STH. The focus should be on generating evidence to inform policymakers and program implementers in the WASH and NTD sectors regarding what kinds of interventions, in what settings, and over what period of time, could be expected to have an impact on STH infections. Particularly important research priorities include investigating the role of WASH in settings with low STH transmission, once regular deworming has ceased (Coffeng et al., 2018), and research into simple, reproducible strategies to measure WASH behaviours, including strategies that involve evaluating environmental contamination with STH infective stages (Gyawali et al, 2016; Steinbaum et al, 2017). Beyond research, fostering collaboration between the WASH and NTD sectors remains crucial as global efforts towards sustainable control continue.

Acknowledgements

This work was supported by a National Health and Medical Research Council (NHMRC; Australia) Partnership project [grant number 1013713] with WaterAid Australia. NEC is supported by an Australian Government Research Training Program Scholarship, ACAC and AJV are supported by NHMRC Senior Research Fellowships, JSM is an Australian NHMRC Practitioner Fellow and DJG is an Australian NHMRC Career Development Fellow. The authors would like to acknowledge the Ministry of Health of Timor-Leste for operational support; Salvador Amaral and the WASH for WORMS field staff, and data clerks for data collection and data entry; WaterAid's and their partner NGOs' field staff for implementation of the WASH intervention; Stacey Llewellyn for laboratory diagnosis; and all of the communities for participating in the trial.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2018.12.006.

References

Anderson, R.M., Turner, H.C., Truscott, J.E., Hollingsworth, T.D., Brooker, S.J., 2015. Should the goal for the treatment of soil-transmitted helminth (STH) infections be changed from morbidity control in children to community-wide transmission elimination? PLoS Negl. Trop. Dis. 9, e0003897.

Bethony, J., Brooker, S., Albonico, M., Geiger, S.M., Loukas, A., Diemert, D., Hotez, P.J., 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 367, 1521–1532.

Bieri, F.A., Gray, D.J., Williams, G.M., Raso, G., Li, Y., Yuan, L., He, Y., Li, R.S., Guo, F., Li, S., McManus, D.P., 2013. Health-education package to prevent worm infections in Chinese schoolchildren. New Engl. J. Med. 368, 1603–1612.

Brooker, S., Bethony, J., Hotez, P.J., 2004. Human hookworm infection in the 21st century. Adv. Parasitol. 58, 197–288.

Brooker, S., Clements, A.C., Bundy, D.A., 2006. Global epidemiology, ecology and control of soil-transmitted helminth infections. Adv. Parasitol. 62, 221–261.

Campbell, S.J., Savage, G.B., Gray, D.J., Atkinson, J.M., Magalhaes, R.J.S., Nery, S.V., McCarthy, J.S., Velleman, Y., Wicken, J.H., Traub, R.J., Williams, G.M., Andrews, R. M., Clements, A.C.A., 2014. Water, sanitation, and hygiene (WASH): a critical component for sustainable soil-transmitted helminth and schistosomiasis control. PLoS Negl. Trop. Dis. 8, e2651.

Campbell, S.J., Nery, S.V., D'Este, C.A., Gray, D.J., McCarthy, J.S., Traub, R.J., Andrews, R.M., Llewellyn, S., Vallely, A.J., Williams, G.M., Amaral, S., Clements, A.C.A., 2016. Water, sanitation and hygiene related risk factors for soil-transmitted helminth and *Giardia duodenalis* infections in rural communities in Timor-Leste. Int. J. Parasitol. 46, 771–779.

Campbell, S.J., Biritwum, N., Woods, G., Velleman, Y., Fleming, F., Stothard, J.R., 2018. Tailoring water, sanitation, and hygiene (WASH) targets for soil-transmitted helminthiasis and schistosomiasis control. Trends Parasitol. 34, 53–63.

Clasen, T., Boisson, S., Routray, P., Torondel, B., Bell, M., Cumming, O., Ensink, J., Freeman, M., Jenkins, M., Odagiri, M., Ray, S., Sinha, A., Suar, M., Schmidt, W., 2014. Effectiveness of a rural sanitation programme on diarrhoea, soil-transmitted helminth infection, and child malnutrition in Odisha, India: a cluster-randomised trial. Lancet Glob. Health 2, e645–e653.

