WASTEWATER AND EXCRETA USE IN AGRICULTURE IN NORTHERN VIETNAM: HEALTH RISKS AND ENVIRONMENTAL IMPACTS

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Dekan

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LIST OF ABBREVIATIONS

ADB	Asian Development Bank
AF	Attributable Fraction
AIDS	Acquired Immunodeficiency Syndrome
APHA	American Public Health Association
CDC	Centers for Disease Control and Prevention
DALYs	Disability Adjusted Life Years
DEC	Diarrheagenic *Escherichia coli*
EAWAG	Swiss Federal Institute of Aquatic Science and Technology
EPG	Eggs Per Gram
FECT	Formalin-Ether Concentration Technique
GEE	Generalised Estimating Equations
IFA	Immunofluorescent Antibodies
IWMI	International Water Management Institute
JACS	Joint Area Case Studies
MBD	Maximum Burden of Diarrhoea
MFA	Material Flow Analysis
MOH	Vietnamese Ministry of Health
MONRE	Vietnamese Ministry of Natural and Resources Environment
MPN	Most Probable Number
NCCR	National Center of Competence in Research
NIHE	National Institute of Hygiene and Epidemiology
PCA	Principal Component Analysis
PDF	Probability Density Functions
QMRA	Quantitative Microbial Risk Assessment
SANDEC	Department of Water and Sanitation in Developing Countries
SDC	Swiss Agency for Development and Cooperation
SES	Socioeconomic Status
SNSF	Swiss National Science Foundation
STH	Soil-transmitted Helminths
Swiss TPH	Swiss Tropical and Public Health Institute
UNICEF	United Nation Children's Fund
USEPA	United States Environmental Protection Agency
WHO	World Health Organisation

SUMMARY

Background

Wastewater is commonly used in agriculture and aquaculture in developing countries but also in developed countries due to the growing water scarcity. In Vietnam, the use wastewater and excreta in agriculture has a long tradition. While this practice has clear advantages (fertiliser, economic impacts etc.), it harbours potential risks for health and environment. The aim of the thesis was to understand the health and environmental risks related to wastewater and excreta reuse in an agricultural community in Northern Vietnam, with a focus on intestinal parasitic infections and diarrhoeal diseases and nutrients flow. This work is to optimizing the benefit of wastewater and excreta reuse in agriculture in from health and environmental perspective, which allows proposing potential interventions for health and environmental improvement.

Methods

In the study area - 2 communes of Hoang Tay and Nhat Tan in Hanam province, Northern Vietnam - wastewater (i.e. Nhue River and local pond), human and animal excreta are commonly used as water irrigation and fertilisers: (i) Cross-sectional, cohort and nested case-control studies were conducted to assess the relative importance of exposure to wastewater and excreta for parasitic infection and diarrhoeal episodes. Exposure data were obtained from household and individual interviews. Stool examinations were used to assess infection status. (ii) Quantitative microbial risk assessment (QMRA) of *Escherichia coli, Giardia lamblia* and *Cryptosporidium parvum* infection due to the exposure to wastewater and excreta was conducted using multi-trial Monte Carlo simulations to estimate diarrhoeal risks. (iii) Material flow analysis (MFA) was used to analyse nitrogen (N) and phosphorus (P) flows in the environmental sanitation and agricultural systems.

Results

Helminth infections were prevalent (e.g. *Ascaris lumbricoides* 24%, *Trichuris trichiura* 40%, and any helminth infections 47%). Risk of infection increased for people having direct contact with Nhue River water (OR = 2.1, 95% CI 1.4-3.2), and using human excreta as fertiliser (OR = 1.5, 95% CI 1.0-2.3). Tap water use in household was a protective factor against *T. trichiura* infection (OR = 0.6, 95% CI 0.4-0.9). *Entamoeba histolytica* infection was not associated with contact with Nhue River and pond water, and human and animal excreta, but with close contact with domestic animals (OR = 5.9, 95% CI 1.9-18.9), never or rarely washed hands with soap (OR = 3.4, 95% CI 1.1-10.0) and average socioeconomic ststus (OR = 4.3, 95% CI 1.3-14.0). Diarrhoeal incidence in adults was 0.28 episodes per person per year (pppy). The direct contact with water from the Nhue River (OR = 2.4, 95% CI 1.2-4.7) and local ponds (OR = 2.3, 95% CI 1.3-4.3), handling practices of human excreta (OR = 5.4, 95% CI 1.4-21.1), and animal excreta (OR = 3.3, 95% CI 1.8-6.0) as fertilisers were important risk factors for diarrhoeal diseases. Furthermore, inadequate use of protective measures (OR = 6.9, 95% CI 3.5-13.9), close contact with people having diarrhoea (OR = 3.7, 95% CI 1.4-10.3), never or rarely washed hands with soap (OR = 3.3, 95% CI 1.8-6.3), eating raw vegetables the day before (OR = 2.4, 95% CI 1.2-4-6), and rainwater use in household for drinking (OR = 5.4, 95% CI 2.4-12.1) were also associated with increased the risks of diarrhoeal diseases.

QMRA revealed that the most hazardous exposures included direct contact with Nhue River, local pond and field water, household sewage, and composted excreta. The annual infection risks due to exposure to wastewater exceeded the WHO reference level (10^{-4}, i.e. ≤ 1 infection per 10,000 individuals), e.g. in scenario of growing rice, *G. lamblia* caused an infection risk of 0.75, *C. parvum* (0.39), and *E. coli* (0.96). The annual diarrhoeal risks were much greater than the WHO threshold values of 10^{-3} (i.e. 0.001 pppy), e.g. due to *G. lamblia* (0.50), *C. parvum* (0.15) and DEC (0.24) in scenario of growing rice.

MFA simulations highlighted that the sanitation system is an important source of nutrients entering the surface water. Every year, 109 tonnes of N and 35 tonnes of P (75% N and 65% P from on-site sanitation system effluents) are discharged into the drainage system; and 118 tonnes of N and 25 tonnes of P released into surface water. Furthermore, simulations revealed that if nutrient management is not improved, levels of nutrients due to wastewater, faecal sludge, and organic solid waste will double until 2020.

Conclusions

In the agricultural settings, where wastewater and excreta are commonly used, important health and environmental impacts were documented. For mitigation purposes, personal hygiene practices and safe water and food consumption must be further addressed. Adequate on-site sanitation system technologies are warranted to assure waste treament and reduce nutrients discharge to the environment. Further investments in this direction are warranted to improve benefit-risk ratio for the agricultural community and increase sustainability of this agricultural system.

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1. INTRODUCTION

1.1. The overview of wastewater and excreta use in agriculture

Wastewater can encompass a wide range of potential contaminants and concentrations resulting from the mixing of wastewaters from different sources, e.g. from domestic effluent consisting of blackwater (excreta, urine and faecal sludge, i.e. toilet wastewater), greywater (kitchen and bathing wastewater), industrial and agricultural effluents, hospitals, and stormwater [1]. The use of wastewater is a widespread practice with a long tradition in many countries around the world, it is estimated that more than 4-6 million hectares are irrigated with wastewater or polluted water [2, 3]. In many European and North American cities, wastewater was disposed of in agricultural fields before the introduction of wastewater treatment technologies to prevent pollution of water bodies [4]. In developing countries like China, Mexico, Peru, Egypt, Morocco, India and Vietnam, wastewater has been used as a source of crop nutrients over many decades [2, 5]. Over the years, the use of wastewater and excreta in both agriculture and aquaculture continues to be common in China, South and South East Asia as well as various places in Africa [6-8]. The majority of wastewater used in developing countries does not receive any conventional treatment before being directly applied to the agricultural land. A rapid and uncontrolled urban sprawl combined with limited financial resources and capabilities for wastewater collection and treatment contribute to this situation [9]. In contrast to many industrialized countries, the use of wastewater has become less popular with the improvement of treatment technologies and increased awareness of the environmental and health issues associated with practice. For example, in France or Israel only secondary treated wastewater is used, and usually as a sprinkle system [10-12]. The use of wastewater has been successful for irrigation of a wide array of crops, and has increase in crop yield [13]; and wastewater used to increase fish production through aquaculture in Asian countries [14]. Wastewater is usually used in agriculture because it is an available water source and nutrient concentrations allows for the cultivation of crops without the use of chemical fertiliser [15], saving in fertiliser costs resulting in higher farm incomes in wastewater farmers [16]. Where vegetables grown in wastewater are the main commodity, there can be a significant aggregate benefit for the society in terms of a more balanced diet. For example, more than 200,000 people eat vegetables produced with wastewater every day in Ghana [17]. While farmers and their families are direct beneficiaries, there are also indirect beneficiaries along the supply chain including farm labourers, transporters, vendors, processors, input suppliers and consumers [18]. With low investments and quick returns, this practice is profitable and enables many farmers to overcome the poverty line [19].

Excreta are important source of nutrients for crops on many farms. Reuse of excreta on agricultural land secures valuable fertilisers for crop production and limits the negative impact on water bodies [20].Use of excreta is rarely made public, but is known to have been practised for centuries in Asia, in particular in China and Vietnam [20-22] in both agriculture and aquaculture: this practice has lead to a strong economic linkage of urban dwellers and urban farmers. In the current context of environmental sanitation system, nutrient flows are mainly linear. Closing nutrient flows and keeping out problematic substances such as drugs, antibiotics, hormones, and heavy metals separate from the plant nutrients cycle must be promoted [23, 24]. The concentrations of phosphorus and potassium are high in human excreta and may significantly increase the crop yield [25]. Use of excreta can help to improve food production, especially for subsistence farmers who otherwise might not be able to afford artificial fertilisers [20]. As indicated by Jensen and colleagues, 2010 that if Vietnamese farmers was to replace human excreta with imported fertiliser, it would involve an extra national expenditure of at least US$ 83 million a year [26]. In the light of the global phosphorus crisis, excreta and wastewater can be critical sources of phosphorus [27]. The increased productivity and related income or food supply gains allow farmers a more reliable livelihood with indirect benefits of using the income for education and improving health conditions [28].

1.2. The health risks related to the use of wastewater and excreta

In developing countries, excreta-related diseases are very common, and faecal sludge and wastewater contain high concentrations of excreted pathogens such as viruses, bacteria, protozoa cysty, and helminths eggs that may cause gastrointestinal infections in humans. The pathogens most commonly found in the wastewater are faecal viruses, including enteric viruses, hepatitis A virus, and rotavirus, bacteria (e.g., *Campylobater* spp., *Shigella* spp., *Salmonella* spp., *Escherichia coli* and *Vibrio cholerae*), protozoa (*Cyclospora cayetanensis, Cryptosporidium parvum, Entamoeba histolytica,* and *Giardia intestinalis*), and soil-transmitted helminths (*Ascaris* spp., *Trichuris* spp., and hookworm) [6].

1.2.1 Excreted-pathogens transmission routes

When these pathogens are introduced into the environment some can remain infectious for long periods of time, and under certain conditions, they may be able to replicate in the environment. The presence of pathogens presents a potential threat to human health. However, for an actual risk of disease an infectious dose of the excreted pathogen must reach a human host [29]. Disease transmission is determined by several pathogen-related factors including: an organism's ability to survive or multiply in the environment; latency period, and an organism's ability to infect the host [30]. Disease transmission is also affected by host characteristics and behaviour, including: immunity, nutritional status, health status, age, sex, personal hygiene, and food hygiene [29].

The transmission routes, important pathogen and host related transmission factors and also possible barriers to transmission for excreted pathogens are described in Figure 1.1, as its sanitation is the primary barrier for preventing faecal-oral disease transmission [29].

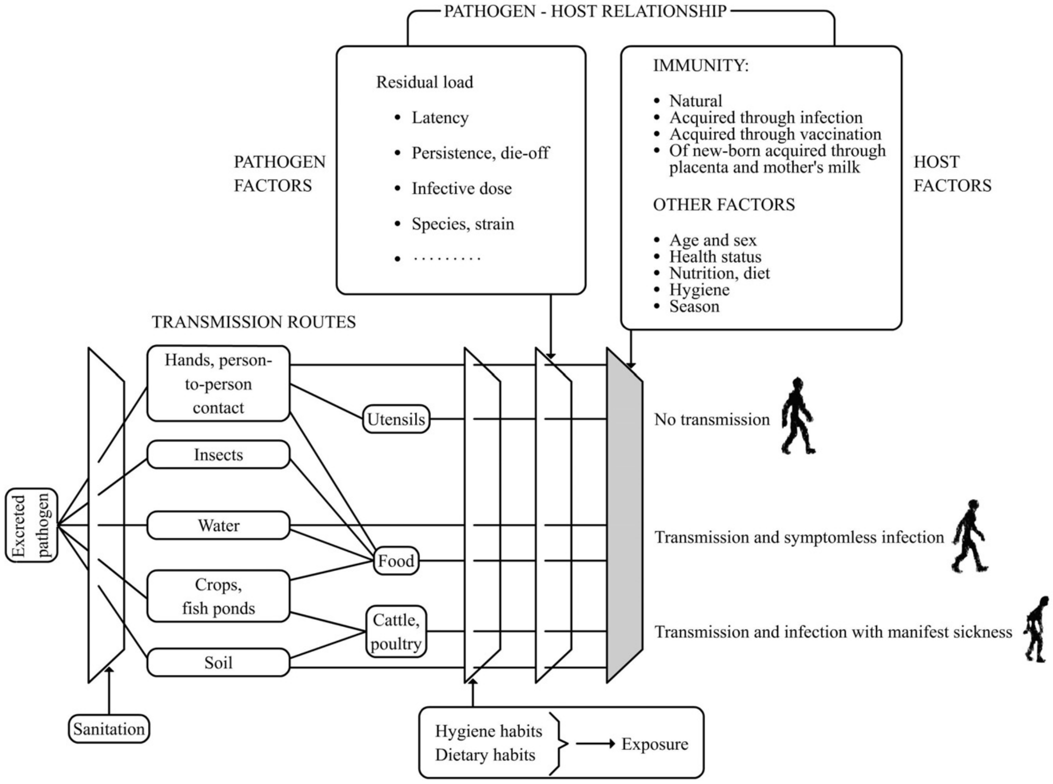

Figure 1.1 Faecal-oral pathogen transmission routes

Source: Carr, R. (2001)

When untreated or inadequately-treated wastewater or excreta is applied to soil and crops, disease transmission can occur. Main health hazards associated with wastewater use in agriculture and aquaculture include enteric diseases caused by excreta-related pathogens (primarily diarrhoeal diseases and intestinal helminth and protozoan infections). The groups of people that are at the highest risk of these diseases are farm workers with prolonged wastewater contact, their families, crop handlers, consumers of crops or meat and milk coming from cattle grazing on polluted fields, and nearby communities exposed to wastewater, sludge or excreta [14, 20, 31].

1.2.2 Enteric virus pathogens

Of the different viruses that may be excreted in faeces, the most common are members in the enterovirus, rotavirus, enteric adenovirus and human calicivirus (novovirus) groups [32]. In developed countries, enteric viruses are considered to be the cause of the gastrointestinal infections [33]. When applying excreta in the fields hepatitis A virus is considered a risk for both waterborne and food-borne outbreak, especially when the sanitary standards are low.

1.2.3 Enteric bacterial pathogens

Worldwide, enteric bacterial pathogens are still a concerned, in particular in developing countries, where epidemics of cholera, typhoid and shigellosis are more frequent in both peri-urban and urban areas. In the areas where there is inadequate sanitation, cholera caused by *Vibrio cholerae* and typhoid fever caused by *Salmonella typhi* constitute major risks through the resulting drinking water contamination. The use of polluted water for irrigation or to unsafe disposal of excreta has strongly been associated with cholera. Other diarrheagenic diseases related to unsafe agricultural practices are shigellosis and gastric ulcers caused by *Helicobacter pylori* [34]. *Escherichia coli* (*E. coli*) of many different serotypes are categorized into four major groups according to virulence mechanisms: enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), and enteroaggregative (EAgg EC). Other groups (e.g., diffusely adherent *E. coli*) are less well established as pathogens [35]. Enterotoxigenic *E. coli* is often related to diarrhoea of travellers in developing countries [36]. Diarrheagenic *E. coli* may be transmitted by a number of routes, but water is a well-proven route of infection, based on available outbreak data [37]. Zoonotic agents transmitted between humans and animals through contamination from faeces or manure, have included diarrheagenic *E. coli*, *Campylobacter* and *Salmonella.*

1.2.4 Intestinal protozoan parasites

The intestinal protozoa *Entamoeba histolytica* causes an amoebiasis and *Giardia intestinalis* is a causative agent of giardiasis. They occur worldwide and constitute a considerable public health burden in countries with low socio-economic conditions where the barriers between human faeces and food and water are inadequate. *E. histolytica* and *G. lamblia* life cycles are illustrated in Figure 1.2.

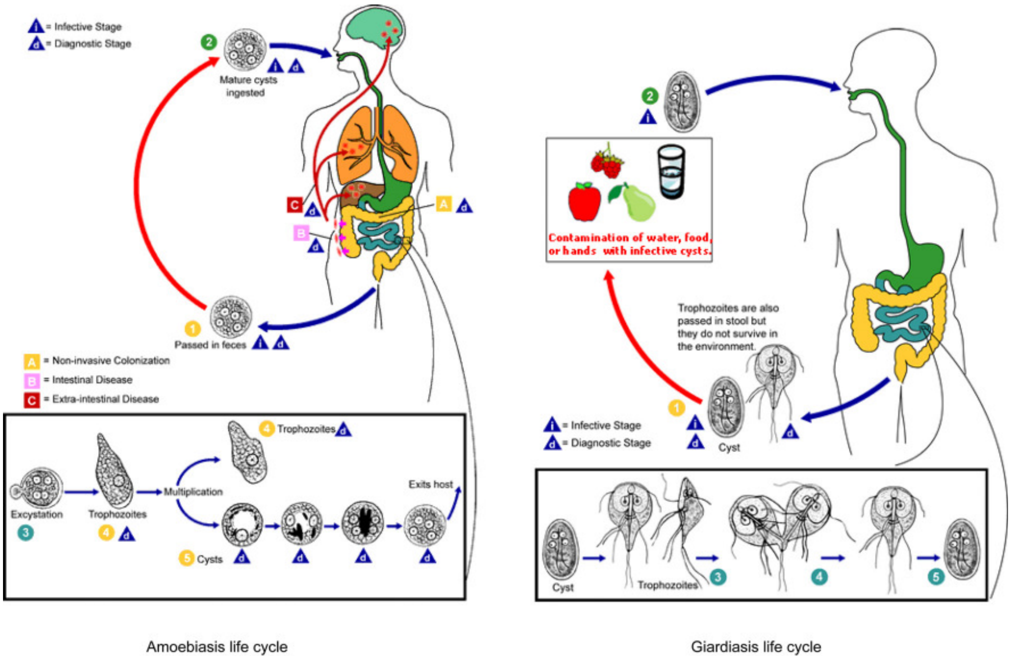

Figure 1.2 Life cycle of Amoebiasis and Giardiasis

(Source: www.cdc.gov)

E. histolytica natural hosts are humans only. Cysts and trophozoites are passed in faeces and ingested from contaminated food, water or hands. After excystation in the small intestine, released trophozoites migrate to the large intestine where they multiply and produce cysts that are passed in the faeces. *G. lamblia* cysts that occur in contaminated water and food, are ingested by drinking, eating, or via the faecal-oral route. After passing to the small intestine, they move to the colon where they encyst again and are released with the faeces. Responsible for transmission of both protozoa are the resistant cysts in the external environment. *E. histolytica* affects an estimated 480 million people worldwide [38], causing severe tissue damage, mostly of the intestinal mucosa and in the liver, and is responsible for 40,000-100,000 people death every year [39]. *G. lamblia* is estimated to be responsible for 2.8 million human infections every year and contributes to nutritional deficiencies in children. In developing countries, prevalence rates can reach 20-30%. *G. lamblia* occurs with high prevalence as an enteric pathogen [40]. *G. lamblia* cysts are instantly infectious once they leave the host through faeces [41].

