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Risk factors for *Entamoeba histolytica* infection in an agricultural community in Hanam province, Vietnam

Phuc Pham Duc^{1,2,3,5*}, Hung Nguyen-Viet^{1,2,4,5}, Jan Hattendorf^{1,2}, Jakob Zinsstag^{1,2}, Phung Dac Cam³ and Peter Odermatt^{1,2}

Abstract

Background: *Entamoeba histolytica* is an important protozoan intestinal infection in resource-poor settings, including Vietnam. The study objective was to assess risk factors of *E. histolytica* infection in a community in Vietnam, where wastewater and human excreta are used in agriculture. A case-control study was conducted among residents of Hanam province, Northern Vietnam. Cases (n = 46) infected with *E. histolytica* and non-infected controls (n = 138) were identified in a cross-sectional survey among 794 randomly selected individuals and matched for age, sex and place of residence. Potential risk factors including exposure to human and animal excreta and household wastewater were assessed with a questionnaire.

Results: People from households with an average socio-economic status had a much higher risk of *E. histolytica* infection (odds ratio [OR]=4.3, 95% confidence interval [CI]: 1.3-14.0) compared with those from households with a good socioeconomic status. Those individuals who never or rarely used soap for hand washing had a 3.4 times higher risk for infection (OR=3.4, 95% CI: 1.1-10.0), compared to those who used always soap. In contrast, none of the factors related to use of human or animal excreta was statistically significant associated with *E. histolytica* infection. People having close contact with domestic animals presented a greater risk of *E. histolytica* infection (OR = 5.9, 95% CI: 1.8-19.0) than those without animal contact. *E. histolytica* infection was not associated with direct contact with Nhue river water, pond water and household's sanitary conditions, type of latrine or water source used.

Conclusions: Our study suggests that in settings where human and animal excreta and Nhue River water are intensively used in agriculture, socio-economic and personal hygiene factors determine infection with *E. histolytica*, rather than exposure to human and animal excreta in agricultural activities.

Background

Amoebiasis caused by the intestinal parasite *Entamoeba histolytica*, has an estimated worldwide prevalence of 500 million infected people and is responsible for 40,000 - 100,000 deaths each year. It is an important health problems, especially in developing countries [1,2]. The incidence rate of *E. histolytica*-associated diarrhoea was 0.08/child-year [3]. The rate of infection by *E. histolytica* differs among countries, socio-economic and sanitary conditions and populations [4]. It is highly endemic throughout poor and socio-economically deprived communities in

the tropics and subtropics. Environmental, socio-economic, demographic and hygiene-related behaviour is known to influence the transmission and distribution of intestinal parasitic infections [5]. A study in Brazil identified place of residence, age, ingestion of raw vegetables and drinking water quality as important risk factors [6].

Wastewater and human and animal excreta are used as fertilizer for a wide variety of crops, and 10% to 30% increases in crop yields have been reported [7]. The use of wastewater and human and animal excreta in agriculture and aquaculture continues to be common in China, South and South East Asia as well as various areas in Africa [8-10] in particular where water scarcity is becoming more severe. The main sources of water for irrigation in Vietnam are fresh water, wastewater and ground water. In

* Correspondence: phuc.pham-duc@unibas.ch

¹Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, P.O. Box CH-4002, Basel, Switzerland
Full list of author information is available at the end of the article

Hanoi about 80 percent of vegetable production is from urban and peri-urban areas irrigated with diluted wastewater [11]. The use of household sewage, and human and animal excreta in agriculture and aquaculture has a long tradition in Vietnam [12]. Despite the potential health risk for intestinal disease of using excreta and animal waste in agriculture [13], 85% of farmers in northern provinces of Vietnam regularly use human excreta in agriculture [14]. Another study in Vietnam on helminth infections among people exposed to wastewater and human excreta has showed that wastewater exposure was not an important risk factor for parasite infection but that the lack of sanitation facilities and the use of fresh or inadequately composted excreta as fertilizers in agriculture increased the risk of parasite infection [15]. A study in Hanoi, Vietnam, on the epidemiology and aetiology of diarrhoeal diseases in adults engaged in wastewater-fed agriculture and aquaculture has showed that the diarrhoeagenic *Escherichia coli* and *E. histolytica* were the most common pathogens [16,17].

