Parasitology International 62 (2013) 172–180

Contents lists available at SciVerse ScienceDirect

Parasitology International

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

Ascaris lumbricoides and *Trichuris trichiura* infections associated with wastewater and human excreta use in agriculture in Vietnam

Phuc Pham-Duc [a,b,c,d,e,*], Hung Nguyen-Viet [a,b,e], Jan Hattendorf [b,c], Jakob Zinsstag [b,c], Cam Phung-Dac [d], Christian Zurbrügg [e], Peter Odermatt [b,c]

[a] *Center for Public Health and Ecosystem Research (CENPHER), Hanoi School of Public Health (HSPH), 138 Giang Vo, Hanoi, Vietnam*
[b] *Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, P.O. Box, CH-4002 Basel, Switzerland*
[c] *University of Basel, P.O. Box, CH-4003 Basel, Switzerland*
[d] *National Institute of Hygiene and Epidemiology (NIHE), 1 Yersin, Hanoi, Vietnam*
[e] *Swiss Federal Institute of Aquatic Science and Technology (EAWAG), Sandec, Department of Water and Sanitation in Developing Countries, P. O. Box, CH-8600 Dübendorf, Switzerland*

ARTICLE INFO

Article history:
Received 3 September 2012
Received in revised form 14 December 2012
Accepted 19 December 2012
Available online 8 January 2013

Keywords:
Helminth infections
Ascaris lumbricoides
Trichuris trichiura
Wastewater
Excreta
Agriculture
Vietnam

ABSTRACT

Background: We assessed the risk of helminth infections in association with the use of wastewater and excreta in agriculture in Hanam province, northern Vietnam. In two cross-sectional surveys, we obtained samples from 1,425 individuals from 453 randomly selected households. Kato-Katz thick smear and formalin-ether concentration techniques were used for helminth diagnosis in two stool samples per person. Socio-demographic and water, sanitation and hygiene related characteristics, including exposure to human and animal excreta and household wastewater management, were assessed with a questionnaire.

Results: Overall 47% of study participants were infected with any helminth (*Ascaris lumbricoides* 24%, *Trichuris trichiura* 40% and hookworm 2%). Infections with intestinal protozoa were rare (i.e. *Entamoeba histolytica* 6%, *Entamoeba coli* 2%, *Giardia lamblia* 2%, *Cryptosporidium parvum* 5% and *Cyclospora cayetanensis* 1%). People having close contact with polluted Nhue River water had a higher risk of helminth infections (odds ratio [OR] = 1.5, 95% confidence interval [CI] 1.1–2.2) and *A. lumbricoides* (OR = 2.1, 95% CI 1.4–3.2), compared with those without contact. The use of human excreta for application in the field had an increased risk for a *T. trichiura* infection (OR = 1.5, 95% CI 1.0–2.3). In contrast, tap water use in households was a protective factor against any helminth infection (i.e. *T. trichiura* OR = 0.6, 95% CI 0.4–0.9). Prevalences increased with age and males had generally lower prevalences (OR = 0.8, 95% CI 0.6–1.0), participants performing agricultural (OR = 1.5, 95% CI 1.1–2.1) and having a low educational level (OR = 1.7, 95% CI 1.2–2.4) were significantly associated with helminth infections. None of the factors related to household's sanitary condition, type of latrine, household's SES, use of animal excreta, and personal hygiene practices were statistically significant associated with helminth infection.

Conclusions: Our study suggests that in agricultural settings, direct contact with water from Nhue River and the use of human excreta as fertiliser in the fields are important risk factors for helminth infection. Daily use of clean water is likely to reduce the risk of worm infection. Deworming policies and national programs should give more attention to these agricultural at risk populations.

1. Introduction

In agricultural production the use of wastewater and excreta is a widespread practice with a long tradition in many countries around the world [1], in particular in China, South and South East Asia as well as various settings in Africa [2–4]. The sources of irrigation water in Vietnam vary from fresh water and wastewater to ground water [5]. Nearly all rural households in north and central regions of Vietnam use excreta as fertiliser in agriculture [6]. Wastewater and excreta have many benefits for agricultural users such as valuable and reliable water resources and nutrients, but they may have negative impacts on human health [7–9]. Most common health risks related with wastewater and excreta use are diarrhoeal diseases and soil-transmitted helminthiases (STH) [9,10].

STH are common worldwide with more than a billion people infected [11,12]. Estimates suggest that *Ascaris lumbricoides* infects over 1 billion people, *Trichuris trichiura* 795 million, and hookworms (*Ancylostoma duodenale* and *Necator americanus*) 740 million [13]. In tropical and sub-tropical countries, distribution of STH is linked with the lack of sanitation and poverty [14,15]. In Vietnam, estimated 39.9 million (44.4%) people are infected with *A. lumbricoides*; 17.6 million (23.1%) with *T. trichiura* [16,17], and 19.8 million (22.1%) with

* Corresponding author at: University of Basel, P.O. Box, CH-4003 Basel, Switzerland.
E-mail addresses: phuc.pham-duc@unibas.ch (P. Pham-Duc), hung.nguyen@unibas.ch (H. Nguyen-Viet), jan.hattendorf@unibas.ch (J. Hattendorf), jakob.zinsstag@unibas.ch (J. Zinsstag), cam@fpt.vn (C. Phung-Dac), christian.zurbrugg@eawag.ch (C. Zurbrügg), peter.odermatt@unibas.ch (P. Odermatt).

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http://dx.doi.org/10.1016/j.parint.2012.12.007

hookworm [17]. High prevalence of helminth infection is found in rural areas of northern Vietnam, which is possibly related with the common use of excreta as fertiliser in the fields [18–23]; and also associated with the high population density, differences in climatic condition and humidity [24].