Cryptosporidium are small coccidian parasites that infect the gastrointestinal and respiratory tracts of a wide variety of humans and animals [42]. Two major pathogens affecting humans are *C. parvum* and *C. hominis.* The life cycle of *Cryptosporidium* is described in Figure 1.3. Oocysts, the environmentally resistant transmission stage of the parasite, are shed by infected hosts with their faeces and are immediately infectious [43]. Oocysts may remain in the environment for very long periods with infectivity. When a new host ingests an oocyst, excystation opening of the suture in the oocyst wall is triggered by the body temperature and the interaction with stomach acid and bile salts. Four motile sporozoites are released, which infect the small intestine epithelial cells. The parasite infects the epithelial cell apex, residing beneath the cell membrane but outside the cytoplasm. The sporozoites undergo several transformations in an asexual and a sexual reproduction cycle; it is the latter that generates the oocysts. *Cryptosporidium* is transmitted by ingestion of faecal contaminated food or water, by exposure to faecal contaminated environmental surfaces, and by the faecal-oral route from person to person [44]. *Cryptosporidium* causes diarrhoea that is self-limiting for immuno-compromised persons, especially those with acquired immunodeficiency syndrome (AIDS). Infection accounts for up to 6 % of all reported diarrhoeal disease in immuno-compromised persons worldwide [42], and 24% of all the persons with both AIDS and diarrhoea are infected with *Cryptosporidium* spp. [44].

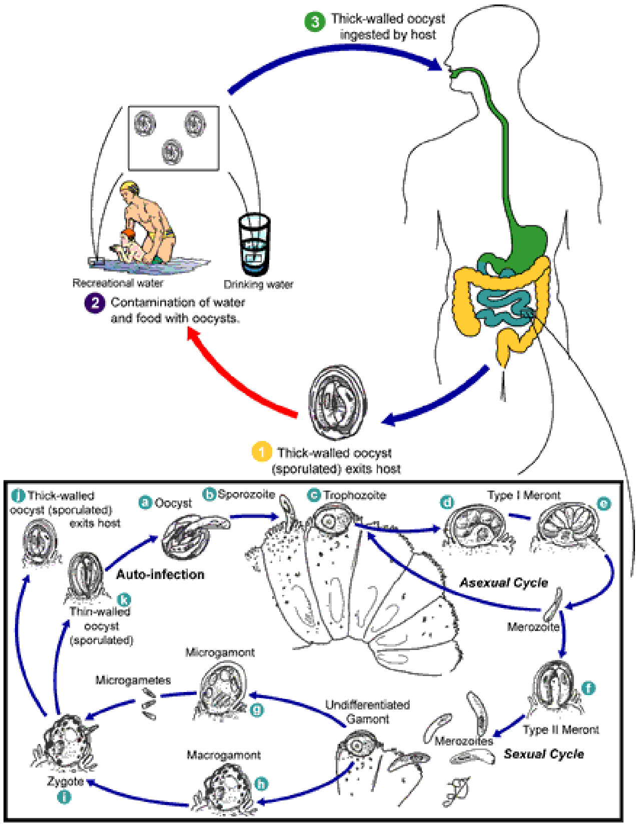

Figure 1.3 Life cycle of *Cryptosporidiosis*

(Source: www.cdc.gov)

1.2.5 Soil-transmitted helminthiasis

Soil-transmitted helminths (STH) are intestinal nematodes which develop partly in the soil and partly in the human body. The main species include the hookworms (*Ancylostoma duodenale* and *Necator americanus*), roundworm (*Ascaris lumbricoides*) and whipworm (*Trichuris trichiura*). Their life cycles are distinctly different. The life cycles of *A. lumbricoides* and *T. trichiura* are shown in Figure 1.4. Eggs of *A. lumbricoides* are passed via stool and maturate in the environment (i.e. on soil) to infective eggs. They are ingested via contaminated food and hatch in the human host. The released larvae migrate via lung and respiratory tract and reach again the intestinal tract where they mature. Released eggs of *T. trichiura* also require a period of maturation in the environment (i.e. on soil, plants) before they become infective. However ingested eggs directly develop into adult worm in the intestine without a migration.

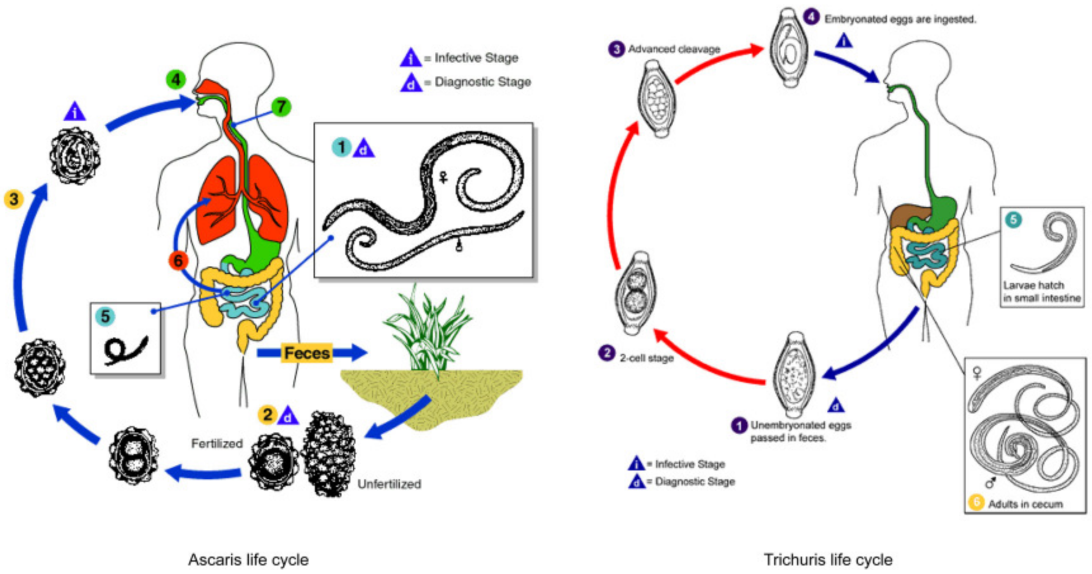

Figure 1.4 Life cycles of *A. lumbricoides* and *T. trichiura*
(Source: www.cdc.gov)

Hookworm eggs leave human with stool and the soil, where larvae are released which develop to an infective larval stages. This filariform larva actively penetrates the human skin and migrates via venous blood vessels, heat, lung and respiratory tract to the small intestine, where they mature into the adult worms (Figure 1.5). Hookworms attach to the intestinal wall, where they draw blood and hence contribute to anaemia [45].

The most important health hazards among farmers and their families exposed to wastewater irrigation in agriculture and aquaculture are parasitic infections, including protozoa and STHs [46-52]. Helminthiases are common in regions where poverty and poor sanitary conditions prevail; under these conditions they can affect up to 90% of the population [53]. Ascariasis is the most common one and is endemic in Africa, Latin America, and Southeast Asia. It is estimated that the high intensity of ascariasis infections approximate 133 million people. Even though the mortality rate of helminthiases is low, i.e. for ascariasis nearly 10,000 persons per year, most of the people affected are children under 15 years old. Approximately 1.5 million of these children never attain expected growth, even if treated [54]. Most studies have found a higher prevalence of helminth infections in the exposed than unexposed people.

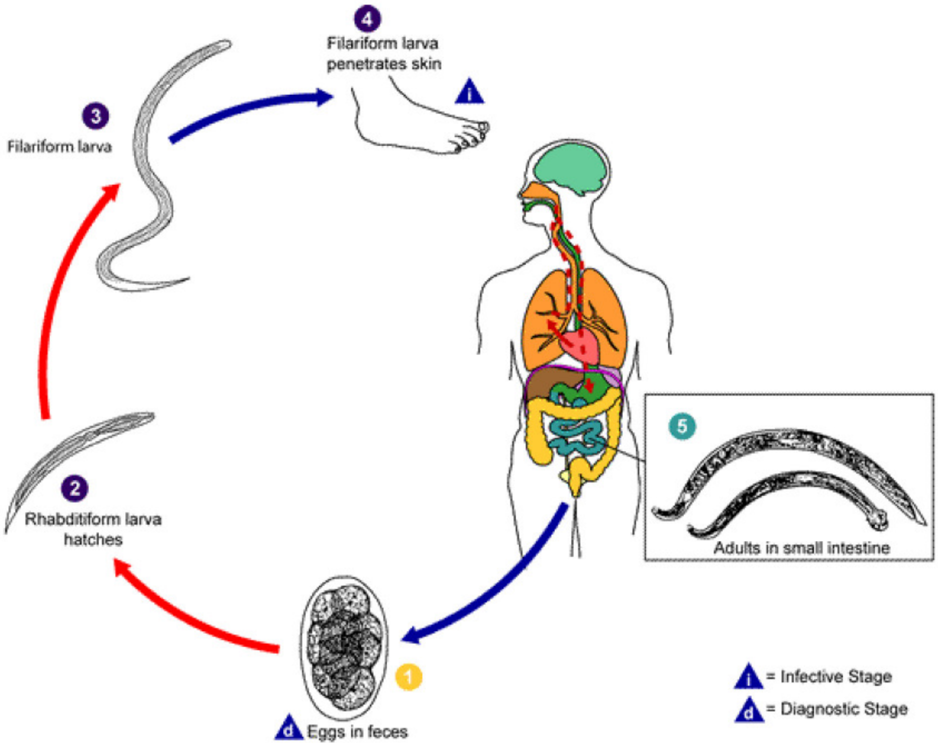

Figure 1.5 Life cycle of hookworm

(Source: www.cdc.gov)

In particular a statistically increased prevalenceof infections with *A. lumbricoides* and hookworm was observed among people with direct contact with wastewater the effect was more obvious in children than in adult farmers [50, 55-58]. It was suggested that the wastewater needs to be treated to achieve a concentration of below one helminth egg per litre where people are exposed; at the same time, there should be restriction of children's contact with wastewater [31].

1.2.6 Diarrhoea related to the use of wastewater and excreta

There are worldwide estimates of about two billion cases of diarrhoeal disease every year. Diarrhoeal disease is a leading cause of child mortality and morbidity, and mostly results from contaminated food and water sources [59]. Approximate 1 billion people lack access to improved clean water and 2.5 billion have no access to basic sanitation. Diarrhoea is widespread throughout developing countries [60]. It is caused by waterborne bacterial pathogen is one the health problems associated with the use of wastewater in agriculture, especially in children under five years of age. Diarrhoea was observed with significantly higher prevalence in wastewater exposed people in Pakistan [48]. In Mexico, studies found a higher prevalence of diarrhoeal diseases in children under 5 years of age exposed to untreated wastewater than those who were exposed to wastewater retained in a single reservoir or no irrigated wastewater [50, 51]. The studies showed that partially treated wastewater can reduce the risk of diarrhoeal diseases, and the harmful effect of wastewater exposure was stronger in the dry season than the rainy season [51]. Several diarrhoeal outbreaks have been associated with wastewater-irrigated vegetables [31]. However, in developing countries it is often a challenge to attribute diarrhoeal outbreaks to specific exposure routes due to other contributing factors including poor hygiene and sanitation and reduced access to safe drinking water [28]. In Vietnam, the awareness of the importance of farmers' exposure to human and animal excreta as a cause of diarrhoeal diseases is still lacking. Indeed, Trang and colleagues assessed the risk of diarrhoeal diseases in adults engaged in wastewater-fed agriculture and aquaculture, and found that contact with wastewater was the principal risk factor for diarrhoea [61]. To our knowledge, very few studies have assessed the risk of diarrhoeal diseases associated with combined the exposures to both excreta and wastewater use in agriculture and aquaculture. The importance of our study is to identify which factor is the most important for risk of diarrhoeal diseases in the agricultural settings, where wastewater and excreta are commonly used.

1.2.7 Summary of health risks associated with the use of wastewater

In terms of the above pathogens associated with excreta and wastewater, intestinal nematodes are ranked highest prevalence, followed by bacteria, protozoa and viruses [62]. Table 1.1 summarizes the health risks related to wastewater use in agriculture and aquaculture.

Table 1.1 Summary of health risks associated with wastewater reuse

Pathogens	Group exposed		
	Farm workers and their families	Nearby communities	Consumers
Helminth infections	Significant risk of helminth infection for both adults and children in contact with untreated wastewater, increased risk of hookworm infection for workers who do not wear shoes, risk of helminth infection remains, especially for children, even when wastewater is treated to < 1 helminth egg per litre, adults are not at increased risk at this helminth concentration.	Transmission of helminth infections not studied for sprinkler irrigation, but same as above for flood or furrow irrigation with intensive contact.	Significant risk of helminth infection for both adults and children in contact with untreated wastewater.
Bacterial or virus infections	Increased diarrhoea risk in young children with wastewater contact if water quality exceeds 10^4 thermotolerant coliforms per 100 mL, elevated risk of *Salmonella* infection in children exposed to untreated wastewater, elevated seroresponse to norovirus in adults exposed to partially treated wastewater.	Sprinkler irrigation with poor water quality and high aerosol exposure associated with increased rates of infections, use of partially treated water in sprinkler irrigation is not associated with increased viral infection rates.	Cholera, typhoid and shigellosis outbreaks reported from use of untreated wastewater, seropositive responses for *Helicobacter pylori* (untreated), and increase in non-specific diarrhoea when water quality exceeds 10^4 termotolerant coliforms per 100 mL.
Protozoan infections	Risk of *Giardia intestinalis* infection reported to be significant increased; risk of amoebiasis observed with contact with untreated wastewater.	No direct evidence of disease transmission	No data

Source: WHO (2006)

1.3 Wastewater and excreta use in agriculture and health risks in Vietnam

In Vietnam the use and recycling of wastes, household sewage, human and animal excreta in agriculture and aquaculture has a long history in many centuries. The reuse of excreta and wastewater for crops and fish ponds may provide many positive benefits, such as cheap fertiliser, reliable source of nutrition and water, reduce commercial fertilisers, improve soil-structure and increase productivity. However, transmission of enteric pathogens is a fundamental public health issue associated to theses practices.

The sources of irrigation in Vietnam vary from fresh water and wastewater to ground water. In urban areas wastewater is mainly from domestic origin with high concentrations of nutrients, which farmers appreciate for its fertiliser value [63]. They use it as a cheap and reliable source of supplemental nutrients and water, and for its contribution to the increasing crop yields. However, people in peri-urban areas have recently been concerned about the contamination of domestic wastewater and industrial effluents due to the rapid development of industries [64, 65]. There is evidenced that the application of wastewater in agriculture, particularly fish farming, has brought benefits to the sector as well as profits to farmers, which have resulted from cheap investment and better harvest yields [64, 66-68].

Applying human excreta to agricultural fields has for centuries been part of the agricultural tradition in Vietnam. Despite the potential health risk for intestinal disease when using excreta and animal waste in agriculture [20], the practice of using excreta as fertiliser in agriculture was estimated at 85% in Northern provinces in Vietnam [21]. The economic benefits obtained from human excreta outweigh the hygiene message for most Vietnamese farmers [26]. The farmers need cheap fertiliser and although they know that the excreta content can be harmful to human health, they still believe that it has a very positive nutrient values on their crops [69].

Despite the widespread use of wastewater and excreta in the country, such practices are often informal and only recognized to a limited degree by the authorities, policy makers, and the public at large. Authorities are often reluctant to get involved because of the perceived human health risks associated with the consumption of products from wastewater-fed systems [70]. In Vietnam, there are few policies related health protection for urban wastewater users, even though the use of wastewater in agriculture and aquaculture is widespread [63, 68].

The health risks associated with wastewater use in agriculture and aquaculture were proven in Vietnam. Indeed, an epidemiological study showed that the incidence rate of diarrhoea is 28 episodes per person-year at risk among people engaged in wastewater-fed agriculture and aquaculture [61]. The study also indicated that wastewater contact was associated with the risk of diarrhoeal diseases in adults and appeared to be more important for public health than other risk factors such inadequate hand washing and consumption of unsafe food or water. Another study in Vietnam showed that wastewater exposure was not associated with helminth infections, but that lack of sanitation facilities and the use of fresh or inadequate composted excreta as fertilisers in agriculture increased such risks [55].

Infection with *A. lumbricoides*, *T. trichiura* and hookworm are widespread in Vietnam and show a declining prevalence rate for *Ascaris* and *Trichuris* from the North to the South [71]. General helminth infections are often given low priority by authorities as they are not associated with obvious morbidity and mortality. However, there is strong evidence that helminth infections result in considerable morbidity, reduced growth among children, and have negative impacts on the learning capabilities of children [72, 73]. Nonetheless, to limit the risk of helminth infection via human excreta, the Vietnamese government has recently introduced at set of guidelines for the proper composting of human excreta before its use in agriculture with recommend a minimum composting period of six months inside the latrines [74]. In Vietnam the prevalence of *Cryptosporidium* and *Giardia* is relatively unknown, but a previous study documented the presence of these protozoans in vegetable production sites where either pig manure or untreated wastewater was used [75].

Nowadays, environmental sanitation and agriculture systems in Vietnam face many troubles. Because of untreated wastewater and excreta are still commonly used as a source of water and nutrients for agriculture and aquaculture, but these practices may create health risks from the contamination of food products and people exposure to pathogenic organisms. Environmental sanitation services including management of solid and liquid wastes are still very poor in many areas of Vietnam, in particular in rural areas where access to adequate sanitation is lower than 20% [76]. In recent years, many projects have been implemented with have led to considerable improvements of environmental sanitation and infrastructure systems. However, many problems still happen in these systems, and interventions have created new issues.

1.4 Integrated approach for assessing the health risks and environmental impacts

Through the program of the National Centre for Competence in Research (NCCR) North-South, Nguyen-Viet et al.,(2009), a conceptual framework was developed for integral interventions improving health and environmental sanitation in urban and peri-urban areas (Figure 1.6) [77]. The framework takes into account three main components: (i) health status, (ii) physical environment, and (iii) social, cultural and economic environment. Information on each of these three components can be obtained by using standard disciplinary methods and an innovative combination of these methods.

