Ascaris and hookworm transmission in preschool children from rural Panama: role of yard environment, soil eggs/larvae and hygiene and play behaviours

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SUMMARY

This study explored whether the yard environment and child hygiene and play behaviours were associated with presence and intensity of *Ascaris* and hookworm in preschool children and with eggs and larvae in soil. Data were collected using questionnaires, a visual survey of the yard, soil samples and fecal samples collected at baseline and following re-infection. The presence of eggs/larvae in soil was associated negatively with water storage (eggs) but positively with dogs (eggs) and distance from home to latrine (larvae). Baseline and re-infection prevalences were: hookworm (28·0%, 3·4%); *Ascaris* (16·9%, 9·5%); *Trichuris* (0·9%, 0·7%). Zero-inflated negative binomial regression models revealed a higher baseline hookworm infection if yards had eggs or larvae, more vegetation or garbage, and if the child played with soil. Baseline *Ascaris* was associated with dirt floor, dogs, exposed soil in yard, open defecation and with less time playing outdoors, whereas *Ascaris* re-infection was associated with water storage, vegetation cover and garbage near the home and not playing with animals. Our results show complex interactions between infection, the yard environment and child behaviours, and indicate that transmission would be reduced if latrines were closer to the home, and if open defecation and water spillage were reduced.

Key words: soil-transmitted helminths, *Ascaris*, hookworm, soil eggs and larvae, vegetation cover, water storage, latrines, open defecation, soil contact, landscape of yard.

INTRODUCTION

Infections with *Ascaris lumbricoides*, hookworms and *Trichuris trichiura* are often acquired in areas where children play (Gyorkos *et al*. 1994; Chongsuvivatwong *et al*. 1999; Mohd Zain *et al*. 2014), and are of particular concern as they impair physical and cognitive development in preschool children (Hall *et al*. 2008). As intestinal nematodes enter the environment with fecal matter and thrive in areas with poor sanitary conditions, risk of these infections is often associated with poor sanitation infrastructure, lack of water at the home and dirt floors (Quintero *et al*. 2012; Strunz *et al*. 2014). Also, other aspects of the environment around the home may contribute to the survival of eggs and larvae, and subsequent risk of infection with these parasites. Vegetation and shade cover can increase relative humidity and lower ultraviolet radiation and thus improve survival and development of eggs and larvae (Gaasenbeek and Borgsteede, 1998; Maikai *et al*. 2008). Vegetation can also protect eggs and larvae from the scattering effects of heavy rainfall (Mizgajska, 1993). Domestic animals, particularly pigs, chickens and dogs, may also increase the risk of infection if they disperse human feces around the yard (Olsen *et al*. 2001; Traub *et al*. 2002; Shalaby *et al*. 2010). In addition, fecal matter from pigs can be a source of *Ascaris* (Blaszkowska *et al*. 2011), as occasional transmission of ascarids between humans and pigs has been reported (Peng *et al*. 1996; Zhou *et al*. 2012; Betson *et al*. 2014).

Child behaviours can also influence risk of infection, if they alter the likelihood of fecal contamination of the yard environment or of contact with soil. Open defecation has been shown to increase infection risk (Nishiura *et al*. 2002; Quihui *et al*. 2006; Kattula *et al*. 2014), whereas use of a latrine has been shown to reduce infection risk (Ziegelbauer *et al*. 2012; Strunz *et al*. 2014). Nematode infections are less common or of lower intensity in children who wash their hands after defecation and before eating (Balen *et al*. 2011; Schmidlin *et al*. 2013; Strunz *et al*. 2014) and hookworm transmission is reduced

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by wearing shoes (Alemu *et al*. 2011; Al-Delaimy *et al*. 2014; Muñoz-Antoli *et al*. 2014; Tomczyk *et al*. 2014). There is limited evidence that geophagy (eating soil) is associated with increased nematode infections (Geissler *et al*. 1998; Nishiura *et al*. 2002), as preschool children who intentionally eat soil, who explore their environment by putting soil and other materials in their mouths, or who spend more time outside may be at increased risk of infection.

Surprisingly, there is a remarkable paucity of studies that consider the combined impact of these diverse factors on risk of nematode infection. Although many surveys consider whether presence of a latrine or a water faucet in the yard is associated with risk of soil-transmitted nematode infections (see review by Strunz *et al*. 2014), they do not consider the impact of distance of the latrine or faucet from the home or whether nematode eggs and larvae are present in the soil. Although some surveys consider whether child defecation and hand-washing behaviours influence infection (see review by Strunz *et al*. 2014), they do not also consider the impact of play behaviours or presence of eggs and larvae in soil where children play. The importance of vegetation cover has been linked to broad-scale infection patterns (Saathoff *et al*. 2005*a*, *b*; Oluwole *et al*. 2015), but not to transmission at the scale of the yard. To our knowledge, no one considers all these variables together. Finally, Strunz *et al*. (2014) note that most studies focus on presence or absence of infection rather than intensity of infection, and that few studies directly examine factors influencing transmission by considering short-term re-infection rates following drug treatment. This is especially true for infections in preschool children. The present study addresses these gaps in the literature.

This study was conducted in a subsistence farming setting in rural Panama, and had two objectives: (1) to determine whether the features of the yard (presence and location of latrine and water faucet, vegetation cover near the home and domestic animals) and preschool child defecation practices were associated with the presence of eggs and larvae of parasitic nematodes in soil where the children play and near the yard faucet; and (2) to determine whether the above features of the yard, child hygiene and play behaviours and/or presence of eggs and larvae in soil influenced soil-transmitted nematode infection and re-infection following drug treatment.