Prevalence of and risk factors for helminth infections have been studied in Vietnam [18,19,23,25–29]. However, only a few studies focused on the relationship between helminth infection and exposure to wastewater such as handling practices and use of wastewater and excreta in agriculture, environmental factors, and personal hygiene practices.

This study aimed at determining the prevalence of helminth infections among people living in an agricultural community, where an intensively polluted river (i.e. Nhue River) is used to irrigate fields and where human and animal excreta serve as fertiliser in agriculture and fish breeding. The main focus of the study was relative contribution of exposure to wastewater and excreta to helminth infections. Two cross-sectional studies were performed in these agricultural communities.

2. Material and methods

2.1. Study area

The study was carried out in Nhat Tan and Hoang Tay communes in Kim Bang district, Hanam province (20.32° N, 105.54° E), northern Vietnam, situated about 60 km south of Hanoi (Fig. 1). Nhat Tan and Hoang Tay communes count 10,500 (2700 households) and 5500 (1500 households) inhabitants, respectively. Most households have livestock in their compounds. The residential areas are in the vicinity of fields used for rice cultivation, vegetable planting and fish breeding. The rice fields and local ponds cover about 50% of the surface. The two communes border on the Nhue River. Hanoi City's wastewater from households, industry and other sources such as hospitals is directly and untreated discharged into the river [30]. The Nhue River water is used for crop irrigation and to feed fishponds. Several pump stations located along the river and a system of open and closed canals distribute the water to fields and fish ponds. Wastewater from household (grey water from kitchens and bathrooms, and effluent from septic tanks and sanitation facilities) is directly discharged into the small irrigation canals.

The area has two main rice production cycles per year, one called "spring season" from January to June and the other "autumn season" from July to October. Human and animal excreta are used as fertiliser in Hanam as in many other places in northern and central Vietnam. In general, excreta from double or single vault latrines are not or partially composted. Personal protective measures to prevent contamination are often lacking.

2.2. Study design

Two cross-sectional surveys were carried out in the rainy season from July to October 2008 and in the dry season from April to June 2009. A total of 15 villages in Nhat Tan and 10 villages in Hoang Tay communes were selected to participate in the study. For each cross-sectional survey, 270 households were randomly selected from all 4282 household on the list provided by the Communal People's Committee. None of the household was selected twice. The sample size was calculated based on an 80% expected proportion of household use of wastewater in agriculture, a precision of 5% and a 95% confidence level. All household members above 12 months of age were eligible.

2.3. Data collection

Questionnaires on household and personal level with five sections were administered to all households members: (i) general demographic information and socio-economic status (SES): age, sex, educational level, occupation, household's economic status were assessed with a list of indicators which included surface of household's rice field and fish ponds, number of animals (pig, chickens, ducks, buffalos, cows, dogs and cats), housing characteristics (building materials, number of

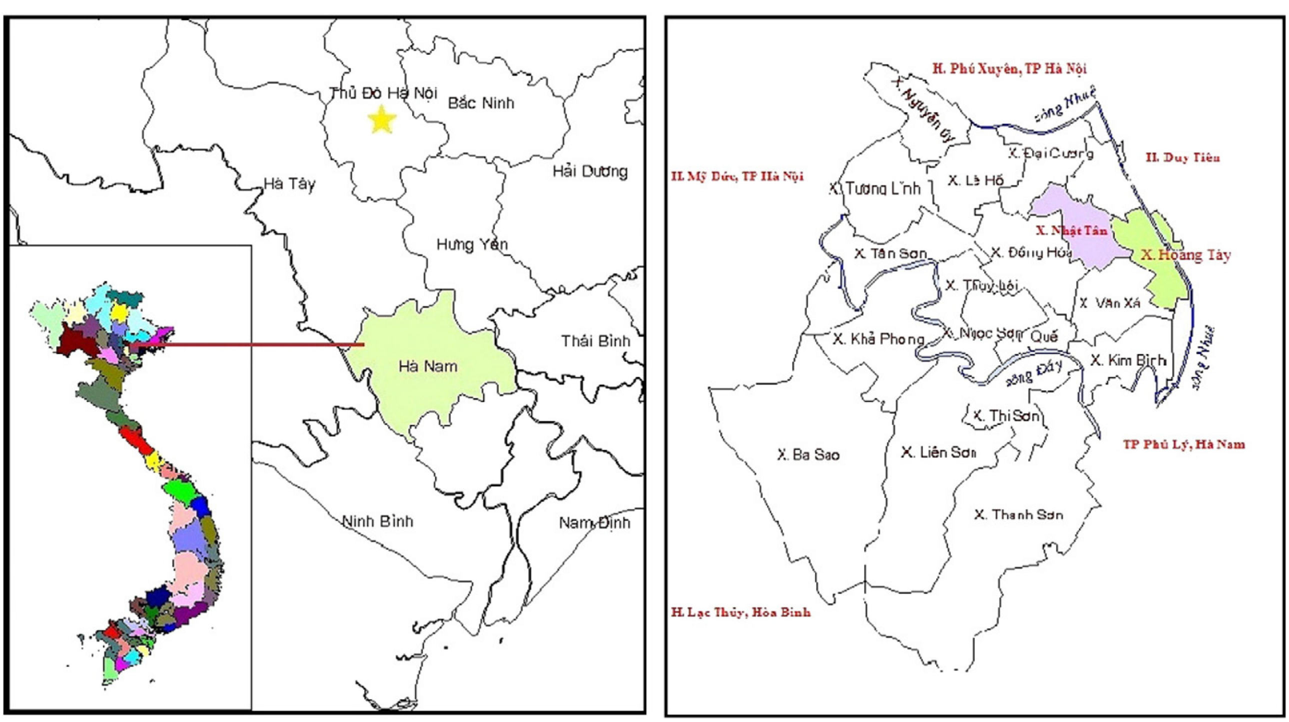

Fig. 1. Map of the study sites in Hoang Tay and Nhat Tan communes, Hanam province, northern Vietnam.