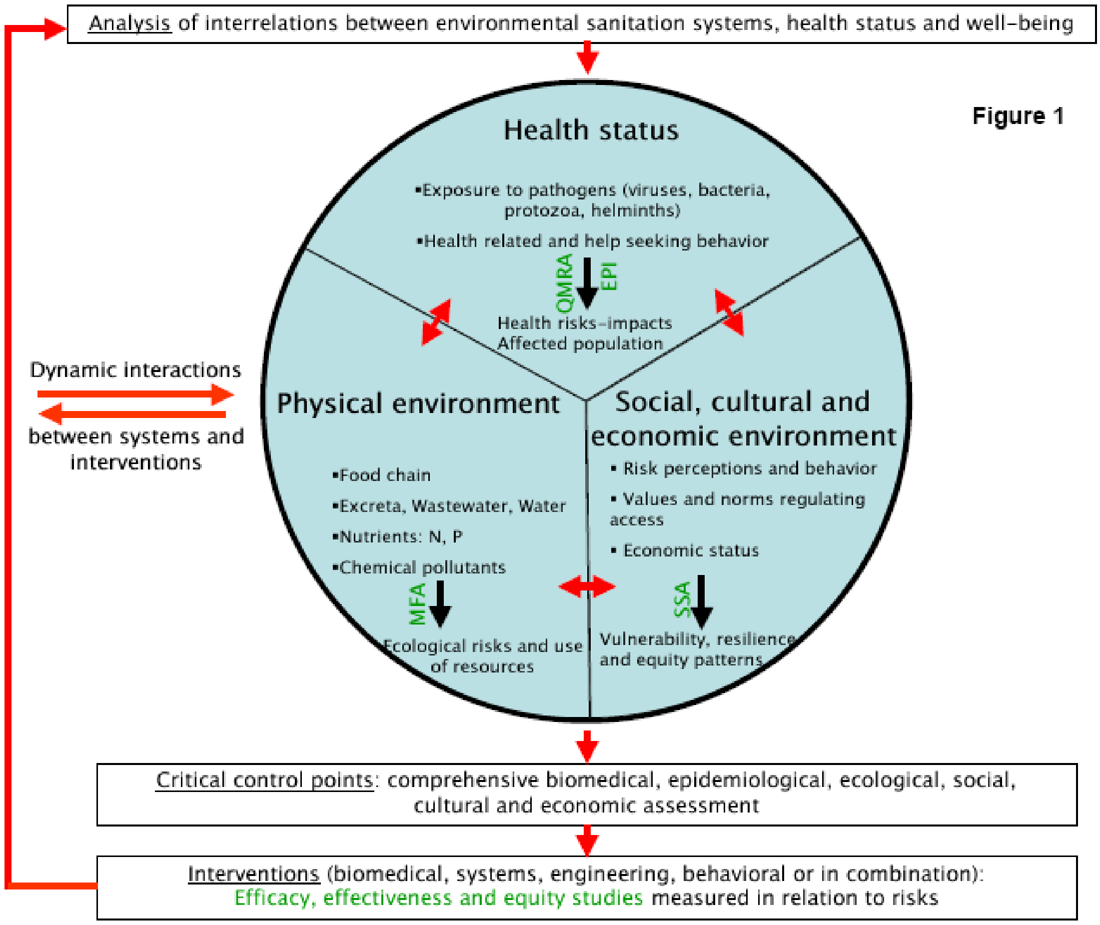

Figure 1.6 Conceptual framework of the combination of health and environmental risk assessment for health and environmental sanitation planning

Source: Nguyen-Viet, H., *et al.* (2009)

In Figure 1.6, green characters refer to methodologies used within the conceptual framework. QMRA is quantitative microbial risk assessment; EPI is epidemiology; MFA is material flow analysis; SSA is social science analysis.

Based on the proposed concept, this PhD study used both epidemiological and quantitative microbial risk assessment (QMRA) methods for assessing the health risks associated with wastewater and excreta reuse. Furthermore, material flow analysis (MFA) was applied for describing the inter-connection between the agricultural system and the environmental sanitation system, identifying relevant nutrient sources in the rural settings in Northern Vietnam.

Epidemiological studies aim to assess the health status and comparing the level of disease in the exposed population with that in an unexposed or control population. As indicated by Beaglehole and colleagues, epidemiological studies are very important to assess health risk associated with food chains and environmental sanitation [78]. The difference in disease levels may then be attributed to the practice of using the wastewater or excreta, provided that the two populations compared are similar in all other respects, such as socioeconomic status and ethnicity [20, 31]. Most of epidemiological studies have been concerned the use of wastewater, whereas very few studies include the use of excreta in agriculture [34]. Epidemiological studies have established that the highest risk to human health of using human excreta and wastewater in agriculture is posed by viruses, bacteria, protozoa and helminth infections. However, in addition to the use of excreta and wastewater, there are other very important environmental, climatic, and human behavioural risk factors for infection with pathogens, of which poor environmental sanitation is probably the most important one. The primary limitation of an epidemiological study is the uncertainty associated with the routine information collection and the influence of confounding factors; both of which reduce the sensitivity of identifying excess risk. Most outbreaks of waterborne disease are therefore not identified by routine case reports unless at least one percent of the population in a community becomes ill within a few months [79]. Not surprisingly therefore, direct epidemiological evidence for excess risk resulting from wastewater use in agriculture is extremely limited [12, 62, 80-82]. The relative importance of the different risk factors is unknown from epidemiological studies. It is difficult to make informed decisions about excreta and wastewater management without detailed knowledge of exposure.

While the epidemiological studies are well known, validated and applied [78], recently QMRA approach has been applied in assessments of health status, and WHO has been recommended this approach to assess health risks related to the consumption of low quality drinking water, and to the use of wastewater, greywater and excreta in agriculture [20, 31, 83]. This methodology has been increasingly used in risk assessment of drinking water [84, 85] and in waste management [86, 87]. Microbial risk assessment model have been developed for a range of waterborne pathogens in drinking water, including *C. parvum* [88], *G. lamblia* [79, 89] and enteric viruses [90, 91]. Also, this model has been applied to practices such as crop irrigation and discharge to recreational impoundments [92, 93]. QMRA methods have been developed in the context of risk of infectious disease transmission related to the use of faeces as fertiliser in Denmark [94]. Recently, QMRA has been used to assess the risk of infection associated with contact with wastewater [95, 96]. QMRA estimates transmission risks of a selected pathogen in a specific exposure pathway. It estimates risks difficult to measure with epidemiological approaches and is therefore a useful complement to epidemiological investigations [97].

MFA methods identify and quantify mass flows of substances (i.e. nutrients) into and in an environmental sanitation system. MFA quantifies charges of nutrients to the system and hence lies a foundation to understand the impact of these nutrients. In recent years, the conventional MFA method was adapted to fit the specificities of developing countries which typically face problems of data scarcity and uncertainty [98]. The adapted MFA method was successfully applied in urban areas of developing countries like in Vietnam [98-100]. However, its applicability in a rural and peri-urban context has not been demonstrated. MFA complements QMRA and epidemiological approaches by evaluating current nutrient discharge trends which in turn indicate potential future systems with regard to resource management, water pollution control and microbial health risks.

2. AIM AND OBJECTIVES

Aim:

The aim of this thesis is to enhance our understanding on health and environmental impact of wastewater and excreta use in agriculture, by combining epidemiological and microbial risk assessment and material flow analysis approaches.

Objectives:

- To assess risk factors for intestinal helminth infections and diarrhoea among people working and living in agricultural settings, where wastewater and excreta are intensively used, by using epidemiological approaches (EPI);
- To assess the infection risks of diarrhoea-related pathogens in an environmental sanitation and agriculture system by using quantitative microbial risk assessment (QMRA);
- To analyse nutrient fluxes of nitrogen and phosphorus in an environmental sanitation and agricultural system by using material flow analysis (MFA), and identify critical control points of nutrient loading for pollution control.

3. DESCRIPTION OF THE 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 (Figure 3.1). Hanam province is part of the Nhue-Day River catchment area in Northern Vietnam, where all the internal rivers flow into Nhue and Day Rivers and carry a heavy load of pollutants [101]. The catchment's water environment is under a pressure of severe pollution from high population density and urbanization as well as industrial, agricultural and aquacultural productions. Among these sources of pollution, Hanoi City's wastewater from households (54%) is directly discharged untreated into the river [101].

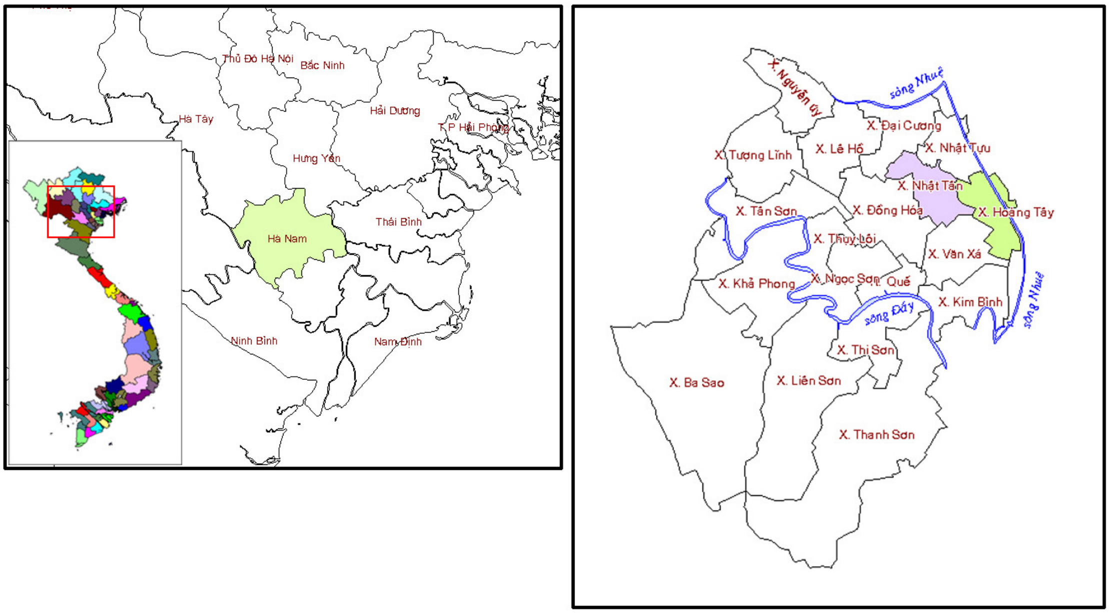

Figure 3.1 Maps of the study sites in Hoang Tay and Nhat Tan communes, Hanam province, Northern Vietnam

Source: National Institute of Agricultural Planning and Protection, Vietnam (2008)

The two communes border the Nhue River and the farmers commonly use water from this river for crop irrigation and to feed fishponds. Several pumping stations are located along the river and a net of open and closed canals distribute the water to the local fields and fish ponds (Photo 3.1). The sub-tropical climate of the Red River Delta has a main rainy season from April to September and year-round high humidity ranging between 80 and 90% [102]. The rice fields and local ponds cover about 50% of the community's lands. The area has two main wetland rice production cycles per year, one called "spring season" from January to June and the other "autumn season" from July to October. People also grow vegetables which are eaten raw or cooked by the local population and are sold to neighbouring towns and Hanoi. Most households have a limited space and do not have a small garden. The residential areas are in the vicinity of fields where agriculture (rice and vegetables) and aquaculture (fish breeding) takes place.

Photo 3.1 Nhue River and water pumping station for irrigation to the fields in Nhat Tan and Hoang Tay communes, Northern Vietnam

(Photo: Hung Nguyen-Viet)

The sanitary conditions in the households under survey are illustrated in detailed in Chapter 5. Overall, the households had poor sanitary conditions, with 33% smelling badly and flies around the latrine, dirty-looking water storage facility from outside, and waste, mud, and animal faeces in the yard. Most households had access to a latrine, and the most common types were single or double vault, but it did not meet the standardised sanitary latrine proposed by the Vietnamese Ministry of Health (MOH) [74]. The number of households with a hygienic latrine (septic tank and biogas) in both of the two communes was approximately 22%. Most households raised livestock in their compounds (pigs, cows, water buffalos and poultry) that is generally situated in the garden area and close to water sources. Wastewater from households (grey water from kitchens and bathrooms, and effluent from septic tanks and sanitation facilities) is freely discharged untreated into the small irrigation canals and local ponds (Photo 3.2).

Photo 3.2 Households raise livestock and breed fish in their compounds in Hoang Tay and Nhat Tan communes, Northern Vietnam

(Photo: Hung Nguyen-Viet)

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 only partially composted. 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 recommended by MOH [74]. All households used collected rainwater from a roof and gutter system as the main source for drinking which was often boiled before consumption, while water used for washing, bathing, and cleaning the yards originated from drilled and dug wells [103, 104]. A survey conducted by the Provincial Department of Health in 2006 showed that households used rainwater (85%), tap water or tube well water (15%) for drinking in both of the two communes. Analysis of 30 samples of drinking water in this commune showed that 100% of samples were contaminated by organic-substances and faecal bacteria [105]. According to the Provincial Department of Health and Communal People’s Committees statistics in 2008, the estimated number of diarrhoea cases in Nhat Tan was 547 cases/100,000 people and Hoang Tay was 814 cases/100,000 people. There also are very high percentages of people infected helminth parasites such as *Ascaris* (86%), *Trichuris* (76%), and hookworm (9%). High percentages of people had eye (25%), skin (14%), and gynaecological (12%) infection [103-105].

According to surveys in the two communes in 2009 [106], people perceived wastewater as smelly and black in colour, and report that contacts with it can cause skin problems (e.g. itching). Farmers felt that fish from the Nhue River and vegetables irrigated with wastewater were potential causes for diarrhoea. When working with wastewater, women used protective wear more often than men. This was attributed to the fact that women spent more time in the fields than men and paid more attention to their skin and beauty.

The environmental sanitation and agriculture system in the study sites were described in detailed in Chapter 8 & 9. Generally, the system of surface water for irrigation was closely connected between the irrigation systems and agricultural fields and local ponds. Water from the Nhue River is pumped into the irrigation systems and then distributed into the rice and vegetable fields and fish ponds. Within the households, wastewater from the kitchens, toilets, and animals are freely discharged into the sewage, pond, and irrigation system. Human excreta are composted in the garden or inside the latrine (Figure 3.2).

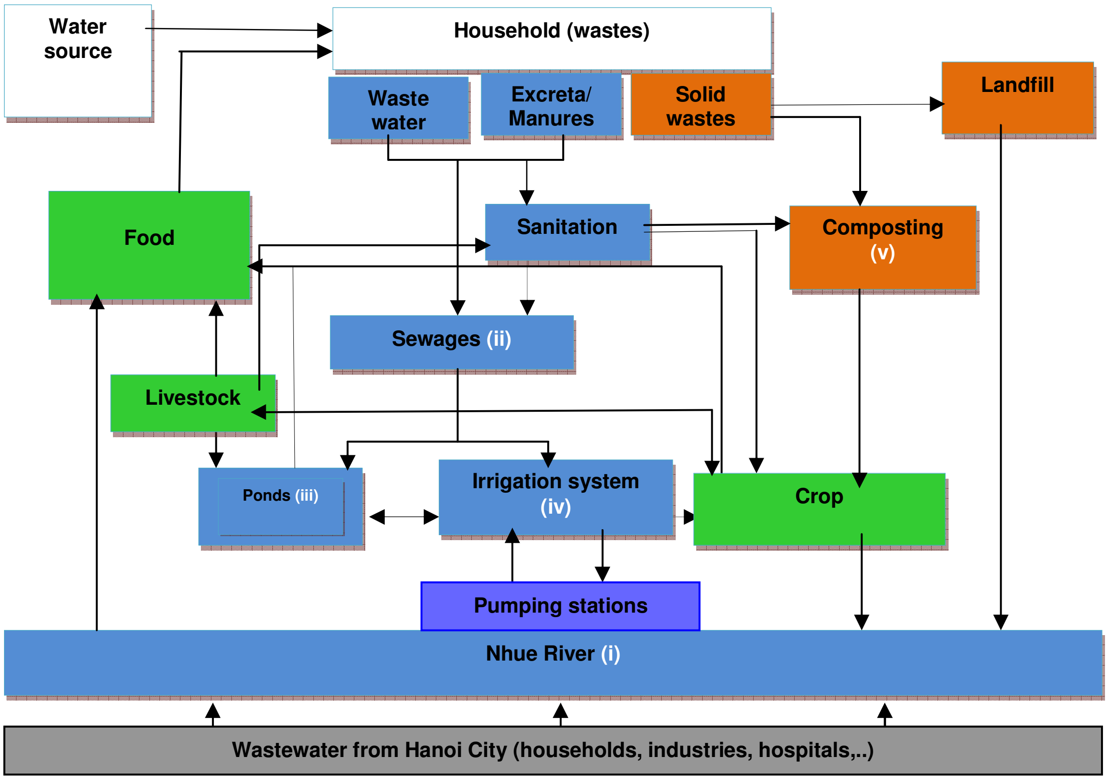

Figure 3.2 The environmental sanitation and agricultural systems in Hoang Tay and Nhat Tan communes, Northern Vietnam. The five sampling points are i, ii, iii, iv and v.

Source: Adapted from Montangero, A. (2006)

At the time we conducted the studies, the number of inhabitants is about 16,200 (52% female), with approximately 4,100 households in both of the two communes. Of the households included in the study, 10% were children under 6 years old, and 59% of the population belonged to the labour force (18-65 years old) which mainly participated in agricultural activities, in particular, rice and vegetable cultivation and fish farming. Each commune had one kindergarten, a primary school, a secondary school, and a health station. About 1% the adult population never attended school; most adults had attended schools but a few attained high school and university education. There was a de-worming program in implemented every year for children primary school aged children, when they entered school [107]. The main source of income in the communes is from agricultural productions and a few of families have an extra income from carpentry. The socio-economic status (SES) of households under survey is described in detail in Chapter 5. Generally, the study households had a good SES, with 33% owning assets such as a television, refrigerator, other electronic equipment and a motorbike, as well as had permanent house construction.

4. METHODOLOGY

The studies used a combination of three approaches, i.e. epidemiological studies and QMRA to assess the health risks, and MFA to assess environmental impacts in terms of nutrients (nitrogen [N] and phosphorus [P]) charge and flows.

4.1 Epidemiological studies

Epidemiological studies were conducted to assess health risks of wastewater and excreta reuse: two cross-sectional surveys, a case-control study, and a nested case-control study with a follow-up (cohort) were carried out. In the cross-sectional and case-control studies intestinal parasitic infection was the outcome assessed with stool examination. In the nested case-control study and the follow-up diarrhoeal episodes of adult farmers were the outcome of interest. In all epidemiological studies the exposures were assessed by questionnaire or by direct observations.