METHODS

Study area and population

The study was conducted in 15 rural communities selected in the province of Veraguas, central Panama because of their participation in an agricultural intervention (*VERASAN*) of the Panama Ministry of Health (a necessary condition for our larger research investigation). All communities were accessible only by 4-wheel drive vehicle and were 45 min – 2 h away from the provincial capital of Santiago. Homes were 1 or 2 room buildings, constructed of concrete block or mud walls, with tin or palm roofs and dirt or concrete floors. Average per capita income earned was <$5/month. Households practiced subsistence agriculture, primarily growing rice, maize, cassava, plantains, a variety of local tubers and vegetables including cucumbers, tomatoes, squash and peppers. Each community had a primary school, and a few communities had a secondary school and/or a health post which was visited by medical staff on a regular basis.

In 2012, average temperature in the region was 27·6 °C (range: 26·6–29·2 °C), average relative humidity was 76·8% (range: 63·1–84·0%), annual rainfall was 2238·3 mm and there were 138 days of rain, with a daily average rainfall of 6·1 mm (INEC, 2014). The normal rainy season is from April to early December with the heaviest rainfall usually in November; however, in 2012 the rainiest month was June, during which 347·8 mm of rain fell (INEC, 2014).

Study design

This was a longitudinal observational study. Questionnaires provided information on household demographics and hygiene and play behaviours of one randomly selected index preschool child per household. A visual survey was used to describe the physical environment of the yard. Fecal samples collected from the index child in February–March and September–October 2012 provided data on prevalence and intensity of *Ascaris, Trichuris* and hookworm infections at baseline and at re-infection following anthelmintic treatment in June–July. Soil samples collected from the play area and the faucet area were examined for nematode eggs and larvae during the re-infection period.

Participant recruitment and ethics

Sample size estimation for multiple regression analysis was based on an alpha of 0·05, a power of 0·80 and an effect size of 0·10 for models including 12 independent variables. Assuming 25% loss to follow-up, we attempted to recruit 250 households in order to achieve the required sample of 184 households.

Households were included, if they had at least one child between 6 months and 5 years at the beginning of the study and if at least one member of the household had participated in the *VERASAN* agricultural program, a criterion relevant to other components of the larger research study. All preschool children were enrolled in the study, and a single index child was chosen randomly after data collection for inclusion in the statistical analyses.

The study was approved by the Internal Review Board of the Faculty of Medicine of McGill University in Canada, and the National Research Bioethics Committee of the Gorgas Commemorative Institute for the Study of Health in Panama. Permission from the national and regional directors of the Ministry of Health was obtained before visiting the study communities to explain the proposed research, answer questions and obtain verbal permission from the community. Households were formally recruited during the first visit to collect data, and then the research was again explained, any remaining questions were answered and signed consent was obtained from the responsible adult present on behalf of the household and the participating preschool children.

Questionnaires on socio-demographic information and child play behaviours

Questionnaires administered to the primary caregiver provided information about preschool child age and sex, caregiver age and education level, number of people and number of children ⩽12 years in the household, presence of a latrine and water faucet in the yard, and household wealth as quantified by an asset-based Household Wealth Index, following methods developed by Filmer and Pritchett (2001) and Vyas and Kumaranayake (2006). Caregivers were asked to report on each preschool child's hygiene practices which included: where they defecated, whether they regularly wore shoes while outside, whether they washed their hands before eating and whether they washed with soap. They were also asked how much time each preschool child spent playing outside each day, whether they sat in the soil while playing, whether they played with animals or soil and whether they were ever seen putting soil in their mouth.

Visual survey of home and yard characteristics

Home construction materials, the distance of the latrine and water faucet from the home, and whether water or animal manure was stored in the yard were recorded. The area within 5 m of the home was characterized by the number of trees and items of garbage, by coverage with vegetation (low-growing herbaceous plants and taller shrubs) or exposed soil (not covered with vegetation, large stones or concrete), and area shaded at noon, each estimated by eye and recorded as 0, 1–5, 6–25, 26–50, 51–75, 76–95 or 96–100%. For the purpose of data analysis, a binary variable (⩽50 *vs* >50% coverage) was used.

Nematode eggs and larvae in soil samples

One soil sample was collected from a place where the preschool children often played, typically hard-packed soil within 5 m of the home. A second soil sample was collected from the damp area within 50 cm of the yard water faucet (if present). Samples were 100 g in weight ($10 \times 10 \times 1$ cm^3 deep), collected using a hand trowel and placed in individually-labelled ziplock bags that were immediately refrigerated. They were stored at 4 °C prior to analysis at the University of Panama for helminth eggs and larvae using formal-ether flotation of a subsample of 10 g of soil. Samples that could not be analysed within a week were preserved in formalin and stored at 4 °C for up to 4 weeks. *Ascaris* and *Toxocara* eggs were identified to the level of genus. Larval nematodes were identified (Eaton *et al*. 2005) as hookworms (rhabditiform and filariform larvae of the Family Ancylostomidae), parasitic filariform larvae of the genus *Strongyloides*, and unidentified parasitic filariform larvae, all of which were included in counts of parasitic larvae. Presence of unidentified rhabditiform larvae was also recorded, but was not included in our statistical analyses because they may have been free-living or parasitic larvae.