bedrooms), and household assets (motorbike, bicycle, refrigerator, television, radio, telephone, bed, cupboard, electric fan and electric devices); (ii) household's general sanitary conditions were assessed by the following indicators: the condition and location of the household's latrine (smell, flies, broken door, mud around the latrine); water storage container with cover and wastes (domestic waste, human/animal faeces) in the yard, type of latrine, type of water used in household and direct contact with animals in the household (i.e., pig, chicken, duck, dog and cat); (iii) exposure to water from Nhue River and local ponds, and irrigation water; (iv) exposure to human and animal excreta at home and in the fields; (v) personal hygiene practices: use personal protection during field work (e.g., gloves, boots, etc.), hand washing with or without soap after work.

The questionnaire was developed in English, translated into Vietnamese, backs-translated for confirmation and pre-tested in villages close to Hanoi. After adaptation the questionnaire was used in face-to-face interviews conducted by five trained and experienced research assistants. Principal researchers accompanied each assistant to three households for quality control (e.g., utilization of same procedures were used and for quality as being precisely followed). The main respondents were household head, or an adult person living permanently in the household (e.g., spouse of household head). Each interview lasted approximately 45 min.

2.4. Stool sample collection

Two stool samples were collected from each enrolled individual on two consecutive days. Each family member was provided with a labelled plastic container to collect a stool sample on the following day (day 1). On the collection day, a second labelled container was provided for the stool of following day (day 2). Samples were transported to the laboratory of the Department of Parasitology in Hanoi Medical University within 4 h after collection and stored at 4–8 °C until analysis on the next day.

2.5. Laboratory procedures

The Kato-Katz thick smear technique was used to identify *A. lumbricoides*, hookworm, and *T. trichiura* eggs [31]. In brief, duplicate Kato-Katz thick smears were prepared from each stool sample with 41.7 mg standard plastic template using faeces filtered through a Nylon mesh screen (number 120-sized). Slides were allowed to clear for 30 min prior examination by light microscopy at a magnification of 400×. The number of eggs was counted and recorded in laboratory sheet for each helminth species separately. The duplicate Kato-Katz method with stool samples from two consecutive days provided the highest sensitivity of *T. trichiura* infection when methods were considered alone. This is likely the result of overcoming the combined effect of sporadic shedding and uneven distribution of eggs in the stool [32].

Additionally, the formalin-ether concentration technique (FECT) was used to detect helminth and intestinal protozoan infections [33]. In brief, approximately 1 g of faeces was placed into a tube containing 10 mL of formalin. The sample was mixed thoroughly, and filtered through a funnel with gauze and then centrifuged for 1 minute at 2000 rpm. Supernatants were removed with a pipette, and 7 mL saline solution was added. Then, 3 mL ether was added, the tube closed with rubber stopper and shaken well (about 30 s). Without rubber stopper the tubes were centrifuged for 5 min at 2000 rpm. Supernatant was discarded and the entire sediment examined on presence of helminth eggs and protozoa cysts by microscope at a magnification of 300–500×.

2.6. Data management and statistical analysis

Data were double-entered into a Microsoft Access database and validated. Analysis was performed using STATA 10.1 (StataCorp., College Station, TX, USA). At beginning of the study, we attempted to assess the seasonal variations, in terms of prevalent rates as well as potential risk factors for STH infections. However, it is known that STHs causing human infection through contact with parasite eggs or larvae that develop in the warm and moist soil, and as adult worms, STHs live for years in the human gastrointestinal tract [12]. People living in the area having a poor sanitation condition to be chronically infected with STHs through the years. Therefore, it could not be reflected a true the transmission of parasitic infections associated with the potential risk factors between rainy and dry season during a year. For this reason, we were combined two cross-sectional surveys into a large survey to analyse and assess the prevalence rates of STH infections and their risk factors among people living and working in the community, where wastewater and excreta are commonly used in agriculture.

Only individuals who provided two stool samples of sufficient quantity were included in the subsequent analyses. The helminth infection prevalence rates were calculated. Helminth species egg counts per Kato-Katz slide were multiplied by a factor of 24 to obtain infection intensities, expressed as eggs per gram of stool (EPG) [34]. *T. trichiura*, hookworm and *A. lumbricoides* infections were grouped into light (1–999 EPG, 1–1999 EPG and 1–4999 EPG), moderate (1000–9999 EPG, 2000–3999 EPG and 5000–49,999 EPG) and heavy (≥10,000 EPG, ≥4000 EPG and ≥50,000 EPG) infection intensity categories according to WHO guidelines [35].

Generalised Estimating Equations (GEE) method was used in both univariable and multivariable analyses to adjust for intra-correlation within a household [36]. First, an univariable logistic regression analysis adjusted for age, sex and study season (rainy and dry season) was carried out to associate potential risk factors with outcomes (helminth infections) for which adjusted OR and its 95% CI were calculated. Univariable analyses were divided into three sections: (i) analysed with basic demographic variables (i.e., age, sex, educational level and occupation) for all subjects; (ii) analysed with the household variables for all subjects (i.e., SES and sanitary condition in the household, type of latrine, type of water source, composting human excreta, use of human and animal excreta as fertiliser in the fields, and use of Nhue River water to irrigate fields); and (iii) analysed with the agricultural exposure variables (i.e., handling human excreta in field work, direct contact with Nhue River water during field work, use of protective measures at work, and hand washing with soap after field work) for only individuals doing field work. Then, variables with adjusted OR ≥1.2 or ≤0.8 in the univariable analysis were included in the multivariable analysis [37].