4.1.1 Cross-sectional surveys

Two cross-sectional surveys were conducted, the first in the rainy season from April to September 2008 and the second in the dry season between October 2008 and March 2009. The main reason for conducting cross-sectional survey twice is to see the difference in health effects of wastewater and excreta reuse between the two seasons for which the pattern of use are distinguished. Indeed, human excreta are mainly used for the crops in the dry season. In addition, the cross-sectional surveys aimed at collecting the demographical and socioeconomic data of the study households and participants, as well as determining the infection status with intestinal parasitic infections (Chapter 5).

The number of households enrolled into each survey was calculated using a formula of sample size for estimating proportions with estimate of standard error 0.05 with 95% confidential level and an expected proportion of household use of wastewater in agriculture was 80%. The total number of households in each survey was 270. All household members, including adults and children above 12 months of age were included in the study. A total 15 villages in Nhat Tan and 10 villages in Hoang Tay communes were selected to participate in the study. For each cross-sectional survey, households were randomly selected from the list of household provided by the Communal People's Committee. None of the household was selected twice. The recruitment of local research assistants, development of questionnaires and observation check list as well as data collection are described in detailed in Chapter 5 & 6 and Annex 2.

In parasitological examinations of stool specimens intestinal protozoan parasites (e.g., *Giardia, Entamoeba, Cryptosporidium* and *Cyclospora*) and helminths (e.g., *A. lumbricoides*, *T. trichiura*, and hookworm) were diagnosed. Each enrolled participant provided two stool samples on two consecutive days. The examination of stool samples was performed at the Parasitological Department in Hanoi Medical University. The Kato-Katz thick smear technique was used for quantitatively determine *A. lumbricoides, T. trichiura* and hookworm eggs per gram stool [108]. The formalin-ether concentration technique (FECT) was used to detect intestinal protozoan infection in addition to helminth eggs [109]. The laboratory procedures are described in detailed in Chapter 5.

4.1.2 Case-control study on risks for *Entamoeba histolytica* infection

In the first cross-sectional study a considerable number patients with an *E. histolytica* infection were diagnosed. A case-control study to assess risk factors for *E. histolytica* infection was developed with a particular interest to assess the relative importance of exposures to wastewater and excreta responsible for the infection. The study was to further the understanding of the relationships between protozoan parasite infections and wastewater and excreta reuse. The case and control definitions, data collection procedures and analysis are specified in Chapter 6.

4.1.3 Cohort study and nested case-control study

The study subjects from the 405 selected households in the two cross-sectional surveys were followed from 1 August 2009 to 31 July 2010. There were a total of 867 adults farmers aged 16-65 years, who were enrolled. During the follow-up time, the study subjects were visited weekly by trained and experienced research assistants who collected their weekly information sheets where the diarrhoeal diseases and exposure information was noted. The recruitment of participants, implementation of the follow-up, identification of diarrhoeal cases and controls, exposure and outcome variables, and data analysis are described in detailed in Chapter 7.

A case-control study was nested into the cohort study [110]. Incident diarrhoeal cases were identified and controls were selected using an incidence density sampling [111]. For cases and controls exposures were assessed. Some exposure variables were transient, i.e. short-lived and temporary [112] and their status may change overtime, according to season, age, personal health, and migration. Nested case-control study can consider transient exposures. A structured-questionnaire was used to obtain the exposure information including the status of contact with excreta and wastewater, food and water consumption, use of personal protective measures, and personal hygiene practices (see Annex 2).

4.2 Quantitative Microbial Risk Assessment

Enteropathogenic bacteria and protozoa were considered in order to be able to link the QMRA assessments with the epidemiological studies.

4.2.1 Selection of pathogens

Diarrheagenic *Escherichia coli* (DEC) is a normal inhabitant of the gastrointestinal tract of human and animals, with cattle being the principal reservoir, but it may also occur in other species such as goats, pigs and chickens [83, 97]. In Vietnam diarrheagenic *E. coli* was isolated from faecal samples of cows [113]. The risk assessment for *E. coli* was developed based on data from the Netherlands [114]. However, in developing countries data on dose-response for *E. coli* is missing.

The protozoan, *C. parvum* and *G. lamblia* have been studied during the last decade partly due to their high environmental persistence and low infection dose. *C. parvum* and

G. lamblia cause gastroenteritis. An infected person might discharge 1-10 billion cysts daily in their faeces for several months. However, ingesting as few as 10 cysts is sufficient for a successful new infection [41, 115]. *C. parvum* infection is caused by ingestion of sporulated oocysts transmitted by the faecal-oral route (direct transmission). In healthy human hosts, the median infective dose of 132 oocysts is required [116]. However, despite the risk assessment for *C. parvum* and *G. lamblia* being the focus of many water quality assessments in developed countries, there has been much less research in developing countries [84].

4.2.2 Main components of QMRA method

QMRA method includes the following four main steps: (i) hazard identification, (ii) exposure assessment, (iii) dose-response assessment, and (iv) risk characterization [97].

(i) Hazard identification is the first QMRA step. It represents the identification of pathogenic organisms of potential significance to human health. We used our findings from the cross-sectional surveys. Critical exposure points were identified by using our current knowledge of the environmental sanitation system, agriculture, aquaculture and livestock in the study sites. The exposure scenarios and sampling points in these study sites are described in Figure 3.2.

(ii) Exposure assessment aimed to determine the exposure of the populations to the pathogen (transmission route). The intensity and duration of exposure was defined. This step requires an estimation of the amount of *E. coli, G. lamblia,* and *C. parvum* in excreta composts, wastewater in each of the exposure points, as well as the amounts of ingested/contacted materials per exposure. This information was obtained from the cross-sectional surveys. The concentration of *E. coli, G. lamblia,* and *C. parvum* in waster water was obtained in analysis performed at the National Institute of Hygiene and Epidemiology laboratory, Hanoi, Vietnam. Numbers of thermotolerant coliforms on multiple tubes of different dilutions were enumerated by using the most probable number (MPN) table, according to the American Public Health Association (APHA) [117], and detection of *C. parvum* and *G. lamblia* in water samples was performed by immunofluorescent antibodies (IFA) and microscopy [75]. The details of the

examinations of the environmental samples, their processing and analysing are described in Chapter 8.

QMRA modelling required the quantity of water and soil ingested daily during the farmers' activities in relation to wastewater and excreta reuse. We used literature information as estimations for these values. While voluntary daily consumption of water can easily be obtain the quantity of involuntarily ingested water and/or soil during various farming activities is difficult to measure. In Table 4.1 the result of our literature search is given with estimations of volumes of ingested water per person and event.

Table 4.1 Involuntarily ingestion volumes based on the intensity of water or soil contact

Contact intensity	Intake volumes	Events	References
Full-body immersion	100 ml swallowed/event	Swimming activities Children playing in water Body-washing Fishing (harvesting fish)	Hass (1999) [97] Genthe and Rodda (1999) [118]
Intermediate	50 ml swallowed/event	Repeated immersion during skiing, surfing, canoeing	Medema et al., (2001) [119]
Others	10 ml swallowed/event 10 mg soil swallowed/event	Laundry Washing Ingestion related to irrigation in agriculture and aquaculture	Genthe and Rodda (1999) [118] Medema et al., (2001) [119] Mara (2007) [95]

Source: Steyn et al. (2004) [120]

(iii) Dose-response analysis: is the link between the level of microbial exposure and the adverse health effect using dose-response model which is given for each pathogenic organism. In this study, the dose-response model use for *G. lamblia* and *C. parvum* infection is an exponential model whereas a β-Poisson model is used for *E. coli* (Table

4.2). The equations for each of the models and calculation of risk of infections are presented in Chapter 8.

Table 4.2 Best-Fit Dose-Response Parameters (Human)

Organism	Exponential	Beta-Poisson		References
	r	ID_{50}	α	
Escherichia coli		8.60 x 10^7	0.1778	Haas et al., 1999 [97]
C. parvum	0.00467			Haas et al., 1996 [121]
G. lamblia	0.0198			Rose et al., 1991 [122]

(iv) Risk characterization integrates the outcomes obtained in steps 1 to 3 characterizes the types and magnitudes of the public health risk and evaluates the variability and uncertainty. This working step consisted of the following procedures: Based on the concentrations of *E. coli, G. lamblia* and *C. parvum* in the samples (Chapter 8), risk of infections (mean, and 5th and 95th percentiles) were estimated for each exposure scenarios in the study sites. The results were plotted in a graph for visual appraisal. Factors of uncertainty and variability were identified and discussed and effective measure for risk minimization in the studied region was proposed. Deterministic and probabilistic approaches using Monte Carlo simulations were used for risk characterization. Finally, the infection risk was converted to risk of disease and disability adjusted life years (DALYs) which were compared between exposures and pathogens [83, 114].

4.3 Material Flow Analysis

MFA is a method to quantify the flow of matter (water, food, excreta, wastewater, etc.) and substances (nitrogen, phosphorus, carbon, etc.) through in a system (city, country, etc.) during a defined period. MFA is based on the law of matter conservation; flows are expressed in kg/year or in kg/capita/year. The method allows identification of environmental problems and quantifying the impact of potential measures on resource recovery and environmental pollution reduction. It can be used to compare different sanitation technology options regarding their environmental and health impacts and may support decision-making with regard to different sanitation options [123]. We adapted MFA from Montangero 2006 [98] and applied for the method for first time in an agricultural community to identify relevant nutrient sources and to assess nutrients (N and P) flow (Chapter 9).

Terms and definitions for MFA research activities were taken from Brunner and Rechberger [123] (Annex 3). The MFA steps were described by Brunner and Rechberger, 2004 [123]. In summary, (i) System understanding and information collection: set up system boundary and survey (semi-structured interviews and key interviews) as well as a review of documents were conducted in interesting area in order to define the system (relevant processes and goods) as well as gather quantitative information on mass and concentration of substance flows; (ii) Review of information on transfer coefficients: transfer coefficients describe the percentage or ratio of a transferred specific output into a total input of amount of substances is called transfer coefficient; (iii) Review of information on sanitation scope: substance flows were measured. Calculations per capita or per area flows were determined in a comparable context. The reduction of number of parameters was quantified through field investigations; (iv) Calculation of mass balance over process: many processes in the sanitation management are only transformation but not storage processes. For the processes selected flows were quantified by mass balance over the processes. This again was reduced the required means on data collection. (v) Data analysis, interpretation and recommendation: once all flows were estimated, the possibility of selected key flows was assessed. The potential of the current flows were analysed. Formation of improvement measures aiming at improving sanitation management in the interesting area was formulated and quantified. The whole processes of MFA method is presented in Figure 4.1 [99].

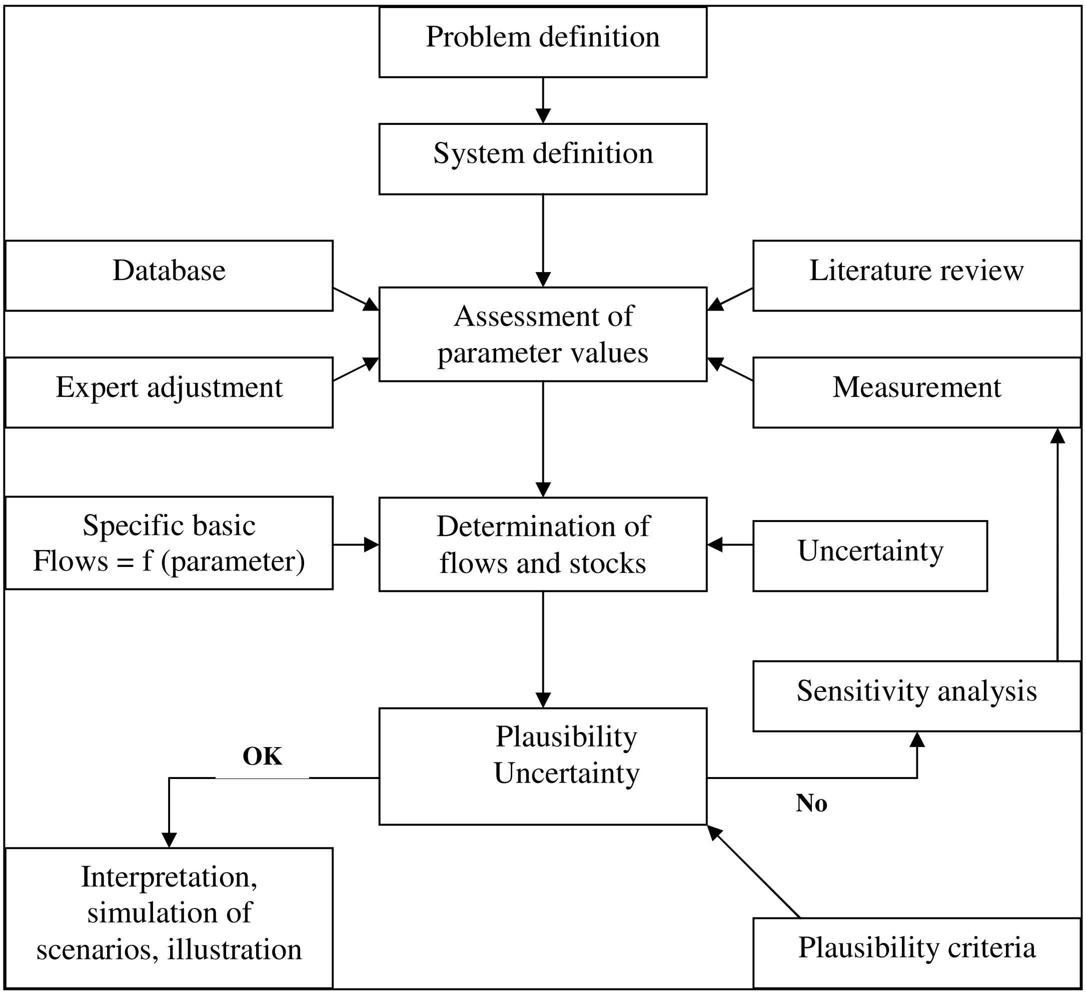

Figure 4.1 Diagram of MFA process

Source: Adapted from Montangero, A. (2004)

4.4 Data analysis approaches

Data analysis approaches used in this study comprised:

- Data entry and management using Microsoft Access database and Microsoft Excel
- Statistical data analysis using STATA 10.1 (StataCorp., College Station, TX, USA)
- Monte Carlo simulations using @Risk version 5.7 student editions (Palisade Corp.) added on to Microsoft Excel.

4.5 Ethical considerations

The Ethical Research Committee at the National Institute of Hygiene and Epidemiology (NIHE, number 149/QĐ-VSDTTƯ-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 all field studies. Before field work the Provincial and District Health Offices were informed and asked for permission. Detailed information on study objectives and procedures were provided and working procedures explained. 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 (Annex 1). All individuals were made aware that they can withdraw from the study any time without any consequences. All individuals with parasitic infections were treated free of charge with anti-parasitic drugs based on the treatment protocol of the Vietnamese Ministry of Health.

5. *ASCARIS LUMBRICOIDES* AND *TRICHURIS TRICHIURA* ASSOCIATED WITH WASTEWATER AND HUMAN EXCRETA USE IN AGRICULTURE IN VIETNAM

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5.1 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 fertilizer 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.

Keywords: Helminth infections, Ascaris lumbricoides, Trichuris trichiura, wastewater, excret, agriculture, Vietnam.

5.2 Introduction

In agricultural production the use of wastewater and excreta is a widespread practice with a long tradition in many countries around the world [28], in particular in China, South and South East Asia as well as various settings in Africa [6-8]. The sources of irrigation water in Vietnam vary from fresh water and wastewater to ground water [63]. Nearly all rural households in north and central regions of Vietnam use excreta as fertilizer in agriculture [22]. 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 [14, 20, 31]. Most common health risks related with wastewater and excreta use are diarrhoeal diseases and soil-transmitted helminthiases (STH) [20, 51].

STH are common worldwide with more than a billion people infected [124, 125]. Estimates suggest that *Ascaris lumbricoides* infects over 1 billion people, *Trichuris trichiura* 795 million, and hookworms (*Ancylostoma duodenale* and *Necator americanus*) 740 million [126]. In tropical and sub-tropical countries, distribution of STH is linked with the lack of sanitation and poverty [127, 128]. In Vietnam, estimated 39.9 million (44.4%) people are infected with *A. lumbricoides*; 17.6 million (23.1%) with *T. trichiura* [71, 129], and 19.8 million (22.1%) with hookworm [129]. High prevalence of helminth infection is found in rural areas of northern Vietnam, which is possibly related with the common use of excreta as fertilizer in the fields [21, 55, 130-133]; and also associated with the high population density, differences in climatic condition and humidity [134].

Prevalence of and risk factors for helminth infections have been studied in Vietnam [55, 130, 131, 135-139]. 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 fertilizer 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.

5.3 Methods

5.3.1 Study area

The study was carried out in Nhat Tan and Hoang Tay communes in Kim Bang district, Hanam province (20.32^o N, 105.54^o E), northern Vietnam, situated about 60 km south of Hanoi. Nhat Tan and Hoang Tay communes count 10,500 (2,700 households) and 5,500 (1,500 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 [101]. 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 fertilizer 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.

5.3.2 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 4,282 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.

5.3.3 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 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 minutes.

5.3.4 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 hours after collection and stored at 4-8° C until analysis on the next day.

5.3.5 Laboratory procedures

The Kato-Katz thick smear technique was used to identify *A. lumbricoides,* hookworm, and *T. trichiura* eggs [108]. 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 minutes prior examination by light microscopy at a magnification of 400x. 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 [140].

Additionally, the formalin-ether concentration technique (FECT) was used to detect helminth and intestinal protozoan infections [109]. In brief, approximately 1 gram 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, three mL ether was added, the tube closed with rubber stopper and shaken well (about 30 seconds). Without rubber stopper the tubes were centrifuged for 5 minutes 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 300-500x.

5.5.6 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 [125]. 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 analyze 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) [141]. In Table 5.1 WHO STH's intensity classes are provided [142].

Generalized Estimating Equations (GEE) method was used in both univariable and multivariable analyses to adjust for intra-correlation within a household [143]. 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) analyzed with basic demographic variables (i.e., age, sex, educational level and occupation) for all subjects; (ii) analyzed 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 fertilizer in the fields, and use of Nhue River water to irrigate fields); and (iii) analyzed 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 [144].

SES and sanitary conditions in the household were calculated according to an asset-based method [145]. 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.

Table 5.1. Classes of intensity for soil-transmitted helminth by stool examination (Montresor, et al. 2002)

Soil-transmitted helminth	Light-intesnity infections (epg)	Moderate-intensity infections (epg)	Heavy-intensity infections (epg)
A. lumbricoides	1 - 4,999	5,000 - 49,999	≥ 50,000
T. trichiura	1 - 999	1,000 - 9,999	≥ 10,000
Hookworm	1 - 1,999	2,000 - 3,999	≥ 4,000

5.3.7 Ethical considerations

The Ethical Research Committee at the National Institute of Hygiene and Epidemiology (NIHE, number 149/QĐ-VSDTTƯ-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. 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 anti-helminthic drugs based on the Vietnamese Ministry of Health guidelines.

5.4 Results

5.4.1 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 (Figure 5.1). A total of 1,655 individuals from 484 households provided stool samples and completed the questionnaire, of which 1,425 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 fertilizer 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.