To further understand the transmission of *E. histolytica* infection, we conducted a case-control study to assess the importance of handling practices of human and animal excreta and wastewater use in irrigation in agriculture and aquaculture, in relation to other potential risk factors, including sanitary conditions, drinking water, food consumption, and personal hygiene practices.

Methods

Study sites

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 located about 60 km south of Hanoi. The number of inhabitants is about 10,500 (2,600 households) and 5,700 (1,500 households) in Nhat Tan and Hoang Tay communes, respectively. Most households have livestock in their compounds. The residential areas are in the vicinity of fields used for agriculture (rice and vegetables) and aquaculture (fish breeding). The rice fields and local ponds cover about 50% of the surface. The two communes border the Nhue River. Hanoi City's wastewater from households, industry and other sources such as hospitals is directly and untreated discharged into the river [18]. 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 the local fields and fish ponds. Wastewater from households (grey water from kitchens and bathrooms, and effluent from septic tanks and sanitation facilities) is 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 "summer season" from July to October. People also grow vegetables which are eaten raw or cooked by

the local population or sold to neighbouring towns and Hanoi. Human 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 only partially composted. The composting procedure does not fully respect the composting guidelines set out by the Vietnamese Ministry of Health which imposes a minimum of 6 months [19]. In practice, farmers utilise the latrine night soil to fertilise crops whenever they need it in the fields, which results often in a shorter storage period than the regulatory 6 months; personal protective measures to prevent contamination are often lacking.

Study design

This study carried out in August 2008 followed the logic of a community based case-control study. A subject was defined as case if diagnosed with an *E. histolytica* infection (at least one of two stool samples positive for *E. histolytica*). Controls were subjects negative for *E. histolytica* in two stool examinations and matched for sex, age groups (i) under 6 years, (ii) 6-15 years, (iii) 16-30 years, (iv) 31-45 years, (v) 45-60 years, and (vi) over 60 years, and place of residence (same commune but different household).

Ascertainment of cases and controls

The cases and controls were identified in a large cross-sectional household survey on intestinal parasitic infections. All patients infected with *E. histolytica* were enrolled as cases. Controls were selected randomly among the non-infected individuals.

Fifteen villages in Nhat Tan and 10 villages in Hoang Tay communes were selected to participate in the cross-sectional study. Households were randomly selected from the household list provided by the Communal People's Committee. Out of the 4282 households living in the two communities, 270 households were selected using random numbers. All household members above 12 months of age were enrolled.

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) by trained personnel. When first container with stool was collected, a second labelled container was provided for the stool of the following day (day 2). Samples were transported to the laboratory of the Parasitological Department in Hanoi Medical University within 4 hours after collection and stored at 4-8°C until analysis.

Laboratory procedures

The formalin-ether concentration technique (FECT) was used for detecting *E. histolytica* [20]. In brief, the preparation process was as follows: a stool sample of approximately 1 gram was placed into a tube containing 10 mL of

formalin. The sample was mixed thoroughly and vigorously, and then the stool solution was filtered using a funnel with gauze and centrifuged for 1 minute at $447 \times g$. Supernatants were removed with a pipette, and 7 mL saline solution were added and mixed with a wooden stick. 3 mL Ether were then added and the tubes closed with rubber stoppers and shaken well (about 30 seconds). The rubber stoppers were then carefully removed and the tubes were centrifuged for 5 minutes at $447 \times g$. The supernatant was discarded and the entire sediment was examined for the presence of protozoa using a microscope at a magnification of 500x.

Sample size

The sample size was calculated for the matched case-control study with a ratio between case and control groups of 1:3 [21]. To detect - at a 95% confidence level - an odds ratio (OR) = 2.5 with a power of 80% and an expected frequency of exposure to excreta and wastewater in the control group of 30%, we require sample sizes of 52 cases and 156 controls.

Data collection

A questionnaire with six sections was administered to all cases and controls: (i) general demographic information and socio-economic status (SES): age, gender, educational level, occupation, household's economic status was assessed with a list of indicators which included surface of household's rice field and fish ponds, number of animals (pigs, buffalos, chickens, ducks, cows, dogs and cats), housing characteristics (building materials, number of bedrooms), and household assets (motorbike, bicycle, refrigerator, television, radio, telephone, bed, cupboard, electric fan and electronic devices); (ii) household sanitary conditions: general sanitary conditions was assessed by 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 human and animal excreta at home and in the fields; (iv) exposure to water from the Nhue River and local ponds, and irrigation water; (v) personal hygiene habits and practices: use personal protection during field work (gloves, boots, etc.), bathing and hand washing after work with or without soap, eating habits, eating leftovers from day before, and source of drinking water; (vi) information related to gastrointestinal symptoms: vomiting, nausea, abdominal pain, watery stools, blood/mucus stools and loose stools.