Nematode infections in preschool children

Caregivers brought fecal samples collected from preschool children to the school at baseline, and following two 3·5-month re-infection intervals. Samples were immediately refrigerated and analysed within 24 h by trained laboratory technicians. Nematode eggs were identified and counted using the FLOTAC flotation technique (Utzinger *et al*. 2008), and the intensity of infection was recorded as eggs per gram (epg) of feces. All children were treated with albendazole, according to WHO guidelines: 200 mg to children 12–23 months, and 400 mg to children ⩾24 months (children <12 months were not treated) (WHO, 2006). We were unable to collect post-treatment fecal samples to confirm that treatment had cleared infection but a single dose of albendazole has been found to be very effective for *Ascaris* (WHO, 2006). Given the lower efficacy of albendazole against hookworm and *Trichuris* (WHO, 2006), all children infected with hookworm or *Trichuris* were retreated for 3 consecutive days, starting within 10 days of the sample collection, according to WHO guidelines (WHO, 2006), to be reasonably sure that all children were cleared of their infections.

Statistical analysis

Statistical analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) with statistical significance established at $P < 0{\cdot}05$. One index preschool child was randomly chosen from each household and used throughout the analyses. Data are reported as means ± S.E. or percentages with 95% confidence intervals.

The presence of parasitic eggs (*Ascaris* spp. and *Toxocara* spp.) and larvae (hookworm, *Stronglyoides* and unidentified filariform larvae) was compared between play and faucet area soil samples using McNemar's test. Logistic regression and contingency tables were used to explore bivariate relationships with environmental variables. Stepwise multiple logistic regression models were developed to explore factors influencing the presence of both parasitic nematode eggs and larvae in soil samples collected both from child play areas and faucet areas. Initial play area models included sanitation infrastructure variables (presence of latrine and water faucet, distance of latrine from home, whether water was stored in the yard), environmental variables (>50% vegetation, exposed soil and shade cover, number of trees and garbage within 5 m of the house, storage of manure and number of domestic animals at the home) and child defecation practices (only used the latrine, defecated in yard sometimes). Initial faucet area models also included the distance of the faucet from the home. Variables with $P < 0{\cdot}15$ were retained in the final models.

Spearman correlation analysis was done to determine if baseline and re-infection epgs were correlated. Multiple logistic regression models adjusted for child age and sex were used as an initial step in exploring the impact of environmental characteristics and child behaviours on presence or absence of *Ascaris* and hookworm in preschool children at baseline and of *Ascaris* re-infection in September–October. Analysis for hookworm re-infection was not done due to very low re-infection rates. In addition, zero-inflated negative binomial multiple regression models were used to examine baseline *Ascaris* and hookworm infections and *Ascaris* re-infection data. This type of model is recommended (Strunz *et al*. 2014) because the logistic component differentiates between infected and uninfected children, and the negative binomial component explores intensity of infection (epg) among those that are infected. Variables initially explored in the models included basic demographic information (child age and sex, caregiver age and education, number of people in the household, number of children 12 years and younger in the household, Household Wealth Index), home sanitation and infrastructure (dirt floors, latrine, water piped to a faucet in the yard, distance of latrine and faucet from the home, water storage in the yard), environmental characteristics of the yard (vegetation cover, exposed soil, shade cover, trees, garbage, manure storage, domestic animals), child hygiene and play behaviours (only used the latrine, defecated in yard sometimes, washed hands, used soap, wore shoes, played outdoors, sat in the soil, played with soil or animals, put soil in the mouth) and occurrence of parasitic nematode eggs and larvae in the soil. Final models included presence of latrine and faucet but not distances from the home. Interaction terms were included for child behaviours that may have been modified by yard characteristics and for variables with counterintuitive estimates. Variables with $P < 0{\cdot}15$ were retained in the final models. No interaction terms were retained in the final models.

RESULTS

Of the 208 households recruited at baseline, 27 (13%) were lost to follow-up; they did not differ in socio-demographic (caregiver age and education, child age and infection status, household size, Household Wealth Index) or infection characteristics from those that completed the study (data not shown). An additional 21 households joined the study in June–July and they were included in the assessment of nematode re-infection. Data from soil samples and a yard survey were obtained from 167 households.

Description of home and yard environment

The majority of homes had dirt floors (84·8%), latrines were present at 82·7% of homes and water was piped to a faucet in the yard at 64·9% of homes. When present, the water faucet was closer to the home (10·4 ± 0·9 m) than the latrine (22·0 ± 1·1 m). Most homes (89·2%) stored water in large plastic buckets (often covered). The ground near the faucet and the water storage buckets was usually damp.

With regard to the area within 5 m of the home, >50% was covered by vegetation (low-growing herbaceous plants and taller shrubs) at 23·4% of homes, >50% was exposed soil at 49·1% of homes, and >50% was shaded at noon at 9·6% of homes (Table 1; Fig. 1). Trees (3·5 ± 0·3) and items of garbage (mostly plastic refuse) (16·1 ± 0·4) were also observed within 5 m of the home (Table 1). Only 17·4% of households stored manure for fertilizer in the yard.

The majority of households owned dogs (82·6%) and chickens (97·6%), and 25·1% owned pigs (Table 1). Chickens and dogs were allowed to run freely, often in and out of the house, whereas pigs were considered a more valuable investment and were securely tied out of the way of daily activities.