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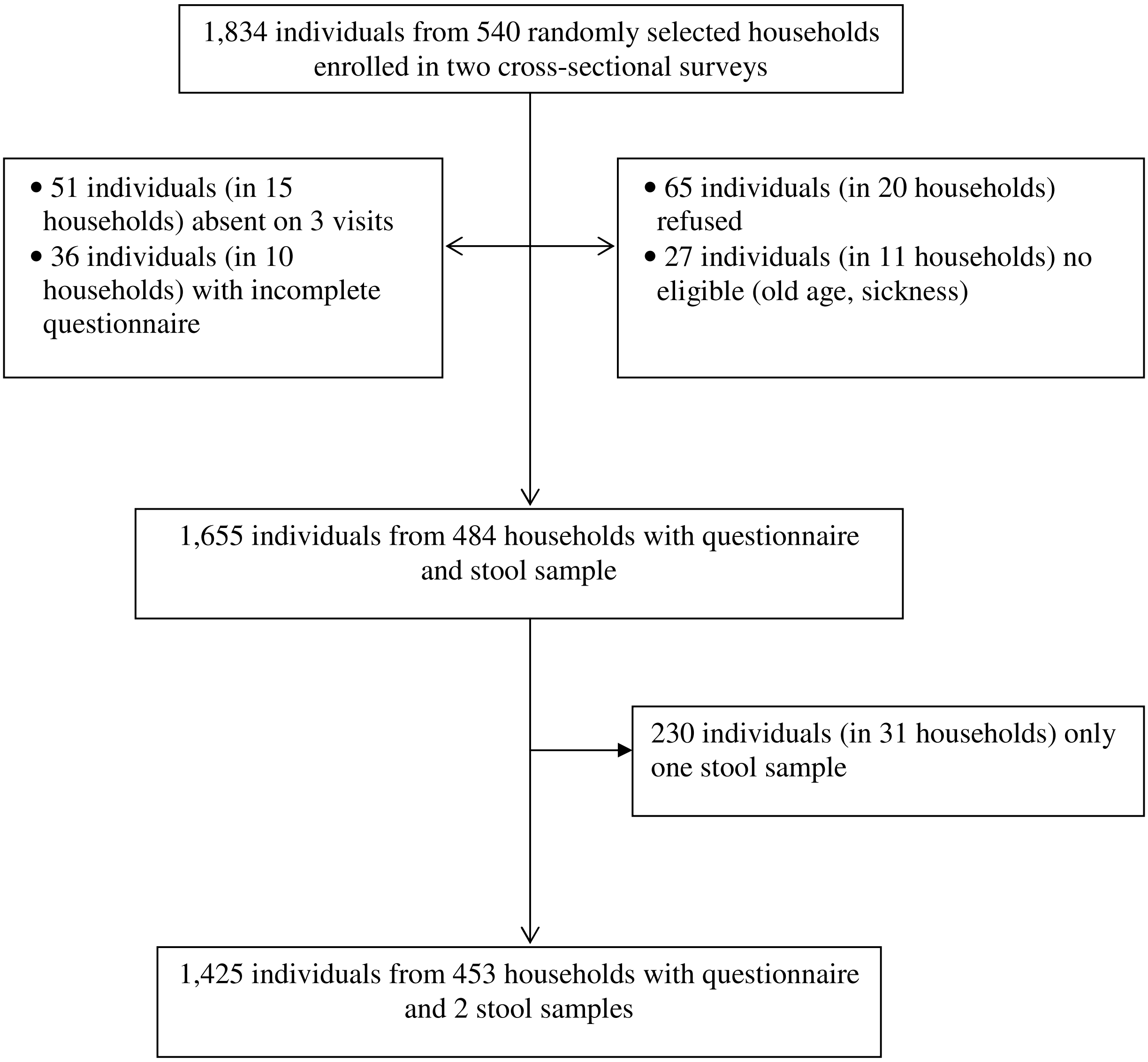

Figure 5.1 Participants' compliance to participate in the study in Hoang Tay and Nhat Tan communes, Hanam province, Northern Vietnam

5.4.2 Prevalence of intestinal parasitic infections

Table 5.2 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 5.2).

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.

Table 5.2 Prevalence of intestinal parasitic infections stratified by sex and age group in Hoang Tay and Nhat Tan communes, Hanam province, Northern Vietnam (N=1,425)

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

5.4.3 Risk factors for helminth infections

The results of the univariable and multivariable logistic regression analysis are presented in Table 5.3 and 5.4, 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 0.4-0.9, 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).

Table 5.3 Risk factors for helminth infection in Hoang Tay and Nhat Tan communes, Hanam (univariable logistic regression analysis)

Risk factors	N (1425)	Any helminth infection				*A. lumbricoides*				*T. trichiura*			
		Positive	OR	OR*	95%CI	Positive	OR	OR*	95%CI	Positive	OR	OR*	95%CI
1. Demographic characteristics and household's economic													
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	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

Table 5.3 continued

Risk factors	N (1425)	Any helminth infection Positive	OR	OR*	95%CI	*A. lumbricoides* Positive	OR	OR*	95%CI	*T. trichiura* Positive	OR	OR*	95%CI
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[a]													
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[a]													
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[a]													
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[a]													
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

* OR model: adjusted for age, sex, and year. GEE were used to account for intra-correlation within a household.

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 fertilizer 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).

Table 5.4 Risk factors for helminth infection in Hoang Tay and Nhat Tan communes, Hanam (multivariable logistic regression analysis)

Risk factors	Any helminth infection			*A. lumbricoides*			*T. trichiura*		
	OR*	**95% CI**	**P-value**	**OR***	**95% CI**	**P-value**	**OR***	**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[a]									
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[a]									
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[a]									
Yes *versus* No	0.9	0.5-1.5	0.66	1.0	0.6-1.7	0.99	N.A.	-	-

Note: N.A: Not applicable; [a] Excluding subjects with non-agricultural work (N = 703).

5.5 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 fertilizer 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 rates reported in previous studies in Vietnam [55, 132, 135, 136, 139, 146, 147]. Several factors can be made responsible for the variation among which climate, types of soils and crops, SES, and human hygiene behavior are of most importance [148]. 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 [149-151]. 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* [51, 52, 56, 152-154]. 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 [55, 139]. 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 in irrigation water, to which farmers have actually an intense and direct contact during a long period during field work [152]. 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 [34]. 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* [55].

Our study showed that the use of human excreta as fertilizer in agriculture had a higher risk of infections with *T. trichiura*. This is consistent with observations made by Trang and colleagues [55].

We observed in our study that composting human excreta before being utilized as fertilizer 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 [21, 133]. These practices are likely to increase the inactivation of helminth eggs and pathogens [155-157]. 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 [158]. 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 [132], 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* [55, 57, 148].

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 [159]. Surprisingly, the use of protective measures during fieldwork and washing hands with soap after field work did not result in a risk reduction. The personal hygiene practices have been widely shown to be important factors in reducing the transmission of helminths and other infectious diseases [48, 57, 139, 148]. 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 [55, 160].

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 [55, 161]. However, our results contradict with some previous studies, where an association between SES and helminth infection was found [139, 162, 163] in a population with a larger variation in SES was included. Our study was clearly defined to an agricultural community.

5.6 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.

5.7 Conflict of interest

All authors declare no conflicts of interest.

5.8 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.

5.9 Acknowledgments

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6. RISK FACTORS FOR *ENTAMOEBA HISTOLYTICA* INFECTION IN AN AGRICULTURAL COMMUNITY IN HANAM PROVINCE, VIETNAM

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6.1 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 4.3 times higher risk of *E. histolytica* infection (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 (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 (odds ratio [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.

6.2 Background

Amoebiasis caused by the intestinal parasite *Entamoeba histolytica* (*E. 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 [164, 165]. The incidence rate of *E. histolytica* - associated diarrhoea was 0.08/child-year [166]. The rate of infection by *E. histolytica* differs among countries, socio-economic and sanitary conditions and populations [167]. 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 [168]. A study in Brazil identified place of residence, age, ingestion of raw vegetables and drinking water quality as important risk factors [169].

Wastewater and human and animal excreta are used as fertiliser for a wide variety of crops, and 10% to 30% increases in crop yields have been reported [13]. 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 [7, 8, 28] 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 Hanoi about 80 percent of vegetable production is from urban and peri-urban areas irrigated with diluted wastewater [170]. The use of household sewage, and human and animal excreta in agriculture and aquaculture has a long tradition in Vietnam [62]. Despite the potential health risk for intestinal disease of using excreta and animal waste in agriculture [20], 85% of farmers in Northern provinces of Vietnam regularly use human excreta in agriculture [21]. 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 fertilisers in agriculture increased the risk of parasite infection [55]. 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 diarrheagenic *Escherichia coli* and *E. histolytica* were the most common pathogens [61, 171].

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.

6.3 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 [101]. 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 [74]. 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) 31-45years, (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 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* [109]. In brief, the preparation process was as follows: a stool sample of approximately 1 gram was places 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 x 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 x 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 [172]. 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, a 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 [144]. 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 [145, 173]. In brief, indicator data were defined by principal component analysis (PCA), with missing values being replaced with the mean of value for 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-VSDTTƯ-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.

6.4 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).

6. *Entamoeba histolytica* infection

Table 6.1 Risk factors of 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
Use of animal excreta as fertiliser in the fields					
No	25 (54.3)	69 (50.0)	Reference		

Table 6.1 continued

Variables	Case N (%)	Control N (%)	Matched OR	95% CI	P-value
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

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.

Table 6.2 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 (*versus* good)			
- average	4.3	1.3-14.0	0.02
- poor	2.2	0.6-7.4	0.22
Household's sanitary conditions (*versus* good)			
- average	0.8	0.3-2.3	0.68
- poor	1.6	0.6-4.6	0.38
Household with tap water (yes *versus* no)	1.3	0.5-3.1	0.57
Close contact with domestic animals in household (yes *versus* no)	5.9	1.9-18.9	0.003
Handling animal excreta in field work (yes *versus* no)	0.2	0.1-0.7	0.01
Direct contact with Nhue river water during field work (yes *versus* no)	0.4	0.1-1.1	0.07
Use of Nhue river water to irrigate fields (yes *versus* no)	3.7	0.4-33.1	0.24
Washing hands with soap after field work (*versus* frequently)			
- sometimes	1.7	0.5-5.8	0.40
- rarely	3.4	1.1-10.0	0.03

6.5 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 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 [174]. 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 fertilisers, 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 was found on any of the washed samples of vegetables [175, 176].

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. lamblia* and *E. histolytica,* are unlikely to survive more than 10 days in soil as they are susceptible to desiccation [30]. 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 [177, 178] and therefore, it is unlikely that cysts are present in the stool. Secondly, all excreta are stored before being utilised in agriculture. The time period and conditions of the storage often do not meet full safety regulations [179]. However, they are sufficient to eliminate an important portion of the infectious agents, including *E. histolytica* cysts [20, 30]. 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 [61, 171]. 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 [180].

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 [63]. 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 [181-184].

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 [185].

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 [186]. An other study in Thailand found that the lack of regular water-treatment practices was also a risk factor [187].

The diagnostic method we used (FECT) does not allow the distinction between pathogenic *E. histolytica* from non-pathogenic *E. dispar* [188] which can be made by by isoenzyme analysis and molecular technique [189]. 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 [61, 186].

6.6 Conclusion

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.

6.7 Conflict of interest

All authors declare no conflicts of interest

6.8 Acknowledgments

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6.9 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.

7. DIARRHOEAL DISEASES IN ADULTS IN AN AGRICULTURAL COMMUNITY, WHERE WASTEWATER AND EXCRETA WAS USED IN HANAM PROVINCE, VIETNAM

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7.1 Abstract

Objectives: This study was carried out to determine the diarrhoeal incidence and associated risk factors among adult population exposed to wastewater and excreta used in agriculture in Hanam province, Northern Vietnam.

Methods: An open cohort of 867 adults, aged 16-65 years, was followed weekly for 12 months to determine the incidence of diarrhoea. A nested case-control study was used to assess the risk of diarrhoeal diseases. Two hundred and thirty-two pairs of cases and controls were identified and exposure information related to wastewater, human and animal excreta, personal hygiene practices, and food and water consumption were collected.

Results: The diarrhoeal incidence rate was 0.28 episodes per person-years at risk. This rate in the dry season was lower than in the rainy season (risk ratio [RR] = 0.77, 95% confidence interval [CI] 0.60-0.99). The risk factors for diarrhoeal diseases included direct contact with the Nhue River water (odds ratio [OR] = 2.4, 95% CI 1.2-4.7, attributable fraction [AF] 27%), local pond water (OR = 2.3, 95% CI 1.2-4.3, AF 14%), composting of human excreta for a duration less than 3 months (OR = 2.4, 95% CI 1.4-4-3, AF 51%), handling human excreta in field work (OR = 5.4, 95% CI 1.4-21.1, AF 7%), handling animal excreta in field work (OR = 3.3, 95% CI 1.8-6.0, AF 36%), lack of protective measures while working (OR = 6.9, 95% CI 3.5-13.9, AF 78%), never or rarely washing hands with soap (OR = 3.3, 95% CI 1.8-6.3, AF 51%), use of rainwater for drinking (OR = 5.4, AF 77%) and eating raw vegetables the day before (OR = 2.4, 95% CI 1.2-4.6, AF 12%).

Conclusions: Our study shows that the direct contact with polluted water from the Nhue River and local ponds, handling practices of human and excreta as fertilisers, poor personal hygiene practices, and unsafe food and water consumption were associated with the risk of diarrhoeal diseases in adults. In the agricultural settings, the wastewater and excreta are commonly used. It is important to find the ways to reduce public health risks in these settings, such as use of protective measures while doing field work and safe composting of human excreta should be promoted. The health and hygiene education program should also give more intensions to improve hygiene behaviours.

Keywords: Diarrhoeal disease, wastewater, excreta, agriculture, Vietnam

7.2 Introduction

In developing countries, including Vietnam, wastewater has been used as a source of crop nutrients and reliable irrigation source for many decades [2, 3, 5, 15, 190]. In some Asian countries, the use of excreta for increasing crop yields and fish production is indeed most common [14]. Despite the potential health risks of excreta use as fertiliser in agriculture, it is still a widespread Northern and central Vietnam [21, 22]. Health hazards associated with the use of wastewater and excreta in agriculture and aquaculture is one the most significant concerns in developing countries [14, 20, 31]. Amongst these health hazards, diarrhoeal diseases remain one of the most important environmental health problems in developing countries [20, 31]. Diarrhoeal diseases was the third leading cause of death in low-income countries, killing an estimated 1.8 million people every year, most of which occur in children under the age of five [191]. The occurrence of gastrointestinal diseases, including diarrhoea, has been associated with the consumption of wastewater-irrigated vegetables [34]. The high-risk groups of people for these diseases are farmers with prolonged wastewater contact, their families, and nearby communities exposed to wastewater irrigation[31]. Indeed, diarrhoeal diseases were observed with significantly higher prevalence in wastewater exposed people in Pakistan [48]. A study in Mexico found a higher prevalence of diarrhoeal diseases in children under 5 years of age exposed to untreated wastewater than those who were exposed to wastewater retained in a single reservoir or no irrigated wastewater [51]. In Vietnam, an epidemiological study showed that close contact with wastewater was associated with the risk of diarrhoeal diseases in adults [61]. However, in Vietnam, the awareness of the importance of handling practices of human and animal excreta as a cause of diarrhoeal diseases is still lacking. To our knowledge, very few studies have assessed the risk of diarrhoeal diseases associated with combined the exposures to both excreta and wastewater use in agriculture and aquaculture. The importance of our study is to identify which factor is the most important for risk of diarrhoeal diseases. The study results could useful proposes a potential intervention to prevent the health impacts of such exposures.

In the concept towards an integrated approach combining assessment of the health status and the status of the physical, social, cultural and economic environment was developed by Nguyen-Viet and colleagues [77]. For this concept the epidemiology is proposed as the key methodology to assess health and identify the determinants of disease burdens. The present study is part of a large research project aiming at validating the integrated framework and assessing the environmental and health risks associated with wastewater and excreta reuse in agriculture Hanam province in Northern Vietnam [77, 192, 193]. Using a consecutive outcome of the follow-up study was served as a sampling frame for the selections of cases and controls in a nested case-control study; we assessed the incidence of diarrhoeal disease among adults living and working in a agricultural community, where the human and animal excreta and wastewater to irrigate field and fish feeding are intensively used. The study also assessed the other potential risks factors, including sanitary condition, drinking water, food consumption, and personal hygiene practices.

7.3 Methods

7.3.1 Study sites

The study was carried out in Nhat Tan and Hoang Tay communes in King Bang district, Hanam province (20.32^{o} N, 105.54^{o} E), Northern Vietnam, situated about 60 km south of Hanoi (Figure 3.1). The number of inhabitants was about 10,500 (2,700 households) and 5,700 (1,600 households) in Nhat Tan and Hoang Tay communes, respectively. Most households raise livestock in their compounds (e.g., chickens, ducks, and pigs). 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 residential areas. The two communes border on the Nhue River. Hanoi’s wastewater originating from households, industry, and other sources such as hospitals, is directly discharged untreated into the river [194]. The Nhue River water is used for crop irrigation and to feed into fish ponds. Several pumping stations are 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 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 only partially composted. Personal protective measures to prevent contamination are often lacking.

7.3.2 Study design

a) Cohort study

The recruited study subjects were adults of both sexes, aged 16-65 years from the 405 selected households, who participated in the baseline surveys in 2008 and 2009 [193]. A total 867 subjects participated in a cohort study and were followed from August 2009 to July 2010. It was an open cohort where people could join or withdraw from the study at any time during the study period. The participants were followed from enrolment to the end of the cohort study or until their withdrawals due to various reasons (e.g., deaths, mobility, or unwillingness to continue). Each participant was assigned an identification number carrying all related household characteristics (i.e., socio-economic status [SES] and sanitary condition; type of water source; latrine type in the household; and animal husbandry); and personal characteristics (i.e., age, sex, education, occupation) collected in the baseline surveys and these were maintained throughout the study. The participants were visited weekly by trained village health workers who collected the past week information of diarrhoea status.

An episode of acute diarrhoea was defined as: (i) at least three or more loose (or watery) stools within 24 hours, regardless of other gastrointestinal symptoms; or (ii) two or more loose stools associated with at least one other symptom of gastrointestinal infection (abdominal pain, cramping, nausea, vomiting, and fever); or (iii) passage of a single loose stool with grossly evident blood/mucous [195, 196]. Two independent diarrhoea episodes were separated by at least three days without diarrhoea, and an episode of diarrhoea with duration of 14 days or more was regarded as an episode of persistent diarrhoea [197]. An individual self-reporting sheet to monitoring the subject's exposure to wastewater and excreta was used. The total time of exposure to wastewater and excreta was recorded daily for each study subject; other potential risk factors (e.g., use of protective measures, hand-washing with soap, drinking raw water, and eating raw vegetables) were also recorded. Table 7.1 is described the characteristics of the study households, which were obtained from the two cross sectional surveys.