SES and sanitary conditions in the household were calculated according to an asset-based method [38]. In brief, indicator data were defined by principal component analysis (PCA), with missing values being replaced with the mean value of the respective asset; all assets had a dichotomous character. SES and sanitary conditions in the household were categorised into three levels as good, average, and poor according to their cumulative standardised asset scores.

2.7. Ethical considerations

The Ethical Research Committee at the National Institute of Hygiene and Epidemiology (NIHE, number 149/QĐ-VSDTT -QLKH), Vietnamese Ministry of Health and the Ethics Committee of the State of Basel (EKBB, number 139/09) approved the study. Before field work the authorities in the Provincial Health Office and the District Health Office were informed on study objectives and procedures and working authorization obtained. Written informed consent was obtained from each individual prior to enrolment. The parents or legally guardian signed informed consent for their children aged between 1 year and 18 years. All individuals with helminth infections were treated free of charge with mebendazole (single dose, 500 mg) based on the Vietnamese Ministry of Health guidelines.

3. Results

3.1. Characteristics of the study population

From 540 selected households, a total of 15 households (3%) were absent during the three household visits; 20 households (4%) refused to participate, 10 households (2%) did not complete the questionnaire; and 11 households (2%) had only elderly and sick persons (Fig. 2). A total of 1655 individuals from 484 households provided stool samples and completed the questionnaire, of which 1425 individuals (78%) from 453 households submitted 2 stool samples and had a complete data record. Among them 743 (52%) were female, the mean age was 30 years (range: 1–87 years). One hundred and eighty two participants (13%) attended high school, 520 (36%) secondary school, and 723 (51%) primary school and pre-school. Seven hundred and three participants (49%) had a primary profession related with agricultural activity (e.g. rice cultivation, vegetable farming, and fish cultivation).

The household's SES and sanitary conditions showed that one-third of the study households had good, average and poor conditions. Two hundred and six (45%) households used tap water, 275 (61%) drilled tube well water, and 396 (87%) rainwater. Dry latrine (i.e. single or double vault) was most common type 285 (63%), followed by water-flushed latrine (i.e. septic tank or biogas) of 137 (30%), and no latrine of 37 (7%). There were 246 (54%) households reported that they composted human excreta before using them as fertiliser in the fields, 228 (50%) and 181 (40%) households had used human and animal excreta for application in field, respectively. Most study households 404 (89%) had used Nhue River water for rice and vegetable farming and fish cultivation.

3.2. Prevalence of intestinal parasitic infections

Table 1 shows the prevalence of intestinal parasitic infections stratified by sex and age. Overall 668 participants (47%) were infected with at least one of three helminth species (*A. lumbricoides*, *T. trichiura*, and hookworm). Three-hundred forty (24%), 573 (40%), and 29 (2%) participants were infected with *A. lumbricoides*, *T. trichiura* and hookworm, respectively. *E. histolytica* (6%) was the most common intestinal protozoan diagnosed, followed by *C. parvum* (5%), *E. coli* (2%), *G. lamblia* (1%) and *C. cayetanensis* (1%). The prevalence rates of helminth infections were generally higher in females and increased with age (Table 1).

In our study, the intensity for all helminth species was low; 98% of *A. lumbricoides* infection and all infections with *T. trichiura* and hookworm were light infections.

3.3. Risk factors for helminth infections

The results of the univariable and multivariable logistic regression analysis are presented in Tables 2 and 3, respectively. In comparison with higher educational levels, participants with a lower educational level (attended secondary school and primary school) had higher risk for any helminths (OR = 1.7, 95% CI 1.2–2.3; and OR = 1.7, 95% CI 1.2–2.4, respectively), and with *T. trichiura* (OR = 1.5, 95% CI 1.1–2.2; and OR = 1.6, 95% CI 1.1–2.3, respectively). Higher infection risk of *A. lumbricoides* was observed in participants who had agricultural work (i.e. rice and vegetable cultivating, and fish feeding) than those who had no agricultural work (i.e. teachers, health workers, small traders, or retired or working at home or students and children) (OR = 1.5, 95% CI 1.1–2.1). The effect was smaller for *T. trichiura* and not statistically significant. The helminth infection was not statistically significantly associated with household's SES in both uni- and multivariable analyses.

Tap water use in the household was found to be a protective factor for any helminth infection, and *T. trichiura* in univariable (OR = 0.7, 95% CI 0.5–0.9; and OR = 0.7, 95% CI 0.5–0.8, respectively) and multivariable analysis (OR = 0.6, 95% CI 0.4–0.9; and OR = 0.6, 95% CI 0.4–