Coffeng, L.E., Bakker, R., Montresor, A., de Vlas, S.J., 2015. Feasibility of controlling hookworm infection through preventive chemotherapy: a simulation study using the individual-based WORMSIM modelling framework. Parasit. Vectors 8, 541.

Coffeng, L.E., Nery, S.V., Gray, D.J., Bakker, R., de Vlas, S.J., Clements, A.C.A., 2018. Predicted short and long-term impact of deworming and water, hygiene, and sanitation on transmission of soil-transmitted helminths. PLoS Negl. Trop. Dis. 12, e0006758.

Esrey, S.A., Potash, J.B., Roberts, L., Shiff, C., 1991. Effects of improved water supply and sanitation on ascariasis, diarrhoea, dracunculiasis, hookworm infection, schistosomiasis, and trachoma. Bull. World Health Organ. 69, 609–621.

Freeman, M.C., Clasen, T., Brooker, S.J., Akoko, D.O., Rheingans, R., 2013a. The impact of a school-based hygiene, water quality and sanitation intervention on soil-transmitted helminth reinfection: a cluster-randomized trial. Am. J. Trop. Med. Hyg. 89, 875–883.

Freeman, M.C., Ogden, S., Jacobson, J., Abbott, D., Addiss, D.G., Amnie, A.G., Beckwith, C., Cairncross, S., Callejas, R., Colford Jr, J.M., 2013b. Integration of water, sanitation, and hygiene for the prevention and control of neglected tropical diseases: a rationale for inter-sectoral collaboration. PLoS Negl. Trop. Dis. 7, e2439.

Gyawali, P., Ahmed, W., Sidhu, J.P.S., Nery, S.V., Clements, A.C., Traub, R., McCarthy, J. S., Llewellyn, S., Jagals, P., Toze, S., 2016. Quantitative detection of viable helminth ova from raw wastewater, human feces, and environmental soil samples using novel PMA-qPCR methods. Environ. Sci. Pollut. Res. 23, 18639–18648.

Gyorkos, T.W., Maheu-Giroux, M., Blouin, B., Casapia, M., 2013. Impact of health education on soil-transmitted helminth infections in schoolchildren of the Peruvian Amazon: a cluster-randomized controlled trial. PLoS Negl. Trop. Dis. 7, e2397.

Holland, C.V., Taren, D.L., Crompton, D.W.T., Nesheim, M.C., Sanjur, D., Barbeau, I., Tucker, K., Tiffany, J., Rivera, G., 1988. Intestinal helminthiases in relation to the socioeconomic environment of Panamanian children. Soc. Sci. Med. 26, 209–213.

Hotez, P.J., Alvarado, M., Basáñez, M., Bolliger, I., Bourne, R., Boussinesq, M., Brooker, S.J., Brown, A.S., Buckle, G., Budke, C.M., Carabin, H., Coffeng, L.E., Fèvre, E.M., Fürst, T., Halasa, Y.A., Jasrasaria, R., Johns, N.E., Keiser, J., King, C.H., Lozano, R., Murdoch, M.E., O'Hanlon, S., Pion, S.D.S., Pullan, R.L., Ramaiah, K.D., Roberts, T., Shepard, D.S., Smith, J.L., Stolk, W.A., Undurraga, E.A., Utzinger, J., Wang, M., Murray, C.J.L., Naghavi, M., 2014. The global burden of disease study 2010: interpretation and implications for the neglected tropical diseases. PLoS Negl. Trop. Dis. 8, e2865.

Hürlimann, E., Silué, K.D., Zouzou, F., Ouattara, M., Schmidlin, T., Yapi, R.B., Houngbedji, C.A., Dongo, K., Kouadio, B.A., Koné, S., Bonfoh, B., N'Goran, E.K., Utzinger, J., Acka-Douabélé, C., Raso, G., 2018. Effect of an integrated intervention package of preventive chemotherapy, community-led total sanitation and health education on the prevalence of helminth and intestinal protozoa infections in Côte d'Ivoire. Parasit. Vectors 11, 115.

Jia, T.W., Melville, S., Utzinger, J., King, C.H., Zhou, X.N., 2012. Soil-transmitted helminth reinfection after drug treatment: a systematic review and meta-analysis. PLoS Negl. Trop. Dis. 6, e1621.