Child hygiene and outdoor play behaviours

Nearly half (46·4%) of index preschool children only used a latrine and 33·0% openly defecated in the yard (Table 1). Among hygiene practices, 91·8% of

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Table 1. Characteristics of home and sanitation infrastructure, the environment within 5 m of the home, and hygiene and play behaviours of index preschool children in rural Panama

	N	% (95% CI) or mean ± S.E.
Home and sanitation infrastructure		
Dirt floor (%)	229	84·8% (79·6–89·2)
Latrine in yard (%)	229	82·7% (77·2–87·3)
Water piped to faucet in yard (%)	229	64·9% (58·4–71·1)
Distance from home to latrine (m)	135	22·0 ± 1·1
Distance from home to faucet (m)	114	10·4 ± 0·9
Stores water in yard (%)	167	89·2% (83·5–93·5)
Environment within 5 m of home	167	
>50% vegetation cover (%)		23·4% (17·2–30·5)
>50% exposed soil (%)		49·1% (41·3–56·9)
>50% shaded at noon (%)		9·6% (5·6–15·1)
Trees (#)		3·5 ± 0·3
Items of garbage (#)		16·1 ± 0·4
Stores manure for fertilizer (%)		17·4% (12·0–24·0)
Domestic animals		
Dogs (%; #)		82·6% (76·0–88·1); 1·7 ± 0·1
Chickens (%; #)		97·6% (94·0–99·3); 10·9 ± 0·7
Pigs (%; #)		25·1% (18·8–32·4); 0·4 ± 0·1
Child hygiene behaviours	194	
Always uses latrine (%)		46·4% (39·2–53·7)
Defecates in yard sometimes (%)		33·0% (26·4–40·1)
Washes hands before eating (%)		91·8% (87·0–95·2)
Washes hands with soap (%)		85·6% (79·8–90·2)
Wears shoes (%)		67·0% (59·9–73·6)
Child play behaviours	188	
Plays outside (h/day)		3·6 ± 0·2
Sits in the soil while playing (%)		75·5% (68·7–81·5)
Plays with animals (%)		25·0% (19·0–31·8)
Plays with soil (%)		34·6% (27·8–41·8)
Puts soil in mouth (%)		3·7% (0·2–7·5)

children washed their hands before eating, 85·6% washed their hands with soap, and 67·0% wore shoes.

Children played outdoors an average of 3·6 ± 0·2 h day^{-1}, usually within 5 m of the home (personal observation), and 75·5% sat in the soil while they played. Caregivers reported that 25·0% of the children played with animals and that 34·8% played with soil but that only 3·7% were seen putting soil in their mouth. None of the hygiene or play behaviours were significantly associated with preschool child age (data not shown).

Contamination of soil with nematode eggs and larvae

The per cent of soil samples containing nematode eggs or larvae was similar between samples from the play area and those from the area around the water faucet.

Soil samples from play area. Only a few soil samples from the play area contained eggs of *Ascaris* spp. (4·2%) or *Toxocara* spp. (3·0%) (Fig. 2) and one sample had eggs of *Taenia* spp. (0·4%) The unadjusted odds of finding nematode eggs in soil samples from the play area were lower if the water faucet was farther from the home (OR = 0·819, $P = 0·043$). In the multiple logistic regression model, homes that stored water were less like likely to have nematode eggs in soil samples from the play area (OR = 0·209; Table 2).

Parasitic nematode larvae were found in 28·1% of soil samples near where children played; 16·2% of samples had hookworm larvae, and 2·4% had filariform larvae of *Strongyloides* spp. (Fig. 2). In addition, unidentified filariform larvae were found in 4·2% of soil samples from the play area. None of the socio-demographic, water, sanitation, home environment, animal or defecation behaviour variables altered the unadjusted odds of finding parasitic nematode larvae in the play area samples (data not shown) or emerged in the multiple logistic regression model at the $P < 0·05$ cut-off. However, play area soil samples from yards with >50% exposed soil tended to be more likely to have larvae (OR = 2·024; $P = 0·066$; Table 2).

Soil samples from the water faucet area. Samples from the water faucet area contained nematode eggs (*Ascaris* spp., 8·5%; *Toxocara* spp., 1·1%) (Fig. 2) as well as Fasciolidae eggs (0·4%). The likelihood of finding nematode eggs in soil samples from the water faucet area was higher if the household owned more dogs, both in the simple logistic

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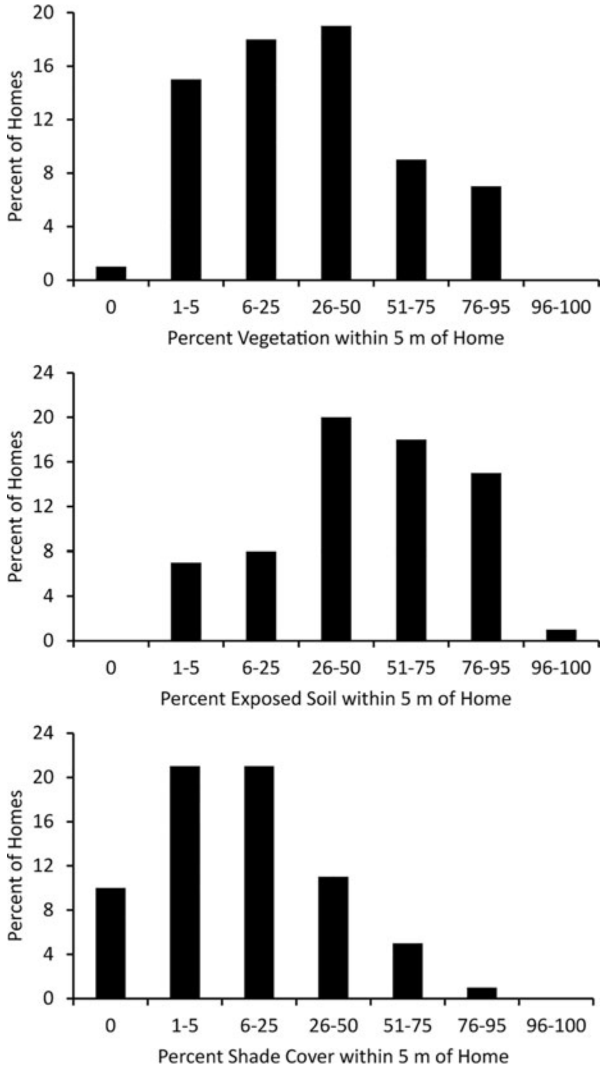