Table 7.1 Characteristics of the study households (N = 405) in Nhat Tan and Hoang Tay communes, Hanam province, Vietnam, 2009-2010

Characteristics	N (%)
Household had a poor socio-economic status	134 (33)
Household had a poor sanitary condition	143 (35)
Household had tap water	180 (44)
Household had drilled tube well water	255 (63)
Household had rainwater	351 (87)
Household had single vault latrine	225 (56)
Household had septic tank	129 (32)
Household composted of human excreta > 3 months before use	131 (32)
Household use of human excreta as fertiliser in agriculture	208 (51)
Households raise animals	341 (84)
Household use of animal excreta as fertiliser in agriculture	175 (43)
Household use of Nhue River water to irrigate field	375 (93)

b) Nested case-control

A case-control study was also conducted as part of prospective monitoring of diarrhoeal disease among all cohort subjects in order to assess the relationship between diarrhoea and exposure to excreta and wastewater (i.e., direct contact with human and animal excreta, Nhue River and local pond water). Other potential risk factor (e.g., personal hygiene aspects, drinking water, food consumption, etc.) determinants of diarrhoeal disease were also obtained. The history of exposure was defined as one week prior to the day of diarrhoeal occurrence or the day of control interviews. For diarrhoeal cases, information was collected on the characteristics of diarrhoea (e.g., duration of episodes, number of stools per day, characteristics of stool, diarrhoeal symptoms, and any related treatment).

Variables on the personal characteristics as well as the household's SES, sanitary conditions, water source and type of latrine use in the household, and animal husbandry obtained from the cross-sectional surveys were also included in the analyses. The assumption that these variables remained unchanged during the study period.

Cases were detected and selected from the active surveillance system (weekly morbidity interview) as well as passive surveillance (self-reporting by a cohort member). We used an incidence-density sampling of cases and controls [111] , which means that controls were sampled concurrently among the cohort. Under the incidence-density sampling scheme, a case could end up as a control later on or vice versa, and the control might be selected by chance for more than one case during the follow-up period. When a case was ascertained, a control (the ratio of cases to controls is 1:1) was randomly selected in the population at risk, e.g. who did not experience diarrhoea in the previous two weeks, living in the community and from a different household that reported a case.

A questionnaire interview was administrated to all cases and controls. The questionnaire was developed in English, translated to Vietnamese, backs-translated for confirmation and pre-tested in villages close to Hanoi. After adaptation the questionnaire was used in face-to-face interview by five trained and experienced research assistants to all cases and controls. Principal researchers accompanied each assistant to three individual interviewees for quality control (e.g., utilization of same procedures were used and for quality as being precisely followed). Each interview lasted approximately 45 minutes.

7.3.3 Data management and analysis

Data was entered into a Microsoft Access data-base, and analyzed using STATA 10.1 Software (STATA-Corporation, College Station, TX, USA).

The diarrhoeal disease incidence was calculated for the cohort study over one year of the follow-up period. The days under surveillance for each participant were recorded, allowing the calculation of an exact number of days at risk between episodes of diarrhoeal disease (person-time at risk). The negative binomial regression model was employed to estimate the relative rate (RR) from the incidence data. In studies where clustering was observed in individuals (e.g. repetitive observations on the same individuals or households), the generalized estimating equations (GEE) were used to account for intra-correlation within a household [143]. For the risk factor analysis, the conditional logistic regression providing odds ratio (OR) was used in both univariable and multivariable analyses from the nested case-control study. First, a univariable conditional logistic regression analysis was carried out to associate potential risk factors with disease outcome (i.e., diarrhoeal disease) 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. Multivariable analyses were performed to evaluate the effect of the explanatory variables, controlling for the effect of other risk factors [144]. The attributable fraction (AF) in the population with an assumption that the exposed proportion in the control group (Pe) is that of the whole population. AF was calculated for the OR of each significant variable in the multivariable model using Levin's formula (AF = Pe(OR-1)/[1+(OR-1)]) [198] for assessing the importance of exposure to the population.

SES and sanitary conditions in the household were calculated according to an asset-based method [145, 173, 199]. 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.

7.3.4 Ethical considerations

The Ethical Research Committee at the National Institute of Hygiene and Epidemiology (NIHE, number 149/QĐ-VSDTTƯ-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. Before field work began, 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.

7.4 Results

7.4.1 Incidence of diarrhoeal disease

A total of 867 people aged 16-65 years (mean 39 years, 53% females) from 384 households participated in the cohort study and were followed for 299,222 days. Diarrhoeal diseases were reported by 142 subjects (16%), with a total of 232 episodes of diarrhoeal disease. This yields an incidence of 0.28 episodes per person-year at risk (95% CI 0.25 -0.32).

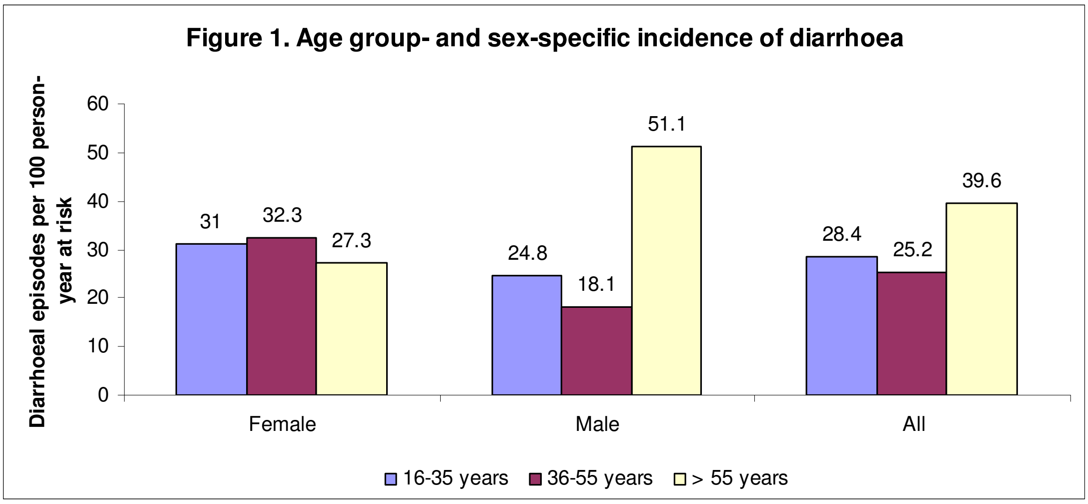

Figure 7.1 Age group- and sex-specific incidence of diarrhoea in 867 adult persons followed for 299,222 person-days at risk, Hanam province, Vietnam, 2009-2010.

Figure 7.1 shows the age- and sex-specific incidence. The lowest diarrhoeal incidence was in participants aged 36-55 years (0.25 episodes per person-year at risk, 95% CI 0.21-0.31); followed by aged 16-35 years (0.28 episodes per person-year at risk, 95% CI 0.23-0.35); and aged 56-65 years (0.40 episodes per person-year at risk, 95% CI 0.29-0.54). There was no difference in diarrhoeal incidence rates between males and females (RR = 0.83, 95% CI 0.54-1.26). There was a trend of seasonality in monthly incidence, with a difference in the rates of diarrhoeal diseases between the dry season (from October to March) and the rainy season (from April to September) (RR = 0.8, 95% CI 0.6-1.0). The peak of diarrhoeal incidence was observed in August, when our monitoring programme was started (Figure 7.2).

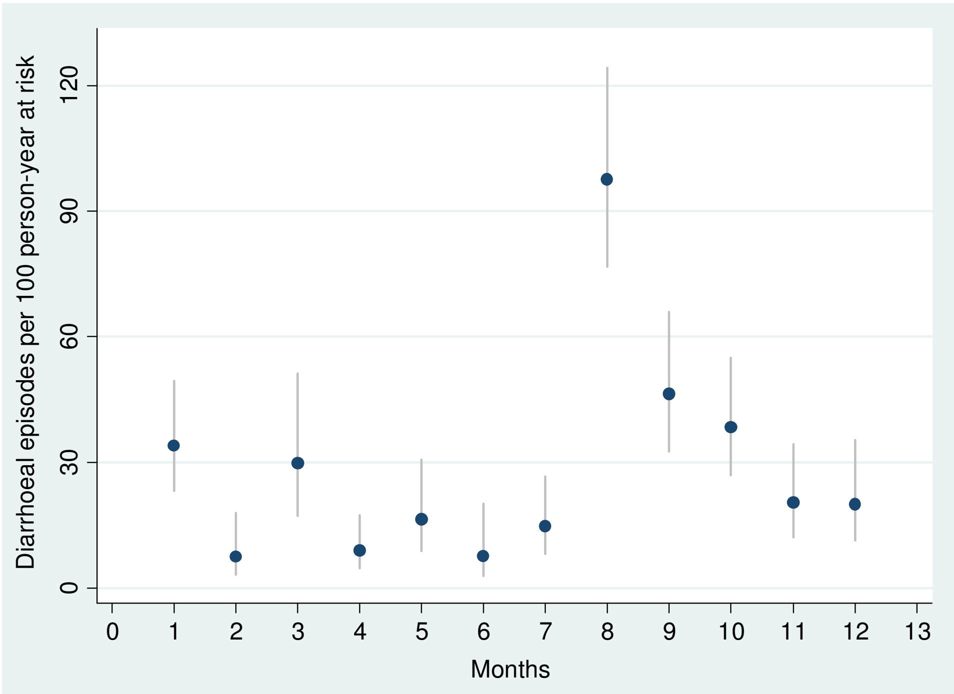

Figure 7.2 Monthly incidence of diarrhoea in 867 adult persons followed for 299,222 person-days at risk, Hanam province, Vietnam, 2009-2010.

7.4.2 Characteristics of diarrhoeal diseases

A total of 232 case/control pairs were recruited, of which 142 subjects were enrolled as cases. Forty-five cases experienced more than one diarrhoeal episode: one person was enrolled as a case ten times; two persons eight times; one person seven times; one person six times; four persons four times; eight persons thrice and 28 persons twice. Of the 167 subjects recruited as controls, seven persons four times, eight persons thrice and 28 persons twice.

The mean duration of diarrhoeal episode was 2 days (interquartile range: 1-6 days). Of all episodes, 3 (1%) had duration of ≥ 7 days, and none of the 232 episodes was persistent. The mean number of stools per day was 3.3 (interquartile range: 2-5 stools). Eight stools (4%) from cases had grossly evident blood, 83 stools (36%) contained mucous and 181 stools (78%) were watery. Study cases reported the diarrhoeal symptoms: abdominal pain (197 cases, 85%); drink more water (82 cases, 79%); fatigue (107 cases, 46%); nausea (58 cases, 25%) and fever (26 cases, 11%). Self-treatment was most common when people have a diarrhoeal disease (104 cases, 45%), followed by pharmacists (74 cases, 32%), private doctor (29 cases, 13%), local health centre (23 cases, 10%) and hospital (2 cases, 1%).

7.4.3 Risk factors for diarrhoeal diseases

The results of univariable and multivariable conditional logistic regression analysis are presented in Table 7.2 and 7.3, respectively.

Among the indicators describing the household sanitary and hygiene conditions, the water sources used for drinking and having a member with diarrhoea in a family were associated with diarrhoeal disease. Participants who lived in household using rainwater to drink had a higher risk of diarrhoea than those living in households with tap water in univariable (OR = 3.9, 95% CI 2.0-7.4) and in multivariable analysis (OR = 5.4, 95% CI 2.4-12.1). However, the use of tube well water was not associated with greater risk of diarrhoea than the use of tap water in both uni- and multivariable analyses (OR = 2.8, 95% CI 0.7-10.9 and OR = 2.2, 95% CI 0.4-12.4, respectively). Contact with persons with diarrhoea also increased the risk of diarrhoea in univariable (OR = 4.7, 95% CI 2.0-11.3) and in multivariable analysis (OR = 3.7, 95% CI 1.4-10.3). The use of dry latrines (single or double vault) and water-flushed latrines (septic tank or biogas) was not statistically significant associated with the risks of diarrhoea in comparison with the households without a latrine (OR = 1.4, 95% CI 0.5-3.7 and OR = 1.2, 95% CI 0.4-3.4, respectively). Close contact with domestic animals in househould increased risk of diarrhoeal diseases (OR = 1.0, 95% CI 0.7-1.5), but not statistically significant.

Composting of human excreta for less than 3 months was associated with the risk of diarrhoeal diseases in both uni- and multivariable analysis (OR = 1.8, 95% CI 1.2-2.8 and OR = 2.5, 95% CI 1.4-4.3, respectively). Household use of human excreta for application in the field was not associated with diarrhoeal diseases (OR = 1.1, 95% CI 0.7-1.6). However, higher risk of diarrhoeal diseases was observed in people who had been handling human excreta in field work than those who had not (OR = 5.1, 95% CI 1.7-15.3) in invariable and (OR = 5.4, 95% CI 1.4-21.1) in multivariable analysis. The risk of diarrhoeal diseases was statistically significant asssociated with the use of animal excreta as fertiliser for application in field in both uni- and multivariable analysis (OR = 1.9, 95% CI 1.3-2.7 and OR = 1.6, 95% CI 1.0-2.6, respectively). In both uni- and multivariable analyses, people who had been handling animal excreta in field work had greater risk of diarrhoeal diseases than those who had not (OR = 2.0, 95% CI 1.3-3.0 and OR = 3.3, 95% CI 1.8-6.0, respectively).

Direct contact with Nhue River water during field work resulted in an risk increasing of diarrhoea (OR = 1.7, 95% CI 1.1-2.6) and (OR = 2.4, 95% CI 1.2-4.7) in the uni- and multivariable analysis, respectively. Close contact with local pond water (i.e., washing clothes, fishing) was statistically significantly associated with an increased the risk of diarrhoea in both the uni- and multivariable analyses (OR = 2.4, 95% CI 1.5-4.0 and OR = 2.3, 95% CI 1.2-4.3, respectively). There was no risk change for a diarrhoea associated with the use of Nhue River water to irrigate fields in univariable (OR = 1.9, CI 0.9-3.8) and in multivariable analysis (OR = 1.0, 95% CI 0.4-2.5).

Risk changes were observed for variables related to personal hygiene. No use of personal protective measures during field work (i.e. gloves and boots), increased the risk of diarrhoea (OR = 1.6, 95% CI 1.1-2.4) in univariable and (OR = 6.9, 95% CI 3.5-13.9) in multivariable analysis. Omitting to wash the hands was a significantly associated with risk of diarrhoea. E.g., People who rarely and sometimes washed their hands with soap had a large odds increase of diarrhoea (OR = 3.3, 95% CI 1.8-6.3 and OR = 2.5, 95% CI 1.3-4.9, respectively) compared to those who frequently washed their hands with soap. Eating raw vegetables the day before was statisitcally significantly associated with an increased risk of diarrhoea (OR = 2.4, 95% CI 1.2-4.6). Diarrhoea was also associated with the consumption of raw water and leftover foods from the day before (OR = 1.4, 95% CI 0.7-2.9 and OR = 1.1, 95% CI 0.7-1.8, respectively) but not statistically significant.

Household's SES was not associated with diarrhoeal disease in both uni- and multivariable analyses (Table 7.2 and 7.3). Cases and controls did not differ in educational levels. Furthermore, no statistically significantly difference was found in occupation (OR = 1.4, 95% CI 0.9-2.3). Approximately three quarters of both groups were farmers (81% of cases versus 75% of controls, P = 0.15). Diarrhoeal disease was not associated with the educational level (OR = 0.9, 95% CI 0.6-1.4).

Analysis of the attributable fractions in the population (Table 3) showed that the lack of protective measures at work was the principal risk factor and may explain about 78 % of diarrhoeal cases; followed the use of rainwater to drink (77%); composting human excreta less than 3 months (51%); never or rarely washing hands with soap (51%); handling animal excreta in field work (36%); direct contact with Nhue River water during field work (27%); close contact with local pond water (14%); eating raw vegetables (12%); close contact with person having a diarrhoea (8%); and handling human excreta in field work (7%).

Table 7.2 Risk factors for adult diarrhoeal diseases in 232 cases and 232 controls in Hanam province, Vietnam (univariable conditional logistic regression analysis and adjusted for age groups and sex)

Risk factors	Case N (%)	Control N (%)	Matched OR	95% CI	P-value
1. Demographic characteristics					
Sex adjusted for age groups					
Female	135 (58)	134 (58)	Reference		
Male	97 (42)	98 (42)	0.9	0.6-1.4	0.74
Age groups (in years) adjusted for sex					
16-35	100 (43)	101 (44)	Reference		
36-55	93 (40)	101 (44)	0.9	0.6-1.4	0.73
56-65	39 (17)	30 (13)	1.3	0.8-2.4	0.31
Educational level					
Pre-school & primary school	79 (34)	74 (32)	Reference		
Secondary & tertiary school	153 (66)	158 (68)	0.9	0.6-1.4	0.71
Occupation					
Non-agricultural work	45 (19)	58 (25)	Reference		
Agricultural work	187 (81)	174 (75)	1.4	0.9-2.3	0.13
Household's socio-economic status					
Poor	68 (29)	68 (29)	Reference		
Average	85 (37)	70 (30)	1.3	0.1-2.2	0.26
Good	79 (34)	94 (41)	0.9	0.6-1.5	0.76
2. Household sanitary and hygiene conditions					
Type of latrine in the household					
No latrine	7 (3)	9 (4)	Reference		
Dry latrine	144 (62)	134 (58)	1.4	0.5-3.7	0.57
Water-flushed latrine	81 (35)	89 (38)	1.2	0.4-3.4	0.74
Water source to drink					
Tap water	15 (7)	45 (19)	Reference		
Rainwater	212 (91)	181 (78)	3.9	2.0-7.4	<0.01
Tube well water	5 (2)	6 (3)	2.8	0.7-10.9	0.14
Close contact with animals in household					
No	113 (49)	114 (49)	Reference		
Yes	119 (51)	118 (51)	1.0	0.7-1.5	0.88
Contact with person with diarrhoea					
No	204 (88)	225 (97)	Reference		
Yes	28 (12)	7 (3)	4.7	2.0-11.3	<0.01
3. Exposed to human and animal excreta					
Composting of human excreta in the household					
Compost > 3 months	44 (19)	66 (29)	Reference		
≤ 3 months or no compost	188 (81)	166 (72)	1.8	1.2-2.8	0.01