The questionnaire was developed in English, translated to Vietnamese and pre-tested in villages close to Hanoi. After adaptation the questionnaire was used in a face-to-

face interview conducted by trained and experienced research assistants. The main researcher accompanied each assistant to three households and supervised him/her to make sure that the procedure was being precisely followed. Each interview took approximately 45 minutes.

Data management and analysis

Data was double-entered in a Microsoft Access database and validated. Analysis was performed using STATA version 10.1 (StataCorp, College Station, TX, USA).

Statistical analysis for the matched case-control study was conducted as follows. First, an univariable conditional logistic regression analysis was carried out to associate potential risk factors with infection status (outcome) for which matched OR and its 95% confidence interval (CI) and P-value were calculated. Then, variables with $P < 0.2$ in the univariable analysis were included in the multivariable conditional logistic regression analysis [22]. Variables related to personal hygiene behaviour were highly inter-correlated. Therefore, we included only one variable (hand washing with soap) in the multivariable model to avoid collinearity.

SES and sanitary conditions in the household were calculated according to an asset-based method [23,24]. 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 categorized into three levels as good, average, and poor according to their cumulative standardized asset scores.

Ethical considerations

Before field work the authorities in the Provincial Health Office and the District Health Office were informed and asked for permission. Detailed information on study objectives and procedures was provided and working authorisation obtained. Written informed consent was obtained from each individual prior to enrolment. The Ethical Research Committee at the National Institute of Hygiene and Epidemiology (NIHE, number 149/QĐ-VSDTTU-QLKH, 22 April 2009), Vietnamese Ministry of Health and the Ethic Commission of the State of Basel (EKBB, number 139/09, 11 May 2009) approved the study.

Results

Description of cases and controls

We identified and enrolled 46 cases and 138 controls. The mean age for cases and controls was 34 years (SD 2.8 years, range: 3-83 years) and 36 years (SD 1.3 years, range: 5-87 years), respectively. Thirty-one cases (67.4%) were found in Nhat Tan and 15 cases (32.6%) in Hoang Tay commune. The mean family size for cases and controls

was 4.1 (SD 1.6) and 4.2 (SD 1.3) persons, respectively, and was not statistically significantly different ($P = 0.46$).

Only few study participants reported gastrointestinal symptoms: eleven cases (23.9%) and 17 controls (12.3%). There was no significant statistical difference between the two groups ($P > 0.20$). The gastrointestinal symptoms were fever (2 cases, 1 control), nausea (1 case, 0 control), abdominal pain (4 cases, 9 controls), and watery stools (4 cases, 7 controls).

Risk factors for *E. histolytica* infection

The results of the univariable and multivariable conditional logistic regression analysis are presented in Table 1 and Table 2, respectively.

Among the indicators describing the general and socio-economic status of the family the general socio-economic status was strongly associated with the *E. histolytica* infection. Participants who lived in households with an average and poor SES had a 3.8 (95% CI: 1.5-9.8) and 2.4 (95% CI: 0.9-6.4) higher risk of infection with *E. histolytica* than those living in households with a good status. The multivariable conditional logistic regression analysis confirmed this finding with the same trend (OR = 4.3, 95% CI: 1.3-14.0). Although in uni- and multivariable analysis the risk increase was high with decreasing general SES, statistically significant risk increase was found only for average versus good SES (i.e. OR = 4.3, $P = 0.02$, Table 2).

Cases and control did not differ in educational levels. Furthermore, no statistically significant difference was found in occupation. Approximately two third of both groups were farmers (65.2% of cases versus 67.4% of controls, $P = 0.79$). Sixteen cases (34.8%) and 45 controls (32.6%) were officers in public services such as teachers, health workers, or small traders, or were retired or working at home.

The sanitary conditions of the household were described with an overall assessment indicator, the type of latrines present and type of water used in the household. In none of these analyses was a significant association between the indicators and the infection status with *E. histolytica* found (Table 1 and 2). However, close contact with domestic animals in the household resulted in a statistical significant two-fold and six-fold risk increase for a *E. histolytica* infection in the univariable (OR = 1.9, 95% CI: 0.8-4.4) and multivariable analysis (OR = 5.9, 95% CI: 1.9-18.9).