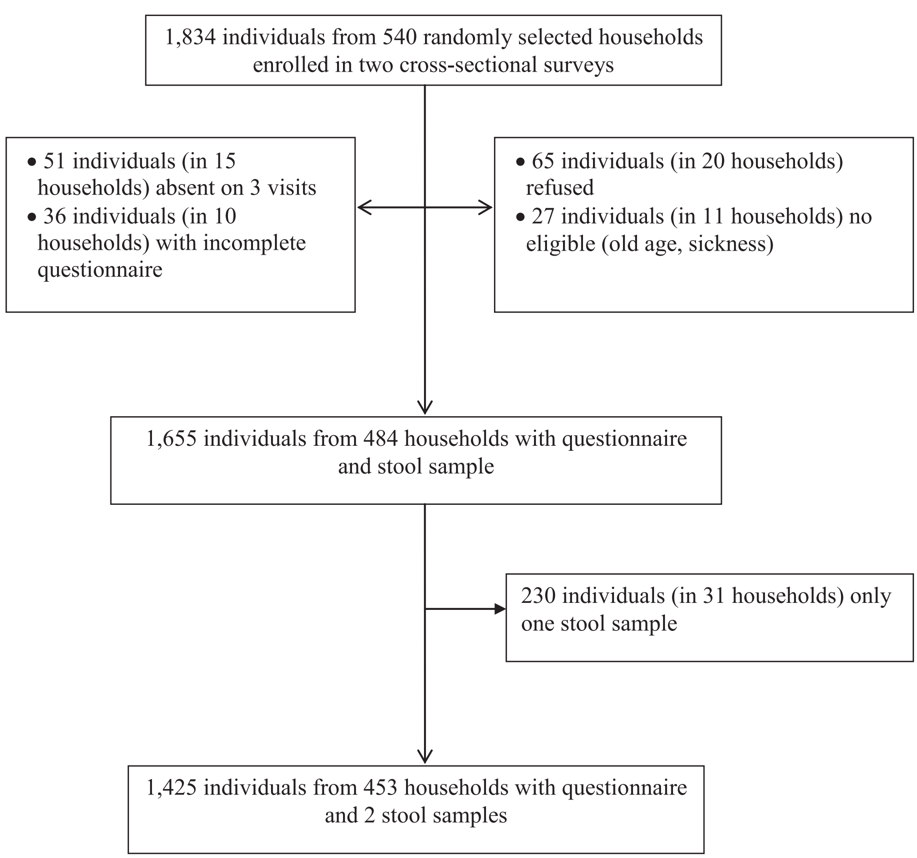

Fig. 2. Participants' compliance to participate in the study in Hoang Tay and Nhat Tan communes, Hanam province, northern Vietnam.

0.9, respectively). In univariable analysis, use of drilled tub well water in the household was a risk factor for any helminth infections and *T. trichiura* (OR = 1.2, 95% CI 1.0–1.6 and OR = 1.3, 95% CI 1.0–1.7, respectively), but not statistically significant in multivariable analysis (OR = 0.8, 95% CI 0.5–1.2 and OR = 0.8, 95% CI 0.6–1.3, respectively). Rainwater use in the household was not a risk factor associated the infection status with helminths (OR = 1.0, 95% CI 0.7–1.4).

The multivariable analysis did not show any significant association between household's sanitary condition and helminth infections. Possessing a dry latrine was a risk factor for any helminth infection (OR = 1.5, 95% CI 1.2–2.0), *A. lumbricoides* (OR = 1.5, 95% CI 1.1–2.1), and *T. trichiura* (OR = 1.4, 95% CI 1.1–1.9) in comparison with having a water-flushed latrine. Multivariable analyses showed that type of latrine in the household was not significantly associated with helminth infections.

Univariable analysis showed that composting of human excreta in the household was associated with increasing risk of any helminth infection (OR = 1.4, 95% CI 1.1–1.7). In multivariable analysis this factor increased risk was not observed (OR = 0.9, 95% CI 0.6–1.4). Helminth species-specific associations with composting of human excreta were not observed. Household use of human excreta for application in fields resulted in a statistically significant risk increase for *T. trichiura* in the univariable (OR = 1.6, 95% CI 1.2–2.0) and in the multivariable analysis (OR = 1.5, 95% CI 1.0–2.3); for any helminth species and *A. lumbricoides* in the univariable analysis (OR = 1.5, 95% CI 1.2–2.0 and OR = 1.5, 95% CI 1.1–2.0, respectively), but not statistically significant in the multivariable analysis. Use of animal excreta as fertiliser in the fields was not a risk factor for helminth infections (OR = 1.1, 95% CI 0.9–1.4).

Among those participants who reported field work (N = 703) the exposure to excreta, direct contact with Nhue River water, and use of protective measures (i.e., gloves, boots, face mask) during field work as well as washing hands with soap after field work was assessed. In univariable analysis, handling human excreta during field work increased the risk for infection with any helminth species (OR = 1.5, 95% CI 1.1–2.1) and *A. lumbricoides* (OR = 1.7, 95% CI 1.2–2.5). However, in multivariable analysis, there was no risk change for infection with any helminth species (OR = 1.4, 95% CI 1.0–2.0), *A. lumbricoides* (OR = 1.4, 95% CI 0.9–2.1), and *T. trichiura* (OR = 1.2, 95% CI 0.9–1.8) observed. Direct contact with Nhue River water during field work resulted in a statistically significantly increased risk for any helminth infection in the univariable (OR = 1.6, 95% CI 1.2–2.3) and multivariable analysis (OR = 1.5, 95% CI 1.1–2.2), and *A. lumbricoides* in the univariable and multivariable analysis (OR = 2.4, 95% CI 1.6–3.5 and OR = 2.1, 95% CI 1.4–3.2, respectively). With regard to *T. trichiura*, there was no significant difference for infection in relation to direct contact with Nhue River water in both univariable and multivariable analysis (OR = 1.2, 95% CI 0.8–1.6 and OR = 1.1, 95% CI 0.8–1.5, respectively).

Using protective measures during field work such as gloves and boots decreased risk for an infection with any helminth species (OR = 0.9, 95% CI 0.5–1.5) but not statistically significant; and no risk change for *A. lumbricoides* infection (OR = 1.0, 95% CI 0.6–1.7) was seen. Washing hands with soap after field work was not significantly reducing for an infection with *T. trichiura* (OR = 0.8, 95% CI 0.6–1.1) or *A. lumbricoides* (OR = 1.3, 95% CI 0.9–2.0).