Fig. 1. Frequency distribution of environmental characteristics of participant yards within 5 m of the home: % vegetation cover (A), % exposed soil (B) and % shaded at noon (C).

regression (OR = 1·678, $P = 0·013$) and in the multiple logistic regression model (OR = 1·616; Table 2).

Among the homes with a water faucet, 36·2% of soil samples from the water faucet area contained nematode larvae: 22·3% of samples had hookworm larvae, 2·1% had filariform larvae of *Strongyloides* spp., and 9·6% had unidentified filariform larvae (Fig. 2). The likelihood of finding nematode larvae in the soil sample from the water faucet area was higher if the latrine was farther from the home both in the simple logistic regression (OR = 1·045, $P = 0·049$) and in the multiple logistic regression model (OR = 1·052; Table 2).

Child intestinal infections

Hookworm, *Ascaris* and *Trichuris* prevalences in index preschool children were highest at baseline at 28·0, 16·9 and 0·9%, respectively (Table 3). Re-infection prevalences following albendazole treatment in June–July were 3·4, 9·5 and 0·7%, respectively. There was no association between baseline and re-infection epg for any of the nematodes. Because of the low prevalence of *Trichuris* throughout the study, it was not investigated further.

Baseline hookworm models. Adjusting for age and sex, index children in households with a water faucet in the yard had lower odds of hookworm infection at baseline (OR = 0·374, $P = 0·012$). The zero-inflated negative binomial model for hookworm at baseline (Table 4) showed that presence of hookworm (logistic component of model) was positively associated with vegetation covering >50% of the area within 5 m of the home ($\beta = 1·644$) and with the child playing with soil ($\beta = 1·169$). The negative binomial component of the model revealed that hookworm epg was higher if the yard had more items of garbage ($\beta = 0·066$) and if parasitic nematode eggs or larvae were found in the soil where the child played ($\beta = 0·839$). Because of the low prevalence of hookworm following re-infection, a model for re-infection was not developed.

Baseline Ascaris *models.* At baseline, adjusting for age and sex, index children who openly defecated at least sometimes in the yard had higher odds of infection with *Ascaris* (OR = 3·422, $P = 0·012$). The zero-inflated negative binomial model (Table 4) showed that the presence of *Ascaris* infection (logistic component of model) was positively associated with a dirt floor in the home ($\beta = 2·202$) and the number of dogs ($\beta = 1·078$), and negatively associated with the time the child spent playing outdoors ($\beta = -0·376$) whereas *Ascaris* epg (negative binomial component of model) was higher if >50% of the area within 5 m of the home was exposed soil (not covered with vegetation, large stones or concrete) ($\beta = 2·654$), if the household owned more dogs ($\beta = 0·982$), and if the index child openly defecated at least sometimes in the yard ($\beta = 1·884$).

Re-infection Ascaris *models.* Following re-infection, the adjusted odds of presence of *Ascaris* was higher if >50% of the area within 5 m of the home had exposed soil (OR = 9·505, $P = 0·041$). The zero-inflated negative binomial model (Table 4) showed that *Ascaris* re-infection (logistic component) was positively associated with water storage at the home ($\beta = 3·053$) and that *Ascaris* epg following re-infection (negative binomial component) was higher if >50% of the area within 5 m of the home was covered with vegetation (low-growing herbaceous plants and taller shrubs) ($\beta = 3·721$) and in yards with more garbage ($\beta = 0·162$), but lower if the child played with animals ($\beta = -2·812$).

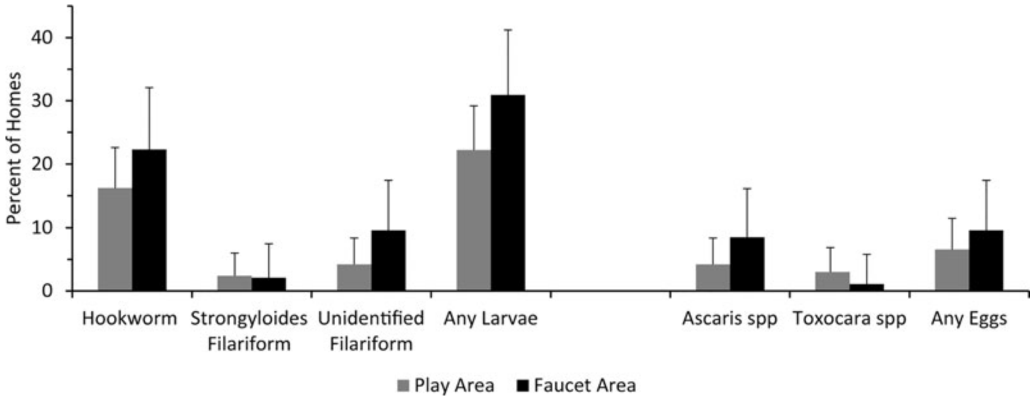

Fig. 2. Occurrence of different types of parasitic nematode larvae and eggs in yard soil samples collected from where preschool children play ($n = 167$) and from around the water faucet ($n = 93$). Error bars are the upper 95% confidence interval.