In univariable analysis, none of the variables related to human excreta showed an increased risk of *E. histolytica* infection. For example, composting of human excreta (OR = 0.8, 95% CI: 0.4-1.7), or use of human excreta as fertiliser for application in field (OR = 1.3, 95% CI: 0.6-2.6), or handling human excreta in field work (OR = 0.8, 95% CI: 0.4-1.8). No association was found for composting of animal excreta (OR = 0.7, 95% CI: 0.3-1.3) and use of

animal excreta as fertiliser for application in field (OR = 0.8, 95% CI: 0.4-1.7). On the contrary, handling animal excreta in the field was found to be a protective factor in the univariable (OR = 0.5, 95% CI: 0.2-0.9) and multivariable analysis (OR = 0.2, 95% CI: 0.1-0.7).

Direct contact with Nhue River water during field work resulted in a substantial risk reduction in the uni- (OR = 0.6, 95% CI: 0.3-1.2) and multivariable analysis (OR = 0.4, 95% CI: 0.1-1.1). Using the Nhue River water to irrigate fields increased the risk (OR = 4.6 and OR = 3.7 in the uni- and multivariable analysis, respectively) but it was not statistically significant. There was no risk change for *E. histolytica* infected individuals associated with close contact and use of local ponds (OR = 1.0, 95% CI: 0.5-2.1, $P = 0.92$).

Risk changes were observed for variables related to personal hygiene. Using personal protective conditions during field work such as gloves and boots reduced the risk (OR = 0.5, 95% CI: 0.3-1.1) and omitting to bath and shower after field work increased the risk (OR = 2.3, 95% CI: 1.0-5.6) for an infection with *E. histolytica*. However, these associations were not statistically significant. Omitting to wash the hands was a significant risk. E.g., People who rarely washed their hands with soap after field work had a large risk increase of an *E. histolytica* infection (OR = 3.0, 95% CI: 1.2-7.4) compared to those who frequently wash their hand with soap after work. This risk increase remained statistically significant in the multivariable analysis (OR = 3.4, 95% CI: 1.1-10.0).

Consuming leftover foods from the day before (OR = 1.1, 95% CI: 0.5-2.3), eating raw vegetables (OR = 0.7, 95% CI: 0.1-3.7) and type of water source used for drinking water (OR = 1.4, 95% CI: 0.4-4.9) were not associated with *E. histolytica* infection.

Discussion

We have studied risk factors associated with *E. histolytica* infection in a semi-rural community where human and animal excreta are intensively used as fertiliser in agriculture and where household wastewater is directed into irrigation channels. We identified lower economic status of households (OR = 4.3), poor hand washing practices after work (OR = 3.4) and close contact with animals in the household (OR = 5.9) as major risk factors for *E. histolytica* infection. None of the factors measuring exposure to human and animal excreta such as composting excreta in the household or using excreta as fertilisers in the field resulted in an increased risk. On the contrary, those who reported handling animal excreta during field work had a substantial risk reduction (OR = 0.2). In addition, close and frequent exposure to Nhue River water reduced the risk (OR = 0.4).

E. histolytica developing in humans is transmitted directly following faecal-oral transmission routes. The

Table 1 Risk factors for *E. histolytica* infection in Hanam province, Vietnam (univariable conditional logistic regression analysis)