4. Discussion

We investigated the helminth infection prevalence rates and their infection risks in rural agricultural communities in northern Vietnam with two large cross-sectional studies in the dry and rainy season. Our particular interest was to evaluate the importance of risk factors associated with the use of wastewater, and composting and use of animal and human excreta in agriculture, practices which are highly prevalent in these settings.

We found that helminth infections were highly prevalent, in particular with any helminth infection (47%), *A. lumbricoides* (24%) and *T. trichiura* (40%). Furthermore, our study shows an increased risk of helminth infection, especially with *A. lumbricoides* in people who had direct contact with Nhue River water during field work, and/or used human excreta as fertiliser in agricultural field. The use of tap water was a clear protective factor against helminth infections.

The prevalence rates of helminth infection we found in our study lied within the range of prevalence rates reported in previous studies in Vietnam [21,23,25,26,29,39,40]. Several factors can be made responsible for this variation among which climate, types of soils and crops, SES, and human hygiene behavior are of most importance [41]. Moreover, in Vietnam auto-medication is quite common, and also anthelminthic drugs are sold over the counter [42], which might have further influenced our observed infection rates. It is however striking that we did not find any food-borne trematodes infection in our study population. In rural populations in Vietnam and Southeast Asia infections can be of major importance [43–45]. They are determined by the consumption of raw or insufficiently cooked fish which is in our study population not a frequent habit.

Our results are in line with other previous studies, which found that people who were exposed to wastewater had a higher risk of helminth infections, especially with *A. lumbricoides* [10,46–50]. However, the findings are in contrast to other studies in peri-urban Hanoi and Nam Dinh province, which revealed that direct exposure to wastewater did not pose a major risk factor for helminth infections [23,29]. In contrast, the risk of helminth infections observed for those who lived in the households using Nhue River water to irrigate field was not statistically different than those who did not. The most probable explanation for the high risk of helminth infections in our study would be associated with the concentration of helminth eggs

Table 1
Prevalence of intestinal parasitic infections stratified by sex and age group in Hoang Tay and Nhat Tan communes, Hanam province, northern Vietnam (N = 1425).

Parasitic infection	Prevalence % (95% CI)	Sex				Age group (in years)						
	(N = 1425)	Female (n = 745)	Male (n = 680)	χ^2	P-value	1–5 (n = 150)	6–11 (n = 167)	12–19 (n = 247)	20-–45 (n = 513)	>45 (n = 348)	χ^2	P-value
Nematodes												
Ascaris lumbricoides	24 (22–27)	26	22	2.32	0.12	15	21	20	26	29	13.79	0.01
Trichuris trichiura	40 (38–43)	43	37	4.42	0.04	33	41	36	39	47	12.66	0.01
Hookworm infection	2 (1–3)	2	2	0.10	0.75	1	0	2	3	3	6.75	0.15
Any helminth infection *	47 (44–50)	49	44	3.98	0.05	37	45	41	47	56	20.84	<0.01
Protozoan												
Entamoeba histolytica	6 (5–7)	6	6	0.11	0.74	4	8	5	6	7	3.63	0.46
Entamoeba coli	2 (1–3)	2	3	3.58	0.06	3	2	2	2	3	0.71	0.95
Giardia lamblia	2 (1–2)	2	2	0	0.99	1	4	2	1	1	7.20	0.13
Cryptosporidium parvum	5 (4–7)	6	5	0.09	0.77	5	6	6	5	6	0.75	0.95
Cyclospora cayetanensis	1 (1–2)	2	1	0.91	0.34	1	2	1	2	1	1.52	0.82

Note: CI: confidence interval; * *A. lumbricoides* and/or, *T. trichiura* and/or hookworm).

Table 2
Potential risk factors for helminth infection in Hoang Tay and Nhat Tan communes, Hanam province (univariable logistic regression analysis).