Table 2. Stepwise multiple logistic regression of parasitic nematode larvae and eggs in soil samples from the play area and around the faucet

	Play area		Faucet area	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Model for larvae	*n* = *162*		*n* = *78*	
>50% exposed soil within 5 m of home	2·024 (0·954–4·294)	0·066	NS	
Stores water (Y/N)	NS		0·180 (0·020–1·655)	0·130
Distance of latrine to home (m)	NS		1·052 (1·002–1·104)	0·041
Model for eggs	*n* = *129*		*n* = *81*	
Stores water (Y/N)	0·209 (0·047–0·935)	0·041	NS	
Dogs (#)	1·351 (0·930–1·961)	0·114	1·616 (1·018–2·567)	0·042
Trees (# within 5 m of home)	NS		1·166 (0·959–1·418)	0·124
Garbage (# within 5 m of home)	NS		1·218 (0·943–1·575)	0·131

NS, non-significant; OR, odds ratio.
Larvae include hookworm filariform and rhabditiform larvae, filariform larvae of *Strongyloides* spp., and unidentified filariform larvae. Eggs include *Ascaris* spp. and *Toxocara* spp.
Other variables explored that did not emerge in any models: water faucet in yard, distance of faucet from home, latrine in yard, >50% vegetation or >50% shaded at noon within 5 m of home, stores manure in yard, presence and number of chickens and pigs, child defecates in yard sometimes.

DISCUSSION

The present study had several strengths, compared with others that have attempted to identify risk factors of *Ascaris* and hookworm infection in children. As highlighted in recent reviews (Ziegelbauer *et al*. 2012; Cundill *et al*. 2013; Strunz *et al*. 2014), few previous studies have considered landscape features in the yard around the home, most have distinguished between infected and uninfected children rather than also considering intensity of infection, and very few have examined factors affecting re-infection following drug treatment. We included not only socio-demographic variables and presence of a latrine and water faucet but also landscape features in the yard, the location of the latrine and water faucet, child hygiene and play behaviours and presence of domestic animals. Data were obtained on both prevalence and intensity of infection and re-infection in preschool children and zero-inflated multiple regression models were used to account simultaneously for presence of infection and epg. Finally, the relationship between these variables and estimates of soil contamination with nematode eggs and larvae was considered.

Several key findings emerged. (1) The presence of a latrine did not lower the prevalence or intensity of *Ascaris* or hookworm infection or the presence of eggs and larvae in soil samples. Instead, transmission was associated with open defecation, children playing with soil, eggs/larvae in soil and dirt floors. In addition, greater distance of the latrine from the home increased the likelihood of finding nematode larvae near the water faucet. (2) Transmission was not associated with the presence of a water faucet in the yard. Instead, storage of water in the yard was positively associated with infection but negatively associated with finding nematode eggs where children played. Garbage also increased hookworm and *Ascaris* epgs

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Table 3. Preschool index child infection prevalence (% and 95% CI), intensity (mean ± S.E.) and maximum eggs per gram (epg) at baseline (February–March 2012) and after 3·5 months re-infection between June–July and September–October 2012

	Baseline	Re-infection
n	*189*	*199*
Hookworm		
Prevalence (%)	28·0 (21·8, 35·0)	3·4 (1·1, 7·7)
Intensity (epg)	85 ± 25	4·3 ± 2·2
Maximum (epg)	3744	192
Ascaris		
Prevalence (%)	16·9 (11·9, 23·1)	9·5 (5·3, 15·4)
Intensity (epg)	267 ± 115	1530 ± 1307
Maximum (epg)	14 312	193 248
Trichuris		
Prevalence (%)	0·9 (0·1, 3·3)	0·7 (0·0, 3·7)
Intensity (epg)	0·5 ± 0·5	0·5 ± 0·5
Maximum (epg)	96	80

Table 4 Zero-inflated negative binomial regression models for hookworm and *Ascaris* infection in index preschool children at baseline (February–March 2012) and for *Ascaris* re-infection between June–July and September–October 2012