Table 7.2 continued

Risk factors	**Case N (%)**	**Control N (%)**	**Matched OR**	**95% CI**	**P-value**
Use of human excreta for application in field					
No	131 (57)	137 (59)	Reference		
Yes	101 (43)	95 (41)	1.1	0.7-1.6	0.69
Handling human excreta in field work					
No	214 (92)	228 (98)	Reference		
Yes	18 (8)	4 (2)	5.1	1.7-15.3	<0.01
Use of animal excreta as fertiliser in the fields					
No	122 (53)	157 (68)	Reference		
Yes	110 (47)	75 (32)	1.9	1.3-2.7	<0.01
Handling animal excreta in field work					
No	145 (63)	175 (75)	Reference		
Yes	87 (38)	57 (25)	2.0	1.3-3.0	<0.01
4. Exposed to Nhue River water and pond water					
Use Nhue River water to irrigate fields					
No	13 (6)	23 (10)	Reference		
Yes	219 (94)	209 (90)	1.9	0.9-3.8	0.08
Direct contact with Nhue River water during field work					
No	149 (64)	171 (74)	Reference		
Yes	83 (36)	61 (26)	1.7	1.1-2.6	0.02
Close contact with local pond water (washing, fishing)					
No	173 (75)	202 (87)	Reference		
Yes	59 (25)	30 (12)	2.4	1.5-4.0	<0.01
5. Personal hygiene habits					
Not use of protective measures (gloves, boots and face mask) at work					
No	67 (29)	90 (39)	Reference		
Yes	165 (71)	142 (61)	1.6	1.1-2.4	0.02
Hand washing with soap in general					
Frequently	35 (15)	71 (30)	Reference		
Sometime	62 (27)	57 (25)	2.2	1.3-3.8	<0.01
Never or rarely	135 (58)	104 (45)	2.7	1.6-4.3	<0.01
Eating raw vegetables the day before					
No	185 (80)	208 (90)	Reference		
Yes	47 (20)	24 (10)	2.6	1.5-4.6	<0.01
Eating leftover foods from day before					
No	86 (37)	127 (55)	Reference		
Yes	146 (63)	105 (45)	2.1	1.5-3.1	<0.01
Drinking raw water the day before					
No	194 (84)	202 (87)	Reference		
Yes	38 (16)	30 (13)	1.4	0.8-2.3	0.25

Notes: OR: odds ratio; CI Confident Interval

Table 7.3 Risk factors for adult diarrhoeal diseases in 232 cases and 232 controls in Hanam province, Vietnam (multivariable conditional logistic regression analysis and adjusted for age groups and sex)

Determinants	**Matched OR**	**95% CI**	**AF***	**% exposure among controls**
Agricultural work				
Yes *versus* No	1.1	0.6-2.0	0.04	75
Water source to drink (*versus* tap water)				
Rainwater	5.4	**2.4-12.1**	**0.77**	78
Tube well water	2.2	0.4-12.4	0.03	3
Contact with person with diarrhoea				
Yes *versus* No	3.7	**1.4-10.3**	**0.08**	3
Composting of human excreta in the household				
≤ 3 months *versus* > 3 months	2.4	**1.4-4.3**	**0.51**	72
Handling human excreta in field work				
Yes *versus* No	5.4	**1.4-21.1**	**0.07**	2
Use of animal excreta as fertiliser in the fields				
Yes *versus* No	1.6	1.0-2.6	0.16	32
Handling animal excreta in field work				
Yes *versus* No	3.3	**1.8-6.0**	**0.36**	25
Use Nhue River water to irrigate fields				
Yes *versus* No	1.0	0.4-2.5	0.00	90
Direct contact with Nhue River water during field work				
Yes *versus* No	2.4	**1.2-4.7**	**0.27**	26
Close contact with local pond water				
Yes *versus* No	2.3	**1.2-4.3**	**0.14**	13
Not use of protective measures at work				
Yes *versus* No	6.9	**3.5-13.9**	**0.78**	61
Eating raw vegetables the day before				
Yes *versus* No	2.4	**1.2-4.6**	**0.12**	10
Eating leftover foods from day before				
Yes *versus* No	1.1	0.7-1.8	0.06	45
Handwashing with soap in general (*versus* frequently)				
Sometime	2.5	**1.3-4.9**	**0.27**	25
Never or rarely	3.3	**1.8-6.3**	**0.51**	45

Notes: *AF: Attributable fraction in the population with an assumption that the exposed proportion in the control group is that of the whole population.

7.5 Discussion

In the rural agricultural communities in Northern Vietnam, we assessed the incidence of diarrhoeal diseases, and their risks using a nested case-control approach. We found that the diarrhoeal incidence in adults was lower than the global estimate for developing regions. The handling practices of wastewater, human and animal excreta in agriculture, as well as poor personal hygiene practices, such as the lack of protective measures, infrequent handwashing with soap, consumption of unsafe water or raw vegetables were associated with a high risk of diarrhoeal diseases.

Participants, who were in direct contact with water from Nhue River and local ponds during field work, had 2.4- and 2.3-fold, respectively, greater risk of diarrhoeal diseases than those who were not. Our result was similar to that found from the other studies in Hanoi and Mexico, where the farmers and their families exposed to wastewater had an excess risk of diarrhoeal diseases [49, 51, 61]. In our study, 27% of diarrhoeal cases could be explained by exposure to Nhue River water; this finding was the same as the study results by Blumenthal et al [51]. However, a study in Hanoi indicated that wastewater exposure to be the principal risk factor for diarrhoeal diseases, as it accounted for 35% of the cases [61]. It could be explained that the frequency of exposure to wastewater may be affected to diarrhoeal diseases incidence, because of the farmers exposed to wastewater was often associated with different agricultural activities (e.g. soil preparation, planting, fertilising, irrigating, excreta application, harvesting, fish feeding and catching) and they also wore protective measures while doing field work.

In the present study, the risk of diarrhoeal diseases was substantially associated with the handling practices of excreta in agricultrue. Human excreta composted less than 3 months before fertilising was associated with a risk of diarrhoeal diseases (OR = 2.4, 95% CI 1.4-4.3) and 51% of the cases could be explained by that factor. It seems that the composting procedure does not fully comply with the composting guidelines set by the Vietnamese Ministry of Health which imposes a minimum of 6 months [74]. Many intervention studies demostrated that impoving the disposal of human excreta has been effective in reducing risks of diarrhoeal diseases up to 36% [200-202]. This finding indicated that the safe composting of excreta should be intensively promoted. As indicated by Jensen et al.,

[158] approximate compost duration 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. Such length of compost could allow for destroying of enteric pathogens, thereby reducing the risk of diarrhoea. People handling human and animal excreta in field work had 5.4- and 3.3-fold, respectively, higher risk of diarrhoeal diseases than those who did not have contact. Handling human excreta in agricultural work, as it accounted only 7% of diarrhoeal cases, whereas handling animal excreta could be explained 36% of the cases. This corresponds with a larger number of farmers handling animal excreta in field work (25%) in comparison with human excreta (2%) in the communities. The occurrence of diarrhoeal diseases was not associated with type of latrine used in the household. A similar observation was made in Ethiopia [203].

Our study shows the risk of diarrhoeal diseases was significantly associated with the use of rainwater for drinking in the household, and as it accounted 77% of diarrhoeal cases. Our results contradict with previous studies in Kenya and Vietnam, which reported that use of rainwater reduced diarrhoeal risks [204, 205]; and also consumption of rainwater did not increase the risk of gastroenteritis among children in South Australia [206]. The most probable explanation for that would be associated with most of households in the communities (87%) using rainwater to drink, whereas the proportion of the household use of tap water and tube well water for drinking is low (6% and 8%, respectively). Furthermore, we observed during households visited that the roofs and gutters collected rainwater with sludge layer, which may be favorable conditions for the growth of mico-organisms. Otherwise, the rainwater may be contaminated with dirt, leaves, and the feaces from domestic animals (e.g. chicken and birds) that travel on the roofs. In addition, the rainwater stored by container which above the ground did not have a lid or this was not frequently closed. As indicated by Daoud and colleagues [207], stored rainwater was significantly contaminated with bacteria (67% of rainwater samples were contaminated with feacal coliforms) resulting in significant human health risk from infectious diseases.

The lack of protective measures (i.e. gloves, boots and face mask) while doing field work and the lack of frequent handwashing with soap had a substantial increase in risk of diarrheoal diseases. These factors are common faecal oral routes in the transmission of common enteric pathogens [208]. When there is a lack of washes the hands before eating, after defecation, especially contact with person has diarrhoea, the pathogens can easily transmitted from person to person within the households [209]. Indeed, personal hygiene practices have been proven to be an important factor in reducing the transmission of infectious diseases; and also it can reduce diarrhoeal diseases by 42-47% [61, 160]. Our study results are in line with a study in Hanoi, which showed that having a family member with diarrhoea also increased the risk of diarrhoeal dieases [61]. The diarrhoea odds ratio was higher in people who ate raw vegetables the day before. This is consistent with observations made by Kaindi and colleagues [210], which found that consumption of vegetables pose the greater risks for symptoms of food-borne gastrointestinal diseases. It may be interpreted that the vegetables were grown in fields irrigated with wastewater highly contaminated with feaces, as indicated by high concentrations of thermotolerant coliform and the presence of protozoan parasites [211]. Regarding the consumption of foods, it has been found that the improperly storage of food for later consumption is a risk factor for diarrhoeal diseases [61, 112, 197]. However, our results shows the risk of diarrhoeal diseases did not differ between people who ate leftover foods and those who not. This could be explained through our observation, that people in the study sites usually reheated leftover food before eating.

In the present study, there was no significant link between diarrhoeal diseases and participants' level of education. Our study population was relatively well educated. Two-third of our study participants attended secondary or high school and were generally very knowledgeable. A similar observation were made in Saudi Arabia, Vietnam and Uganda [61, 212, 213]. Our result in line with the previous study in Hanoi that showed no association between diarrhoeal diseases and household's SES [61]. This finding in constrast to the other studies, which found that people had diarrhoea came from lower SES group [214, 215]. This study results were similar to that found by Trang and colleagues [61] conducted in Hanoi where the different age groups and sex were not associated with the risk of diarrhoeal diseases.

In our study, the diarrhoeal incidence in adults was low (0.28 episodes per persons per year), which was similar to that found as an investigation in Hanoi [61]. However, it is much lower than the global estimated incidence of diarrhoeal diseases for age above 5 years in developing regions, which ranged between 0.40 - 0.60 episodes per persons per year [216]. Our results may have been affected by the under-reporting of diarrheoal episodes because of the unwillingness of some subjects to participate in the study. We also observed that people usually self-treatment by experience from family members or neighbours at home when they had diarrhoea (45%). Moreover, for diarrhoeal disease, local people perceived it as a private issue which was not to be shared to others, especially strangers. Therefore the study may have underestimated the true rate of diarrhoeal incidence.

The incidence of diarrhoeal disease was lower in the dry season than in the rainy season. This is in contrast to the other studies, which found that the diarrhoeal incidence did not differ much between two seasons, although the diarrhoeal episodes were more frequent in the dry and cool season [61]. Furthermore, Blumenthal and colleagues reported that the untreated wastewater in dry season was a greater risk of enteric infection than in the rainy season [51]. In our study, the peak of diarrhoeal incidence was in August (which is during the rainy season). This finding could be explained by the fact that in the study sites, this is the period during which people usually empty and compost human excreta as fertilisers for the next crop. It is noted that, excreta contains variety of different pathogens, particularly enteric bacteria such as *E. coli*, *Shigella spp*., *Salmonella* and *V. cholera* [30]. Therefore, people may have been exposed to the excreted-organisms causing diarrhoeal diseases.

7.6 Conclusion

In an agricultural community of Hanam province, Northern Vietnam, the incidence of diarrhoeal diseases in adults was associated with the handling of human and animal excreta, contact with water from Nhue River and local ponds during field work, the lack of use of protective measures, as well as consumption of unsafe water sources and raw vegetables. In the rural areas of Vietnam, the appropriate treatment of wastewater remains limited, and human and animal excreta are widely used. Therefore, to reduce the public health risks related to the use of wastewater and excreta, the safe composting of excreta process and use of protective measures while doing field work should be promoted. In addition, improved personal hygiene practices as well as safe water and food consumption should also be promoted.

7.7 Conflict of interest

All authors declare no conflicts of interest

7.8 Acknowledgments

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7.9 Authors´ contributions

PPD, HNV, JZ, PDC, CZ and PO planned and designed the study. PPD, HNV and PDC conducted and supervised field 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.

8. ASSESSMENT OF INFECTION RISKS OF DIARRHOEA ASSOCIATED WITH WASTEWATER AND EXCRETA USE IN AGRICULTURE IN HANAM PROVINCE, NORTHERN VIETNAM

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8.1 Abstract

When untreated wastewater and excreta are used for agricultural production, enteric pathogens may be a primary hazard to human health through different routes of exposure, as in direct contact with wastewater and excreta while doing field work. A quantitative microbial risk assessment of *Escherichia coli, Giardia lamblia* and *Cryptosporidium parvum* infection was conducted using multi-trial Monte Carlo simulations (10,000 iterations) to predict the risk of diarrhoea related to the use of wastewater and excreta for agricultural production in Hanam province, Northern Vietnam. A total of 173 wastewater and excreta samples were collected from 5 critical sampling points. Three pathogens were analyzed quantitatively: *E. coli* by the Most Probable Number method and the protozoan parasites *G. lamblia* and *C. parvum* by immunofluorescent antibodies and microscopy. A survey with 235 households was conducted using a structured-questionnaire to assess people's exposure to wastewater and excreta. The most hazardous exposures included direct contact with the Nhue River and pond water, field water and composted excreta during field work. The highest mean concentration of diarrheagenic *E. coli* (DEC) (6.3 x 10^8 MPN/100 ml) and *C. parvum* (30 oocysts/100 ml) was in household sewage; whereas *G. lamblia* was highest in composted excreta (119 cysts/gram). Estimated annual infection risks in all the exposures were much higher than the commonly proposed thresholds of 10^{-4} (< 1 infection per 10,000 individuals); the estimated annual risks of diarrhea values were at least 3-fold greater than maximal risk of 10^{-3} per person per year (pppy); and the annual burden of diarrhoeal disease was extremely greater than the health target of 10^{-6} DALYs (≤ 1 DALY/million persons) recommended by WHO. The assessment indicated exceeded risks for *G. lamblia, C. parvum* and DEC infections among people exposed to wastewater and excreta. Study results are useful in developing an integrated strategy for pathogen management and public health control in the agricultural settings where wastewater and excreta are intensively used as irrigation water sources and fertilisers and where household wastewater is freely discharged into irrigation channels.

Keywords: pathogens, risk assessment, wastewater, excreta, agriculture, Vietnam.

8.2 Introduction

The use of treated or partially diluted or untreated wastewater is a widespread practice in many countries around the world [28]. In Vietnam, the irrigation water sources vary from fresh water and wastewater to groundwater [63]. The use of excreta in both agriculture and aquaculture has been practised for centuries in Asia [20], in particular in China [217] and Vietnam [21, 179]. Wastewater and excreta provides many benefits for agricultural users such as reliable water resources, valuable nutrients, increased crop yields and reduces use of inorganic fertilisers, but these practices may pose potential health risks [15, 190]. The most common diseases associated with wastewater and excreta are the diarrhoeal diseases [20, 31]. Diseases are linked to the nature of the pathogen concentrations and distributions in the wastewater and excreta, and the risks can be observed in agricultural workers, their families and consumers [31, 218]. However, in developing countries, there are various pathways of disease exposure and the comparative risk contribution from excreta and wastewater irrigation has ever been comprehensively studied [28].

In the third edition of its guidelines for the safe use of wastewater, grey water and excreta in agriculture and aquaculture [14, 20, 31], the WHO promotes the use of a risk-based approach to estimate the required reductions of viral, bacterial and protozoan pathogens in wastewater-irrigated, with the goal of achieving a certain health protection level in an exposed population. Quantitative Microbial Risk Assessment (QMRA) is a technique that has been developed for calculating the risk of infection and disease from a particular pathogen [37, 97]. The approach has been applied to assess the health risks for farmers using wastewater and faecal sludge under different irrigation and technology regimes in Mexico, Thailand and Ghana [95, 96, 219]. QMRA has been used to establish the health risk associated with consuming wastewater-irrigated food crops and vegetables [92, 220-222]. Schönning and colleagues [94] have also applied QMRA to evaluate the transmission risk of infectious disease related to the use of faeces as fertiliser within private households in Denmark. Nguyen-Viet and colleagues, have extended QMRA concept towards an integrated approach combining it with material flow analysis and social, cultural and economic environment assessments [77]. This study is part of a large research project aiming at validating the integrated framework and assessing the environmental and health risks associated with wastewater and excreta reuse in agriculture Hanam province in Northern Vietnam [77, 192, 193]. We presents here is one of the first studies using QMRA in Vietnam to assess the microbial infection risks of diarrhoeal diseases related to the use of wastewater and excreta in agriculture. We have also attempted to identify the major exposure points and plausible disease transmission pathways and mitigate public health risks in the agricultural settings where wastewater and excreta are intensively used for irrigation and as fertilisers.

8.3 Materials and methods

8.3.1 Study sites

The study was carried out in Nhat Tan and Hoang Tay communes, Kim Bang district, Hanam province (20.32^{o} N, 105.54^{o} E), Northern Vietnam, situated about 60 km south of Hanoi (Figure 3.1). Nhat Tan and Hoang Tay communes count 10,500 (2,700 households) and 5,700 (1,600 households) inhabitants, respectively. In Figure 2 we presented a flowchart of the principal material flow investigated in this study. Most households raise livestock in their compounds (e.g., chickens, ducks, and pigs). 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 the Nhue River. Hanoi City's wastewater from households, industry and other sources, such as hospitals, is directly discharged untreated into the river [101]. The Nhue River water is used for crop irrigation and to feed fish ponds. Several pumping stations are located along the river and a system of open and closed canals distribute the water to fields and fish ponds. Wastewater from households (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. In general, un-composted or partially composted human and animal excreta are used as fertiliser in Hanam, as in many other places in Northern and Central Vietnam. Personal protective measures to prevent contamination are often lacking.

8.3.2 Selection of pathogens

Three reference pathogens were selected for this study: *Escherichia coli, Giardia lamblia* and *Cryptosporidium parvum.* All of these have been causes of waterborne disease, are known to occur in Vietnam, and have been detected in wastewater and vegetables grown in wastewater-irrigated fields [211].

Several pathogenic strains of *E. coli* have been identified [97]. Enterotoxigenic *E. coli* is a well-known waterborne pathogen in developed countries and has been the cause of high-profile outbreaks of disease, such as that at Walkerton, Ontario [37]. In our study areas pigs play a more important role in the agricultural system, especially integrated pig-fish farms. Pigs are also a source of *E. coli* [223, 224]. In undertaking the QMRA, the risk from all pathogenic *E. coli* was calculated based on the impact expected from pathogenic *E. coli*. Thermotolerant coliforms were used as a surrogate for pathogenic *E. coli*. We have assumed that 95% of thermotolerant coliforms were *E. coli* [225] and that 8% of *E. coli* were pathogenic [97].

Infections of *Cryptosporidium* in humans are caused by *C. parvum* [226, 227]. *C. parvum* is very common among newborn calves that can excrete oocysts in high numbers, but is also frequently found in adult livestock and other ruminants. The oocysts are extremely resistant to chlorination and have been involved in many waterborne outbreaks [228]. Water is a significant route of infection in developing countries [229].