Risk factors	N (1425)	Any helminth infection				*A. lumbricoides*				*T. trichiura*			
		Positive	OR crude	OR[a] model	95%CI	Positive	OR crude	OR[a] model	95%CI	Positive	OR crude	OR[a] model	95%CI
1. Demographic characteristics and household's economic status													
Age group (in years)													
1–5	150	56				23				50			
6–11	167	75	1.4	–	0.9–2.1	35	1.5	–	0.8–2.6	69	1.4	–	0.9–2.3
12–19	247	100	1.1	–	0.8–1.7	50	1.4	–	0.8–2.4	89	1.2	–	0.7–1.7
20–45	513	243	1.5	–	1.0–2.2	133	1.9	–	1.2–3.1	200	1.3	–	0.9–1.9
>45	348	194	2.1	–	1.4–3.1	99	2.2	–	1.3–3.6	165	1.8	–	1.2–2.7
Sex													
Female	745	368				190				319			
Male	680	300	0.8	–	0.7–1.0	150	0.8	–	0.6–1.1	254	0.8	–	0.6–1.0
Educational level													
High school	182	60				33				52			
Secondary school	520	255	2.0	1.7	1.2–2.3	135	1.6	1.4	0.9–2.1	214	1.7	1.5	1.1–2.2
Primary school or never attended school	723	353	1.9	1.7	1.2–2.4	172	1.4	1.2	0.8–1.8	307	1.8	1.6	1.1–2.3
Occupation													
Non agricultural work	722	304				138				271			
Agricultural work	703	364	1.5	1.1	0.8–1.4	202	1.7	1.5	1.1–2.1	302	1.3	0.9	0.7–1.3
Household's economic status overall													
Poor	478	241				130				204			
Average	473	222	0.9	1.0	0.7–1.3	113	0.8	1.0	0.7–1.3	188	0.9	1.0	0.7–1.3
Good	474	205	0.7	0.8	0.6–1.1	97	0.7	0.8	0.5–1.1	181	0.8	0.9	0.7–1.2
2. Sanitary condition in the household													
Sanitary condition overall													
Poor	476	198				95				171			
Average	475	241	1.4	1.4	1.0–1.9	127	1.5	1.5	1.0–2.1	213	1.5	1.5	1.1–2.0
Good	474	229	1.3	1.3	1.0–1.8	118	1.3	1.4	1.0–2.0	189	1.2	1.2	0.9–1.7
Type of latrine													
Water-flushed latrine	440	176				87				154			
Dry latrine	900	457	1.5	1.5	1.2–2.0	235	1.4	1.5	1.1–2.1	389	1.4	1.4	1.1–1.9
No latrine	85	35	1.1	1.0	0.6–1.8	18	1.1	0.9	0.5–1.9	30	1.0	1.0	0.6–1.7
3. Water source used in the household													
Tap water													
No	716	368				173				324			
Yes	709	300	0.7	0.7	0.5–0.9	167	1.0	1.0	0.7–1.3	249	0.7	0.7	0.5–0.8
Tube well water													
No	583	259				148				214			
Yes	842	409	1.2	1.2	1.0–1.6	192	0.9	0.9	0.6–1.2	359	1.3	1.3	1.0–1.7
Rainwater													
No	181	82				49				71			
Yes	1244	586	1.2	1.0	0.7–1.4	291	0.8	0.8	0.5–1.3	502	1.0	1.0	0.7–1.5
4. Agricultural practices related with excreta													
Composting human excreta in the household													
No	659	281				1.47				245			
Yes	766	387	1.4	1.4	1.1–1.7	193	1.2	1.3	0.9–1.7	328	1.3	1.3	1.0–1.7
Use of human excreta for application in field													
No	693	287				143				243			
Yes	732	381	1.5	1.5	1.2–2.0	197	1.4	1.5	1.1–2.0	330	1.5	1.6	1.2–2.0
Use of animal excreta as fertiliser in the fields													
No	828	381				199				330			
Yes	597	287	1.1	1.1	0.9–1.4	141	1.0	1.1	0.8–1.4	243	1.0	1.1	0.8–1.4
Handling human excreta in field work[b]													
No	286	130				70				114			
Yes	417	234	1.5	1.5	1.1–2.1	132	1.4	1.7	1.2–2.5	188	1.2	1.3	0.9–1.8
5. Agricultural practices related with Nhue River water													
Use Nhue River water to irrigate fields													
No	125	53				24				44			
Yes	1300	615	1.2	1.4	0.9–2.2	316	1.4	1.6	0.9–2.7	529	1.3	1.5	0.9–2.3
Direct contact with Nhue River water during field work[b]													
No	387	180				95				162			
Yes	316	184	1.6	1.6	1.2–2.3	107	1.6	2.4	1.6–3.5	140	1.1	1.2	0.8–1.6
6. Personal hygiene practices related with agricultural work													
Washing hands with soap after field work[b]													
No	377	201				95				172			
Yes	326	163	0.9	0.9	0.7–1.2	107	1.5	1.4	0.9–1.9	130	0.8	0.8	0.6–1.1
Use protective measures (gloves, boots, face mask) at work[b]													
No	614	313				172				263			
Yes	89	51	1.3	1.3	0.8–2.0	30	1.3	1.8	1.1–2.9	39	1.0	1.1	0.7–1.7

Table 3
Risk factors for helminth infection in Hoang Tay and Nhat Tan communes, Hanam province (multivariable logistic regression analysis).

Risk factors	Any helminth infection			*A. lumbricoides*			*T. trichiura*		
	OR[a]	95% CI	P-value	OR[a]	95% CI	P-value	OR[a]	95% CI	P-value
1. Household's economic status overall									
Average *versus* Poor	1.0	0.7–1.4	0.89	1.0	0.7–1.4	0.79	N.A.	–	–
Good *versus* Poor	1.2	0.8–1.8	0.40	0.9	0.6–1.5	0.80	N.A.	–	–
2. Sanitary conditions in the household									
Household's sanitary condition overall									
Average *versus* Poor	1.2	0.8–1.7	0.31	1.4	0.9–2.1	0.15	1.3	0.9–1.9	0.16
Good *versus* Poor	1.1	0.8–1.6	0.58	1.2	0.8–1.9	0.46	1.0	0.7–1.5	0.81
Type of latrine in the household									
Dry latrine *versus* Water-flushed latrine	1.3	0.7–2.5	0.42	1.2	0.6–2.6	0.60	1.0	0.5–1.8	0.96
No latrine *versus* Water-flushed latrine	1.0	0.5–2.0	0.89	0.7	0.3–1.5	0.33	0.9	0.5–1.6	0.68
3. Water source used in the household									
Household use of tap water									
Yes *versus* No	0.6	**0.4–0.9**	**0.01**	N.A.	–	–	**0.6**	**0.4–0.9**	**0.01**
Household use of tube well water									
Yes *versus* No	0.8	0.5–1.2	0.34	N.A.	-	-	0.8	0.6–1.3	0.38
Household use of rainwater									
Yes *versus* No	N.A.	–	–	0.8	0.5–1.2	0.28	N.A.	–	–
4. Agricultural practices related with excreta									
Composting human excreta in the household									
Yes *versus* No	0.9	0.6–1.4	0.64	0.8	0.5–1.3	0.31	0.9	0.6–1.4	0.56
Use of human excreta for application in field									
Yes *versus* No	1.3	0.9–2.0	0.18	1.3	0.8–2.0	0.33	**1.5**	**1.0–2.3**	**0.04**
Handling human excreta in field work[a]									
Yes *versus* No	1.4	1.0–2.0	0.06	1.4	0.9–2.1	0.10	1.2	0.9–1.8	0.20
5. Agricultural practices related with Nhue river water									
Use Nhue River water to irrigate fields									
Yes *versus* No	1.1	0.7–1.8	0.63	1.3	0.7–2.3	0.36	1.1	0.7–1.8	0.62
Direct contact with Nhue River during field work[b]									
Yes *versus* No	**1.5**	**1.1–2.2**	**0.04**	**2.1**	**1.4–3.2**	<**0.01**	1.1	0.8–1.5	0.68
6. Personal hygiene practices related with agricultural work									
Washing hands with soap after field work[b]									
Yes *versus* No	N.A.	–	–	1.3	0.9–2.0	0.11	0.8	0.6–1.1	0.15
Use protective measures at work[b]									
Yes *versus* No	0.9	0.5–1.5	0.66	1.0	0.6–1.7	0.99	N.A.	–	–