	Hookworm baseline		*Ascaris* baseline		*Ascaris* re-infection	
	β	*P*	*β*	*P*	*β*	*P*
N	*111*		*111*		*102*	
Logistic portion						
Intercept	1·570	0·018	−0·504	0·638	4·574	0·021
Dirt floor (Y/N)	NS		2·202	0·011	NS	
Stores water (Y/N)	NS		NS		3·053	0·023
Latrine in yard (Y/N)	0·905	0·131	NS		NS	
>50% vegetation cover near home (Y/N)	1·644	0·014	1·764	0·118	1·935	0·148
Items of garbage near home (#)	NS		NS		−0·182	0·082
Dogs (#)	NS		1·078	0·002	0·662	0·115
Child always uses latrine (Y/N)	NS		1·103	0·108	NS	
Child plays outdoors (h/day)	NS		−0·376	0·010	NS	
Child plays with soil (Y/N)	1·169	0·038	NS		NS	
Negative binomial portion						
Intercept	3·237	0·001	7·464	<0·001	−0·541	0·838
Caregiver age (year)	NS		NS		0·091	0·055
Faucet in yard (Y/N)	NS		1·121	0·131	NS	
>50% vegetation cover near home (Y/N)	NS		NS		3·721	<0·001
>50% exposed soil near home (Y/N)	NS		2·654	<0·001	NS	
>50% shaded at noon near home (Y/N)	−1·131	0·065	NS		NS	
Items of garbage near home (#)	0·066	0·021	NS		0·162	0·025
Dogs (#)	NS		0·982	0·016	NS	
Child defecates in yard sometimes (Y/N)	NS		1·884	0·003	NS	
Child plays with animals (Y/N)	NS		NS		−2·812	0·003
Child plays with soil (Y/N)	−0·853	0·071	NS		NS	
Nematode larvae/eggs in play area (Y/N)	0·839	0·022	NS		NS	

NS, not significant.The zero-inflated negative binomial model distinguishes uninfected from infected children in the logistic portion, and intensity (epgs) among infected children in the negative binomial portion.
The following variables were initially explored but were not included in the final models: socio-demographic information (child age and sex, caregiver education, number of people in the household, number of children ⩽12 years in the household, Household Wealth Index), home sanitation and infrastructure (distance of latrine and faucet from home), environmental characteristics of the yard (trees, manure storage and domestic animals), child hygiene and play behaviours (child washed hands, used soap, wore shoes, sat in the soil, put soil in the mouth).

perhaps because it trapped water. (3) Vegetation cover near the home was positively associated with hookworm infection and with *Ascaris* re-infection epg.

In contrast to recent meta-analyses showing that access to sanitation reduced the odds of *Ascaris* (overall OR = 0·62; CI = 0·44–0·88) (Strunz *et al*.

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2014) and that combined availability and use of sanitation facilities for safe disposal of feces and urine reduced the odds of *Ascaris* (overall OR = 54; CI = 0·43–0·69) and hookworm infection (overall OR = 0·60, 95% CI = 0·48–0·75) (Ziegelbauer *et al*. 2012), presence of latrines did not directly affect presence or intensity of either hookworm or *Ascaris* infection or presence of nematode eggs or larvae in soil samples in our multiple regression analyses. The apparent ineffectiveness of latrines in preventing infection may have been a function of the distance of the latrine from the home. Compared with the WHO recommendation (1996) that latrines be a minimum of 6 m from the home to minimize odours reaching the home but to facilitate ease of access by children and at night (Steinmann *et al*. 2010), latrines were on average 22 m from the home. Although latrines did not enter models, perhaps because over 80% of homes had a latrine, our data show that four indicators of fecal contamination (dirt floors, open defecation, playing with soil, and eggs/larvae in soil) contributed to nematode infections in the preschool children. As reported elsewhere (Gamboa *et al*. 2009; Quintero *et al*. 2012), presence of *Ascaris* at baseline was associated with dirt floors. Also, the positive association between open defecation and *Ascaris* epg at baseline extends previous studies based on presence of infection where open defecation increased the likelihood of *Ascaris* infection in school children in Pakistan (Nishiura *et al*. 2002), of soil-transmitted helminths in school children from south India (Kattula *et al*. 2014) and of intestinal parasites of school children in Mexico (Quihui *et al*. 2006). Although open defecation *per se* did not enter the hookworm model, children who played with soil were more likely to be infected with hookworms, and presence of nematode eggs and larvae in soil samples from the play area was positively associated with hookworm epg. Finally the fact that soil samples taken near the water faucet were more likely to contain nematode larvae if the latrine was farther from the home also suggests the importance of fecal contamination of the yard environment. Given that faucets were generally located in a primary drainage point for the yard, we suggest that nematode larvae may have accumulated around the water faucet.

Several observations indicate that presence of a water faucet did not reduce the risk of nematode infection. First, neither the presence of a water faucet nor distance of the faucet from the home emerged in multiple regression models for hookworm infection or *Ascaris* infection or re-infection. Furthermore, we found no evidence that washing hands before eating or washing hands with soap influenced *Ascaris* or hookworm infection, perhaps because so many children were reported to wash their hands. This is in contrast to a recent meta-analysis that reported that the odds of *Ascaris* infection were reduced both by the presence of a water faucet (overall OR = 0·40; 95% CI = 0·39–0·41) and by hand-washing after defecation (overall OR = 0·45, 95% CI = 0·35–0·58) (Strunz *et al*. 2014). Instead, our results indicate that stored water in the yard promoted transmission, as *Ascaris* re-infection was more likely if households stored water. Even though the large plastic water storage containers were usually covered, the ground around them was often wet. Furthermore, hookworm epgs and *Ascaris* re-infection epgs were positively associated with the number of pieces of garbage in the yard. Garbage has been identified as a risk factor for soil-transmitted nematode infections (Fonseca *et al*. 2010) not only because it attracts flies that disperse *Ascaris* eggs (Adenusi and Adewoga, 2013) but also because it collects water that, if spilt would dampen the soil and prolong survival of nematode eggs and larvae (Smith, 1990; Gaasenbeek and Borgsteede, 1998). Interestingly, both storage of water and having a faucet farther from the home lowered the odds of finding nematode eggs in soil samples from the play area perhaps because water flowed downhill from the play area near the home towards the faucet that was typically 10 m from the home.