Variables	Case N (%)	Control N (%)	Matched OR	95% CI	P-value
1. Socio-economic status					
Educational level					
High school	6 (13.0)	17 (12.0)	Reference		
Secondary school	24 (52.2)	79 (57.3)	0.8	0.7-2.7	0.76
Primary school	16 (34.8)	42 (29.8)	1.1	0.3-3.8	0.87
Occupation					
Non agricultural work	16 (34.8)	45 (32.6)	Reference		
Agricultural work	30 (65.2)	93 (67.4)	0.8	0.3-2.2	0.70
Household's economic status overall					
Good	8 (17.4)	53 (38.4)	Reference		
Average	22 (47.8)	39 (28.3)	3.8	1.5-9.8	0.01
Poor	16 (34.8)	46 (33.3)	2.4	0.9-6.4	0.08
2. Household sanitary and hygiene conditions					
Household's sanitary conditions overall					
Good	20 (43.5)	40 (29.0)	Reference		
Average	12 (26.1)	50 (36.2)	0.5	0.2-1.1	0.08
Poor	14 (30.4)	48 (34.8)	0.6	0.3-1.3	0.21
Type of latrine in the household					
Water latrine (septic tank, biogas)	15 (32.6)	47 (34.1)	Reference		
Dry latrine (single or double vault)	29 (63.0)	87 (63.0)	1.1	0.5-2.2	0.89
No latrine	2 (4.4)	4 (2.9)	1.7	0.2-11.2	0.61
Household use of tap water					
No	24 (52.2)	89 (64.5)	Reference		
Yes	22 (47.8)	49 (35.5)	1.7	0.9-3.4	0.13
Household use of tube well water					
No	16 (34.8)	53 (38.4)	Reference		
Yes	30 (65.2)	85 (61.6)	1.2	0.6-2.5	0.64
Household use of rainwater					
No	5 (10.9)	12 (8.7)	Reference		
Yes	41 (89.1)	126 (91.3)	0.8	0.3-2.4	0.66
Close contact with domestic animals in household					
No	9 (19.6)	42 (30.4)	Reference		
Yes	37 (80.4)	96 (69.6)	1.9	0.8-4.4	0.15
3. Exposed to human and animal excreta					
Composting of human excreta in the household					
No	22 (47.8)	60 (43.5)	Reference		
Yes	24 (52.2)	78 (56.5)	0.8	0.4-1.7	0.59
Use of human excreta for application in field					
No	18 (39.1)	62 (44.9)	Reference		
Yes	28 (60.9)	76 (55.1)	1.3	0.6-2.6	0.48
Handling human excreta in field work					
No	22 (47.8)	61 (44.2)	Reference		
Yes	24 (52.2)	77 (55.8)	0.8	0.4-1.8	0.61
Compound with animal husbandry					
No	6 (13.0)	25 (18.1)	Reference		
Yes	40 (87.0)	113 (81.9)	1.5	0.6-4.0	0.42
Composting of animal excreta in the compound					
No	31 (67.4)	79 (57.3)	Reference		
Yes	15 (32.6)	59 (42.7)	0.7	0.3-1.3	0.23

Table 1 Risk factors for *E. histolytica* infection in Hanam province, Vietnam (univariable conditional logistic regression analysis) (Continued)

Use of animal excreta as fertiliser in the fields					
No	25 (54.3)	69 (50.0)	Reference		
Yes	21 (45.7)	69 (50.0)	0.8	0.4-1.7	0.59
Handling animal excreta in field work					
No	31 (67.4)	69 (50.0)	Reference		
Yes	15 (32.6)	69 (50.0)	0.5	0.2-0.9	0.03
4. Exposed to water from Nhue river and local pond					
Direct contact with Nhue river water during field work					
No	30 (65.2)	72 (52.2)	Reference		
Yes	16 (34.8)	66 (47.8)	0.6	0.3-1.2	0.12
Use local pond for fishing, bathing, washing					
No	32 (69.6)	95 (68.9)	Reference		
Yes	14 (30.4)	43 (31.1)	1.0	0.5-2.1	0.92
Use Nhue river water to irrigate fields					
No	1 (2.2)	13 (9.4)	Reference		
Yes	45 (97.8)	125 (90.6)	4.6	0.6-35.4	0.15
5. Personal hygiene habits					
Use protective measures (gloves, boots and face mask) at work					
No	28 (60.9)	63 (45.6)	Reference		
Yes	18 (39.1)	75 (54.4)	0.5	0.3-1.1	0.07
Showering, bathing (with soap) after field work					
Frequently	9 (19.6)	48 (34.8)	Reference		
Sometimes	19 (41.3)	49 (35.5)	2.2	0.9-5.5	0.09
Rarely	18 (39.1)	41 (29.7)	2.3	1.0-5.6	0.06
Washing hands after field work					
Frequently	30 (65.2)	108 (78.3)	Reference		
Sometimes	3 (6.5)	9 (6.5)	1.6	0.4-7.0	0.50
Rarely	13 (28.3)	21 (15.2)	3.4	1.2-10.0	0.02
Washing hands with soap after field work					
Frequently	9 (19.6)	50 (36.2)	Reference		
Sometimes	14 (30.4)	43 (31.2)	1.8	0.7-5.1	0.24
Rarely	23 (50.0)	45 (32.6)	3.0	1.2-7.4	0.02
Eating leftover food from day before					
No	13 (28.3)	42 (30.4)	Reference		
Yes	33 (71.7)	96 (69.6)	1.1	0.5-2.3	0.78
Eating raw vegetables the day before					
No	44 (95.6)	130 (94.2)	Reference		
Yes	2 (4.4)	8 (5.8)	0.72	0.1-3.7	0.70
Water source for drinking					
Rainwater	42 (91.3)	129 (93.5)	Reference		
Tube well water	4 (8.7)	9 (6.5)	1.4	0.4-4.9	0.61