Note: GEE were used to account for intra-correlation within a household; N.A: Not applicable.
[a] OR model: adjusted for age, sex, and season.
[b] Excluding subjects with non-agricultural work (N = 703).

in irrigation water, to which farmers have actually an intense and direct contact during a long period during field work [46]. We renounced to test environmental samples for parasitic contamination. In our study we concentrated our effort to identify modifiable risk factors associated with parasitic infections. Furthermore, infectious parasite stages (i.e., hookworm larvae) are preferably present in moisty soil close to water bodies such as the Nhue river which lead then to increased infection in those farmers who are frequently in close contacts with the water [51]. Indeed, a study in Hanoi showed that people who frequently had contact with irrigation water throughout the year had a higher risk of infection with *T. trichiura* [23].

Our study showed that the use of human excreta as fertiliser in agriculture had a higher risk of infections with *T. trichiura*. This is consistent with observations made by Trang and colleagues [23].

We observed in our study that composting human excreta before being utilised as fertiliser in agriculture was not associated with a risk increase with helminth infections. In fact, in rural areas of Vietnam people normally add ash and occasionally lime into the vault of dry latrine and during the composting process to reduce bad odor and fly production [20,22]. These practices are likely to increase the inactivation of helminth eggs and pathogens [52–54]. Furthermore, approximate compost duration of 3–4 months under the conditions of high pH and temperature and low moisture could provide a safe compost product to be used for agricultural application [55]. Such length of compost could allow for the degeneration of helminth eggs, thereby reducing the risk of helminth infection.

The overall sanitary condition and type of latrine used in the households were not associated with helminth infections. This findings goes in line a study in Hoa Binh province in northern Vietnam which showed that the presence of latrine alone is not sufficiently reducing helminth infection risk in a rural agricultural community [21], most probably rather its correct and hygienic use. However, our results contradict with some previous studies in Vietnam and Pakistan which reported that absence of a latrine was found to be associated with an increase in helminth infections, especially *A. lumbricoides* [23,41,56].

In our study, the use of tap water source in the household was a protective factor against any helminth infection and *T. trichiura*. Other studies such as an investigation in Ethiopia also documented a risk reduction of STH infection associated with tap water use [57]. Surprisingly, the use of protective measures during fieldwork and washing hands with soap after field work did not result in a risk

Notes to Table 2:
[a] OR model: adjusted for age, sex, and season. GEE were used to account for intra-correlation within a household.
[b] Excluding subjects with non-agricultural work (N = 703).

reduction. The personal hygiene practices have been widely shown to be important factors in reducing the transmission of helminths and other infectious diseases [29,41,56,58]. In this perspective our result might have a limitation. Data on personal hygiene practices were also collected with questionnaire. For this type of information direct observations might have been a more appropriate method [23,59].

We also could not find any association between SES and helminth infection. These results are in line with the other studies in Vietnam and Thailand [23,60]. However, our results contradict with some previous studies, where an association between SES and helminth infection was found [29,61,62] in a population with a larger variation in SES was included. Our study was clearly defined to an agricultural community.

5. Conclusion

Our study further documents that STH infections are of importance in rural communities in Vietnam. Most importantly, agricultural related risk factors such as the exposure to human excreta for fertilizing fields are among the important determinants of infection. Therefore, public health intervention are required to address these risk factors in addition to current strategy of chemotherapy in order prevent infection and re-infection.

Conflict of interest

All authors declare no conflicts of interest.

Authors' contributions

PPD, HNV, JZ, PDC, CZ and PO planned and designed the study. PPD and HNV conducted and supervised field and laboratory work. PPD, JH, PO and HNV analysed and interpreted data together with JZ, PDC and CZ. PPD, HNV and PO prepared the first draft of the manuscript and all authors revised the manuscript critically. All authors read and approved the final version of the manuscript.

Acknowledgements

We thank the staff of the health stations of Hoang Tay and Nhat Tan communes as well as community members for their kind cooperation and participation in the study. Thanks are also given to Dr. Nguyen Van De and the staff of the Department of Parasitology at the Hanoi Medical University who performed the stool examinations for protozoan parasites. This work has been supported by the Swiss National Science Foundation (SNSF) and the Swiss Agency for Development and Cooperation (SDC) through the program of the National Centre for Competences in Research (NCCR) North–south.

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