Our consideration of the natural landscape of the yard provided support for its importance in nematode transmission as children whose yards had >50% vegetation cover within 5 m of the home were more likely to be infected with hookworm and to have higher *Ascaris* re-infection epgs. Large-scale satellite imaging has shown that hookworm and *Ascaris* infections are positively associated with vegetation cover both in South Africa (Saathoff *et al*. 2005*a*, *b*) and in Nigeria (Oluwole *et al*. 2015). Our results complemented these larger-scale studies by demonstrating that the relationship between vegetation cover and child infection also operated at the household level. This relationship can be explained by the better survival of hookworm larvae in the shade (Udonsi and Atata, 1987) and by the persistence of *Ascaris suum* eggs (and presumably *A. lumbricoides* eggs) under protective vegetation cover (Bergstrøm and Langeland, 1981; Mizgajska, 1993). Shade and vegetation provide higher relative humidity, lower ultraviolet radiation, lower land surface temperatures and protect against the scattering effects of heavy rainfall, all which promote survival of eggs and larvae (Gaasenbeek and Borgsteede, 1998; Maikai *et al*. 2008; Schüle *et al*. 2014).

Our results also highlight somewhat unexpected associations between domestic animals and nematode infection in the children. First, the number of dogs present in the home was positively associated with presence of nematode eggs including *Toxocara* (presumably *T. canis*) in soil samples from the faucet area. This is in contrast to findings

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that presence of dogs reduced the odds of finding helminth eggs in soil samples from Nigerian playgrounds (Maikai *et al*. 2008). Second, *Ascaris* re-infection epgs were lower in children who played with animals, but Nigerian preschool children were more likely to be infected with *Ascaris* in households that owned a dog (Kirwan *et al*. 2009). It is possible that contact with zoonotic infections around the home may have provided cross-protection against *Ascaris* as non-specific serological cross-reactivity has been reported between *Ascaris* and the dog ascarid, *Toxocara canis* (Nicholas *et al*. 1984; Fan and Su, 2004). This would be consistent with our finding that children who spent more time playing outside were less likely to be infected with *Ascaris*. This warrants further investigation. Third, we did not find an association of *Ascaris* infection with the number of pigs even though children of families that raise livestock are at higher risk of *Ascaris* infection (Wang *et al*. 2012) and even though cross-transmission of *Ascaris suum* from pigs and *A. lumbricoides* from humans occurs (Zhou *et al*. 2012). This is likely because pigs in these communities were tied up at a distance from the house, limiting the spatial overlap of pigs and children.

We acknowledge the following limitations. Because we only collected one soil sample from each area and because we only sampled two areas in the yard, it is likely that we underestimated the presence of nematode eggs and larvae in soil as their spatial distribution tends to be highly aggregated (Hominick *et al*. 1987). Furthermore, as unidentified rhabditiform larvae from soil samples could not be distinguished as free-living or parasitic and were therefore excluded from our counts of parasitic larvae, the occurrence of parasitic larvae in soil samples was underestimated. Also, neither eggs nor larvae could be identified to species with the diagnostic techniques that were available to us and therefore we could not distinguish between human and zoonotic species of *Ascaris*, hookworms or *Strongyloides*. Despite these limitations, we were able to detect significant relationships between environmental characteristics of the yard and presence of eggs and larvae in soil samples, and between presence of eggs and larvae in the soil and hookworm infections in the preschool children. An additional limitation was the low rate of hookworm re-infection which precluded us from exploring factors influencing hookworm re-infection. Given logistical constraints that prevented us from collecting fecal samples shortly after albendazole treatment, it is possible that some children remained infected. However, we found no correlation between baseline and re-infection epgs, intensity of infections was low, the efficacy of albendazole against *Ascaris* is high (WHO, 2006) and all children with hookworm infections received follow-up treatment. Therefore it is unlikely that children remained infected after treatment. Our inability to detect a relationship of having a latrine at home or washing hands with hookworm or *Ascaris* infection may have been due to the high percentage of households with latrines and of children who washed their hands. On the other hand, the large number of homes with latrines enabled us to show that odds of detecting larvae in soil samples increased with distance of the latrine from the home. This added an important nuance to our understanding of the relationship between sanitation infrastructure and risk of transmission. Finally, data on child hygiene and play behaviour were collected solely through questionnaires to caregivers, and we acknowledge the possibility of reporting bias, particularly for those behaviours that caregivers may have preferred not to mention, such as putting soil in the mouth (Simpson *et al*. 2000).

In summary, fecal contamination is one of the key determinants of transmission of intestinal nematodes, most frequently represented by evidence that children are less likely to be infected in homes with latrines (Ziegelbauer *et al*. 2012; Strunz *et al*. 2014). Our study highlighted the importance of open defecation behaviours and presence of nematode eggs and larvae in the soil rather than presence of a latrine, perhaps because of the long distance of the latrine from the home. Access to piped water in the yard is also considered central in prevention of soil-transmitted nematodes (Strunz *et al*. 2014). Our data showed that presence of stored water in the yard rather than presence of a faucet in the yard or hand washing behaviours, explained transmission in this setting. This, together with the important role of vegetation cover, suggests that a better understanding of transmission dynamics can be obtained by exploring factors such as soil moisture that enhance survival of eggs and larvae in soil. Based on our results, we suggest that transmission in this setting could be reduced by placing latrines closer to the homes, by reducing water spillage and soil moisture in areas where children play, and by reducing open defecation.

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