risk pattern identified in our study follows this logic. In particular, the transmission routes via contaminated hands play a major role, documented in our study with a more than three- fold risk increase if hands are not washed properly. In contrast, the transmission routes via contaminated food are not of relevance. We did not find any association between an *E. histolytica* infection and consumption of raw vegetables, leftover food from previous days and different types of drinking water. Similar

observations were made by Nyarango and colleagues in Kenya [25]. In addition, we observed in our study area that vegetables were grown usually in a garden, very close to the house where wastewater and human excreta were not likely to be used often for irrigation and as fertilizers, probably due to the smell of human excreta. Furthermore, it was frequently seen that vegetables are properly washed before they are consumed. Indeed, a study in Iran indicated that no parasitic contamination

Table 2 Risk factors for *E. histolytica* infection in Hanam province, Vietnam (multivariable conditional logistic regression analysis)

Risk factors	Matched OR	95% CI	P-value
Household's socioeconomic status (<i>versus</i> good)			
- average	4.3	1.3-14.0	0.02
- poor	2.2	0.6-7.4	0.22
Household's sanitary conditions (<i>versus</i> good)			
- average	0.8	0.3-2.3	0.68
- poor	1.6	0.6-4.6	0.38
Household with tap water (<i>yes versus</i> no)	1.3	0.5-3.1	0.57
Close contact with domestic animals in household (<i>yes versus</i> no)	5.9	1.9-18.9	0.003
Handling animal excreta in field work (<i>yes versus</i> no)	0.2	0.1-0.7	0.01
Direct contact with Nhue river water during field work (<i>yes versus</i> no)	0.4	0.1-1.1	0.07
Use of Nhue river water to irrigate fields (<i>yes versus</i> no)	3.7	0.4-33.1	0.24
Washing hands with soap after field work (<i>versus</i> frequently)			
- sometimes	1.7	0.5-5.8	0.40
- rarely	3.4	1.1-10.0	0.03

was found on any of the washed samples of vegetables [26,27].

Interestingly, close contact with domestic animals was associated with an important risk increase. This finding is somehow difficult to explain. But it is well possible that cysts of *Entamoeba* deposited on the surface (fur) of the animals during close contact with humans and then later transmitted to a next person. In order to support this hypothesis, the presence of *Entamoeba* cysts in fur must be documented. Unfortunately, we could not conduct this verification during our field work.

Our study showed that agricultural field practices which involve handling of excreta of humans and animals are not relevant for the transmission although a considerable *Entamoeba* infection prevalence was documented in the faeces. Although *E. histolytica* cysts are quite resistant, they perish in human excreta within a short time period of storage or composting. Protozoan cysts, including those of *G. intestinalis* and *E. histolytica*, are unlikely to survive more than 10 days in soil as they are susceptible to desiccation [28]. On the contrary, we found that those handling animal excreta in the field had a significantly lower risk for an *E. histolytica* infection than those who have no contact with animal excreta. Several points are important with regard to this result. First, animals do not harbour *E. histolytica* infections, it is rarely found in domestic animals, including dog and cat [29,30] and therefore, it is unlikely that cysts are present in the stool. Secondly, all excreta is stored before being utilised in agriculture. The time period and conditions of the storage often do not meet full safety regulations [31]. However, they are sufficient to eliminate

an important portion of the infectious agents, including *E. histolytica* cysts [13,28]. Thirdly, those handling animal excreta are more likely to use personal protective measure and wash their hands with soap after work, i.e. in our study area; the Nhue River is an excellent opportunity for that as it is situated next to the agricultural land. Indeed, 96.4% of those handling animal excreta washed their hands after work compared to 61.0% of those who did not handle animal excreta.

The agricultural area of our study borders the Nhue River. Water from the Nhue River is used intensively for irrigation of fields and personal hygiene of farmers during field work. We found that intensive contact with Nhue river water during field work reduced the risk. This finding is to some degree in contradiction to the results of the study in Hanoi where diarrhoea episodes were significantly associated with contact with river water [16,17]. However, it must be noted that our study area is at a considerable distance to Hanoi and important agglomerations (60 km) where substantial contamination takes place. Hence, the concentration of infectious agents are diluted to a much higher degree [32].