Llewellyn, S., Inpankaew, T., Nery, S.V., Gray, D.J., Verweij, J.J., Clements, A.C., Gomes, S.J., Traub, R., McCarthy, J.S., 2016. Application of a multiplex quantitative PCR to assess prevalence and intensity of intestinal parasite infections in a controlled clinical trial. PLoS Negl. Trop. Dis. 10, e0004380.

Mahmud, M.A., Spigt, M., Bezabih, A.M., Pavon, I.L., Dinant, G., Velasco, R.B., 2015. Efficacy of handwashing with soap and nail clipping on intestinal parasitic infections in school-aged children: a factorial cluster randomized controlled trial. PLoS Med. 12, e1001837.

Manun'Ebo, M., Cousens, S., Haggerty, P., Kalengaie, M., Ashworth, A., Kirkwood, B., 1997. Measuring hygiene practices: a comparison of questionnaires with direct observations in rural Zaire. Trop. Med. Int. Health 2, 1015–1021.

Muller, R., 2002. Chapter 5: The nematodes. In: Muller, R. (Ed.), Worms and, Human Disease. CABI, London.

Nery, S.V., McCarthy, J.S., Traub, R., Andrews, R.M., Black, J., Gray, D., Weking, E., Atkinson, J., Campbell, S., Francis, N., Vallely, A., Williams, G., Clements, A., 2015. A cluster-randomised controlled trial integrating a community-based water, sanitation and hygiene programme, with mass distribution of albendazole to reduce intestinal parasites in Timor-Leste: the WASH for WORMS research protocol. BMJ Open 5, e009293.

Nery, S.V., Traub, R.J., McCarthy, J.S., Clarke, N.E., Amaral, S., Llewellyn, S., Weking, E., Richardson, A., Campbell, S.J., Gray, D.J., Vallely, A.J., Williams, G.M., Andrews, R. M., Clements, A.C.A., 2019. WASH for WORMS, a cluster randomized controlled trial of the impact of a community integrated WASH and deworming intervention on soil-transmitted helminth infections Am. J. Trop. Med. Hyg. 100, 750–761.

Patil, S.R., Arnold, B.F., Salvatore, A.L., Briceno, B., Ganguly, S., Colford, J.M., Gertler, P. J., 2014. The effect of India's total sanitation campaign on defecation behaviors and child health in rural Madhya Pradesh: a cluster randomized controlled trial. PLoS Med. 11, e1001709.

Ram, P.K., Halder, A.K., Granger, S.P., Jones, T., Hall, P., Hitchcock, D., Wright, R., Nygren, B., Islam, M.S., Molyneaux, J.W., Luby, S.P., 2010. Is structured observation a valid technique to measure handwashing behavior? Use of acceleration sensors embedded in soap to assess reactivity to structured observation. Am. J. Trop. Med. Hyg. 83, 1070–1076.

Scolari, C., Torti, C., Beltrame, A., Matteelli, A., Castelli, F., Gulletta, M., Ribas, M., Morana, S., Urbani, C., 2000. Prevalence and distribution of soil-transmitted helminth (STH) infections in urban and indigenous schoolchildren in Ortigueira, State of Paranà, Brasil: implications for control. Trop. Med. Int. Health 5, 302–307.

Steinbaum, L., Kwong, L.H., Ercumen, A., Negash, M.S., Lovely, A.J., Njenga, S.M., Boehm, A.B., Pickering, A.J., Nelson, K.L., 2017. Detecting and enumerating soil-transmitted helminth eggs in soil: New method development and results from field testing in Kenya and Bangladesh. PLoS Negl. Trop. Dis. 11, e0005522.

Strunz, E.C., Addiss, D.G., Stocks, M.E., Ogden, S., Utzinger, J., Freeman, M.C., 2014. Water, sanitation, hygiene, and soil-transmitted helminth infection: a systematic review and meta-analysis. PLoS Med. 11, e1001620.

Udonsi, J., Atata, G., 1987. *Necator americanus*: temperature, pH, light, and larval development, longevity, and desiccation tolerance. Exp. Parasitol. 63, 136–142.

WHO, 2017. Preventive chemotherapy to control soil-transmitted helminth infections in at-risk population groups. World Health Organization, Geneva.

Ziegelbauer, K., Speich, B., Mausezahl, D., Bos, R., Keiser, J., Utzinger, J., 2012. Effect of sanitation on soil-transmitted helminth infection: systematic review and meta-analysis. PLoS Med. 9, e1001162.