Our study has some limitations. First, we had a relative small number of cases which resulted in a relative small overall sample size. Changes of exposure in a few cases may result in a risk change which is statistically not significant. For example, we found a statistical significant increased risk for households with average compared with good socio-economic status. However, those participants with a poor SES had an increased risk which was not statistically significant. Also, the risk increase

observed for those who use Nhue River water to irrigate field was not statistically significant. The small sample size could be a reason for this statistically non-significant observation. Other studies could show an increased risk of protozoan infection associated with Nhue River water [33]. Secondly, in our dataset the variables describing practices and habits of personal hygiene were highly correlated. Therefore, we could retain only one variable for the multivariable analysis. As a consequence, we could not perform a fine tuned multivariable analysis in which the effects of the different hygiene practices could be directly compared.

The association between infection and households' SES indicated that the participants living in households with an average SES presented a more than four fold risk increase (OR = 4.3, 95% CI: 1.3-14.0) compared to those living in households with a good SES. This finding is similar to that found in previous epidemiological studies indicating that unsanitary conditions and low SES were significant risk factors for *E. histolytica* infection [34-37].

However, in our study, there was no significant link between *E. histolytica* infection and participants' level of education. Our study population was relatively well educated. Two-third of our study participants finished secondary or high school and were generally very knowledgeable. A similar observation was made in Pakistan [38].

The fact that the households' water source was not a risk factor for *E. histolytica* infection is not surprising. Indeed, it was commonly observed that boiled rainwater was used for drinking in almost all study households. Nevertheless, a study from central Vietnam showed that river water may be an important source of *E. histolytica* infection [39]. An other study in Thailand found that the lack of regular water-treatment practices was also a risk factor [40].

The diagnostic method we used (FECT) does not allow the distinction between pathogenic *E. histolytica* from non-pathogenic *E. dispar* [41] which can be made by isoenzyme analysis and molecular technique [42]. Therefore, whenever *E. histolytica* is named in this article, it can not be excluded that it is *E. dispar*. *E. histolytica* infection, and resulting intestinal disease and liver abscesses are a public health concern in many tropical areas, including Vietnam. In our study we diagnosed among 794 randomly selected individuals 46 (5.8%) infected persons. Virtually all of them were asymptomatic but contribute to transmission. Even higher prevalence rates were observed in different parts of Vietnam, e.g. in a suburb of Hanoi and in Hue where 10.0% and 11.2% were infected [16,39].

Conclusions

Our study documents that agricultural practice in which human and animal excreta and household waste water are used as fertiliser and for irrigation are not relevant

for the transmission of *E. histolytica*. It confirms that in these settings other transmission routes such as contaminated hand are of importance and provides further arguments that basic personal hygiene measures such as hand washing with soap must be further promoted.

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 Faculty 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 (SCD) through the program of the National Centre for Competences in Research (NCCR) North-South.

Author details

¹Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, P.O. Box CH-4002, Basel, Switzerland. ²University of Basel, P. O. Box CH-4003, Basel, Switzerland. ³National Institute of Hygiene and Epidemiology (NIHE), 1 Yersin, Hanoi, Vietnam. ⁴Department of Environmental Health, Hanoi School of Public Health (HSPH), 138 Giang Vo, Hanoi, Vietnam. ⁵Swiss Federal Institute of Aquatic Science and Technology (EAWAG), Sandec - Department of Water and Sanitation in Developing Countries, Dübendorf, Switzerland.

Authors' contributions

PPD, HNV, JZ and PO planned the study and designed the protocols. PPD, HNV and PDC conducted the field study and supervised the study programme, including the collections of stool samples and data from the questionnaire interviews, as well as the management of collected data. PPD and HNV supervised all the laboratory work. PPD, JH, PO and HNV carried out the data analysis and interpretation. 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.

Conflict of interest

The authors declare that they have no competing interests.

Received: 11 May 2011 Accepted: 10 June 2011 Published: 10 June 2011

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doi:10.1186/1756-3305-4-102

Cite this article as: Pham Duc et al.: Risk factors for *Entamoeba histolytica* infection in an agricultural community in Hanam province, Vietnam. *Parasites & Vectors* 2011 **4**:102.

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