The prevalence of giardiasis typically ranges between 2 and 5 % of people in industrialised nations [230]. In developing countries, giardiasis prevalence can be as high as 20 - 30 % [231] and few studies have been performed to quantify its risks. *G. lamblia* is the most common intestinal protozoan in United States; it also is frequently reported in association with waterborne diseases [40, 97, 122]. Cysts may be found in water as a result of faecal contamination from both humans and animals; it has also been found to be resistant to the disinfectants commonly used in drinking water treatment [228, 232].

8.3.3 Sampling sites

The environmental sanitation and agricultural systems presented in Figure 3.2. The system indicates the high-risk location, from where samples were collected.

From August 2009 to July 2010, a total of 173 wastewater and excreta samples were collected monthly from 5 typical sampling points (Figure 2): (i) the Nhue River (36), (ii) household sewage (36), (iii) local ponds (36), (iv) irrigation system (60), and excreta composts (5). Water samples were directly collected 20 cm from the surface and at midpoints of the river, canal and sewage. A separate 1 litre water sample to test for *E. coli* was collected by a sterile glass bottle with wide-mount and screw-cap; and the other separate 5 litre water sample to test *G. lamblia* and *C. parvum* was collected by a sterile plastic can. Human excreta composts were collected using a 500 gram sterile steel container. The samples were stored on ice (4-8^{o} C) during transport to the laboratory of the Microbiological Department at the National Institute of Hygiene and Epidemiology, Hanoi, Vietnam for further processing within 6 hours.

8.3.4 Laboratory examinations

Detecting and enumerating thermotolerant coliforms in wastewater samples was based on lactose fermentation, gas production and indole production from tryptophane at 44º C within 24h. Numbers of thermotolerant coliforms on multiple tubes of different dilutions were enumerated by using the most probable number (MPN) table, according to the American Public Health Association (APHA) [117].

Detection of *C. parvum* and *G. lamblia* in water samples was achieved by immunofluorescent antibodies (IFA) and microscopy [75]. Briefly, the collected water samples have been sediment in 24 hours, then the supernatant was carefully removed with a vacuum suction pump and approximately 100 ml of sediment was left and poured into two 50 ml centrifuge tubes. Sediment water was concentrated by centrifugation followed by a flotation step, where 10 ml of sample volume was underlaid with 5 ml of flotation fluid (saturated NaCl solution with 500 g of glucose added per liter, diluted 1:1

with sterile distilled water to a final density of 1.13 and centrifuged for 1 min at 100 *G* to clean off larger debris). The sample was subsequently washed with sterile distilled water to remove remains of the flotation fluid before the sample was concentrated to volume of 2 ml. Approximately 200 µl of the final sample volume was air-dried on a Teflon printed diagnostic slide fixed with methanol and stained with fluorescent monoclonal antibodies to *Giardia* and *Cryptosporidium* according to the manufacturer's protocol (*Crypto/Giardia* CEL; Cellabs Pty Ltd, Australia). Each was read at x400 magnification with a standard fluorescence microscope equipped with a UV-filter block (500 nm excitation, 630 nm emission). All cysts and oocysts in a well were counted, and their numbers were estimated for 100 ml sample.

8.3.5 Exposure assessment

A field survey was carried out from April to August 2009 in Hoang Tay and Nhat Tan communes. There were 235 adults from both sexes, ranging from 16 to 65 in age, randomly selected from the household list provided by the Communal People's Committee. A structured questionnaire was used to obtain information to assess people's exposure to the Nhue River water, local ponds, and irrigation water, as well as exposure to human and animal excreta at home and in the fields. This was developed in English, translated into Vietnamese and pre-tested in a village close to Hanoi. After revisions, the questionnaire was used in face-to-face interviews conducted by five trained and experienced research assistants.

8.3.6 Exposure scenarios

Five exposure points (scenarios) were modelled in the assessment for the accidental ingestion of Nhue River water, household sewage, local ponds, irrigation system, and composted excreta, based on the authors' observation and individual interview, as shown in Table 8.1. The accidental ingestion dose associated with each scenario was assumed in reference to Haas et al., 1999 [97] and Mara et al., 2007 [95]. The exposure scenarios involved (i) an accidental ingestion of 10 ml of Nhue river water while harvesting vegetables for single-exposure, and the field survey revealed that farmers were exposed 132 times over a year; (ii) an involuntarily ingestion of 10 ml households' wastewater by individual cleaning the households' sewage 8 times per year; (iii) an involutarily ingestion of 100 ml local pond water by individual fishing 72 times per year; (iv) an accidental ingestion of 10 ml irrigation system water through farmers growing rice 12 times over a year; and (v) an involuntarily ingestion of 10 mg composted excreta by farmers applying fertiliser in the fields 6 times per year.

Table 8.1 Accidental ingestion at each exposure point (scenario) and dose assumptions in Hoang Tay and Nhat Tan communes, Hanam province, Northern Vietnam

Exposure points	Events	Ingestion dose of water
(i) Nhue River water	Harvesting vegetable in Nhue River	10 ml/event 132 events/year
(ii) Household sewage	Cleaning household sewage	10 ml/event 8 events/year
(iii) Local pond	Fishing in the local ponds	100 ml/event 72 events/year
(iv) Canal/field	Growing rice	10 ml/event 12 events/year
(v) Composted excreta	Application of excreta in the fields	10 mg/event 6 events/year

8.3.7 Dose response assessment

The β-Poisson dose response model was used to estimate the risk of DEC infection [97] while the exponential model was used for *G. lamblia* and *C. parvum* [121, 122]. The risk of infection for single exposure models and formulae employed were as follows:

(i) β-Poisson dose response model for *E. coli*, $\mathbf{P_{inf(d)} = 1 - [1+ (d/ID_{50})(2^{1/\alpha} - 1)]^{-\alpha}}$, where $\alpha = 0.1778$ and $ID_{50} = 8.60 \times 10^7$

(ii) Exponential model for *G. lamblia*, $\mathbf{P_{inf(d)} = 1 - exp(-rd)}$, where r = 0.0198

(iii) Exponential model for *C. parvum*, $\mathbf{P_{inf(d)} = 1 - exp(-rd)}$, where r = 0.00467; and $P_{inf(d)}$ is the probability of infection; α, ID_{50} and r are pathogen infectivity constants; d is the dose level (number of organisms).

8.3.8 Risk characterisation

Given the above infection per single exposure, the annual risk of infection ($P_{inf/y}$) was calculated for each scenario using the following equation [97]: $\mathbf{P_{inf/y} = 1 - [1- P_{inf(d)}]^n}$, where n was the number of exposures over one year to a pathogen dose (d). Also the annual risk of diarrhoea disease (P_{ill}) was calculated using the following equation [84]: $\mathbf{P_{ill} = P_{inf/y} \times P_{ill/inf}}$, where $P_{ill/inf}$ was risk of illness per infection by diarrhegenic *E. coli* (0.25) [84]; *Giardia* (0.67) [122]; and *Cryptosporidium* (0.39) [97] were constants.

Having estimated annual risks of infection from each of the exposure scenarios; it is tempting to calculate the combined from these exposure scenarios. Assuming that the annual risks of infection from all the exposure scenarios are independent, combined annual risk of infection (P_{comb}) was estimated by using the following default [40, 97]: $\mathbf{P_{comb} = 1 - (1 - P_{inf/y1})(1 - P_{inf/y2})(1 - P_{inf/y3})(1 - P_{inf/y4})(1 - P_{inf/y5})}$. Where, $P_{inf/y1}$, $P_{inf/y2}$, $P_{inf/y3}$, $P_{inf/y4}$, $P_{inf/y5}$ are the annual risks of infection from the exposure scenario of harvesting vegetables in Nhue River, cleaning household sewage, fishing in the local ponds, growing rice and application of excreta in the fields, respectively. For plausibility, we compared the combined annual risk of diarrhoea by each pathogen with the overall reported diarrhoea incidence, which was found from an epidemiological study in the same study area (Chapter 7).

For all the different exposure categories and consumptions, diarrhea infection risks were calculated using estimated probability distributtion functions (PDF) randomly sampled by Monte Carlo simulation (10,000 iterations). The models were run using @Risk sofware (student version 5.7, Palisade Corpo., Newfield, NY) added on to Microsoft Excel. The mean single and annual risks of infections were reported for each scenario. The 5^{th} and 95^{th} percentiles were also expressed to account for variability in the estimated risks. The estimated annual risk of infection in all the exposure scenarios were compared with the acceptable risk commonly proposed thresholds of 10^{-4} (i.e., $\leq$ 1 infection/10,000 individuals), this criterion has been suggested for drinking water assuming 2 litres daily exposure for 365 days, as defined by the United States Environmental Protection Agency (USEPA) [233]. Risk of diarrhoea was reported as the number of cases per 10,000 people, and compared with the tolerable risk of waterborne disease from drinking fully treated dringking water set by the WHO is 10^{-3} per person per year (pppy) [83]. This mean that either the occurrence of disease is one individual per 1,000 year or 0.1% of individuals in a community are diseased within a year as a result of drinking fully treated drinking water that is unduly stringent [121].

Disability Adjusted Life Years (DALYs) is used as a metric for translating the risk of disease burden a general health burden per diarrhoea case [83]. DALY accounts for the years lived with a disability (YLD) plus the years of life lost (YLL) due to the hazard. DALY is calculated as the produce of the probability of diarrhoea for each pathogen with a severity factor and duration (in years), based primarily on the Global Burden of Disease project have been presented and reviewed by Havelaar and colleagues [114, 234]. However for this risk assessment the equation for calculating the DALY contributation per infection, with the maximum burden of diarrhoea (MBD) for DEC (0.32), *G. lamblia* and *C. parvum* (0.15) was taken from Howard et al., 2006 [84]. The equation is: $\mathbf{DALY = P_{ill} \times MBD \times SF}$, where P_{ill} is the risk of diarrhoeal disease, MBD is the maximum disease burden, and SF is the susceptible fraction. The SF is the proportion of population in the communities involved in the specific agricultural activities (exposure scenarios). The SF was obtained from the field survey.

8.4 Results

8.4.1 Microbiological contamination

The measured concentrations of diarrhegenic *E. coli* (DEC), *G. lamblia* and *C. parvum* are presented in Table 8.2. There highest mean concentration of *E. coli* (6.3×10^8 MPN/100 ml) and *C. parvum* (30 oocysts/100 ml) was in the household sewage; whereas it was *G. lamblia* in composted excreta (119 cysts/gram). The lowest concentration of DEC (2.1×10^5 MPN/gram) was in composted excreta; *G. lamblia* (3 cysts/100 ml) in local ponds; whereas *C. parvum* oocysts was not observed in composted excreta samples.

The human exposure survey revealed that exposure was high during the rice growing seasons (62%), followed by application of excreta in the fields (17%), fishing in the local ponds (7%), harvesting vegetables in the Nhue river (3%), and cleaning the households' sewage (2%). Whereas the number of events over a year was high while harvesting vegetables in the Nhue River (132), this was followed by fishing in the local ponds (72); rice growing (12); cleaning household sewage (8); and application of excreta in the field (6).

Table 8.2 Mean concentrations of pathogens in the exposure points in Hoang Tay and Nhat Tan communes, Hanam province, Northern Vietnam (expressed in probability density functions - PDF)

Exposure points (Scenarios)	Pathogens / 100 mL	Concentrations		
		Mean	Min	Max
Nhue River water	*G. lamblia*	6	0	76
	C. parvum	5	0	61
	DEC[a]	1.1×10^6	78	1.1×10^7
Household sewage	*G. lamblia*	28	0	310
	C. parvum	30	0	295
	DEC[a]	6.3×10^8	5.1×10^4	2.1×10^9
Local pond water	*G. lamblia*	3	0	43
	C. parvum	2	0	32
	DEC[a]	4.1×10^6	23	4.0×10^7
Irrigation system	*G. lamblia*	9	0	125
	C. parvum	1	0	17
	DEC[a]	1.9×10^6	180	2.1×10^7
Composted excreta	*G. lamblia*	119	0	1561
	C. parvum	0	0	0
	DEC[a]	2.1×10^5	14	2.1×10^6

[a] Diarrhegenic *E. coli* is usually 8% of the measured *E. coli* concentration; and *E. coli* is assumed 95% of the Thermotolerant cofiforms (Haas et al., 1999; Howard et al., 2006)

8.4.2 Risks of infections and diarrhoeal diseases

The mean single risks of *G. lamblia, C. parvum* and DEC infections (including the 5th and 95th percentile range) associated with the accidental ingestion of the different exposure points is presented in Table 8.3. The mean single risk of *G. lamblia* infection per individual in all the exposure scenarios ranged between 1.4×10^{-2} and 1.9×10^{-1}, with a highest infection risk in the scenario of application of excreta in the fields. For *C. parvum*, the single risk of infection was highest in the exposure scenario of cleaning household sewage (1.4×10^{-2}) and was not found in the application of excreta in the fields' scenario. The single risk of DEC infection fluctuated between 2×10^{-3} and 4.1×10^{-1}, with maximised risk of infection in the scenario of cleaning household sewage.

Table 8.3 Single infection risks in the different exposure scenarios estimated by 10,000-trial Monte Carlo simulations in Hoang Tay and Nhat Tan communes, Hanam province, Northern Vietnam

Exposure scenarios	Pathogens	Single risks of infection		
		Mean	**5th**	**95th**
Harvesting vegetables in	*G. lamblia*	1.4×10^{-2}	0.0	4.3×10^{-2}
	C. parvum	2.7×10^{-3}	0.0	8.4×10^{-3}
	DEC	1.0×10^{-2}	5.4×10^{-4}	2.9×10^{-2}
Cleaning household	*G. lamblia*	5.4×10^{-2}	2.0×10^{-3}	1.6×10^{-1}
	C. parvum	$\mathbf{1.4 \times 10^{-2}}$	4.7×10^{-4}	4.3×10^{-2}
	DEC	$\mathbf{4.1 \times 10^{-1}}$	1.7×10^{-1}	5.6×10^{-1}
Fishing in the local ponds	*G. lamblia*	7.1×10^{-2}	0.0	2.3×10^{-1}
	C. parvum	1.0×10^{-2}	0.0	3.7×10^{-2}
	DEC	1.6×10^{-1}	1.9×10^{-2}	3.1×10^{-1}
Growing rice	*G. lamblia*	1.9×10^{-2}	0.0	5.8×10^{-2}
	C. parvum	4.7×10^{-4}	0.0	1.9×10^{-3}
	DEC	1.6×10^{-2}	9.4×10^{-4}	4.7×10^{-2}
Application of excreta in	*G. lamblia*	$\mathbf{1.9 \times 10^{-1}}$	1.2×10^{-2}	5.1×10^{-1}
	C. parvum	0.0	0.0	0.0
	DEC	2.0×10^{-3}	1.1×10^{-4}	6.1×10^{-3}

Figure 8.1 shows the highest mean annual risk of *G. lamblia* infection per farmer for the accidental ingestion of local pond water while fishing was 0.75; followed by harvesting vegetables in the Nhue River (0.62); application of excreta in the fields (0.59); cleaning the households' sewage (0.31); and growing rice (0.19). For *C. parvum*, the highest mean annual risks due to the accidental ingestion of water while fishing in the local ponds was 0.39; whereas the annual risk of *C. parvum* infection was not found for the exposed to composted excreta. The mean annual risk of DEC infection was 0.96 for the accidental ingestion of water while fishing in the local ponds; cleaning the households' sewage (0.95); harvesting vegetables in local ponds (0.58); growing rice (0.17); and composting excreta (0.01).

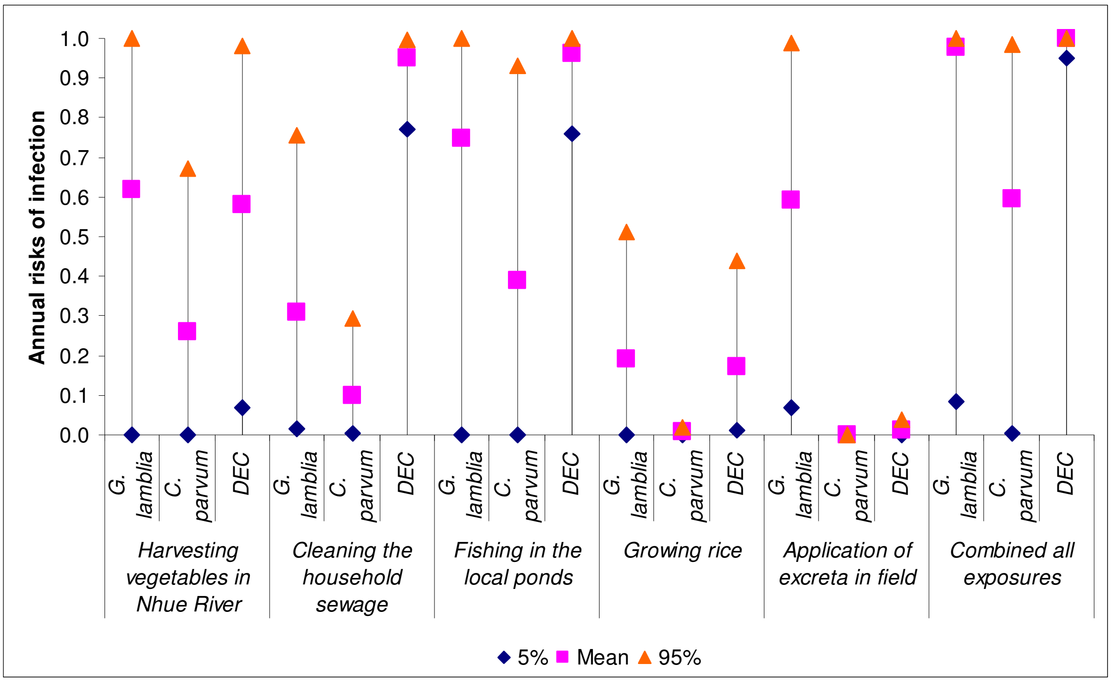

Figure 8.1 Annual infection risks in the different exposure scenarios estimated by 10,000-trial Monte Carlo simulations in Hoang Tay and Nhat Tan communes, Hanam province, Northern Vietnam

Figure 8.2 shows the annual risks of diarrhoeal disease by pathogen and exposure. *G. lamblia* caused higher risk of diarrhea than DEC and *C. parvum*, particularly through fishing in local ponds (0.50), harvesting vegetables in the Nhue River (0.42), and application of excreta in the fields (0.40). The annual risk of diarrhoea by DEC for the involutarily ingestion of water during harvesting vegetables in the Nhue river was 0.15; fishing in the local ponds was the same for cleaning the households' sewage (0.24); growing rice (0.04). The risk of diarrhoeal disease due to DEC was not found in the exposure scenario of human excreta application in the fields. The highest annual risk of diarrhoea by *C. parvum* was 0.15 for the involutarily ingestion of water while fishing in the local ponds; followed by harvesting vegetables in Nhue River (0.10); and cleaning household sewage (0.04). The application of excreta in the fields and growing rice were not a diarrhoea risk of *C. parvum.* In case of combination of all exposure scenarios, the annual risk of diarrhoea due to *G. lamblia* was 0.66, DEC (0.25) and *C. parvum* (0.23).

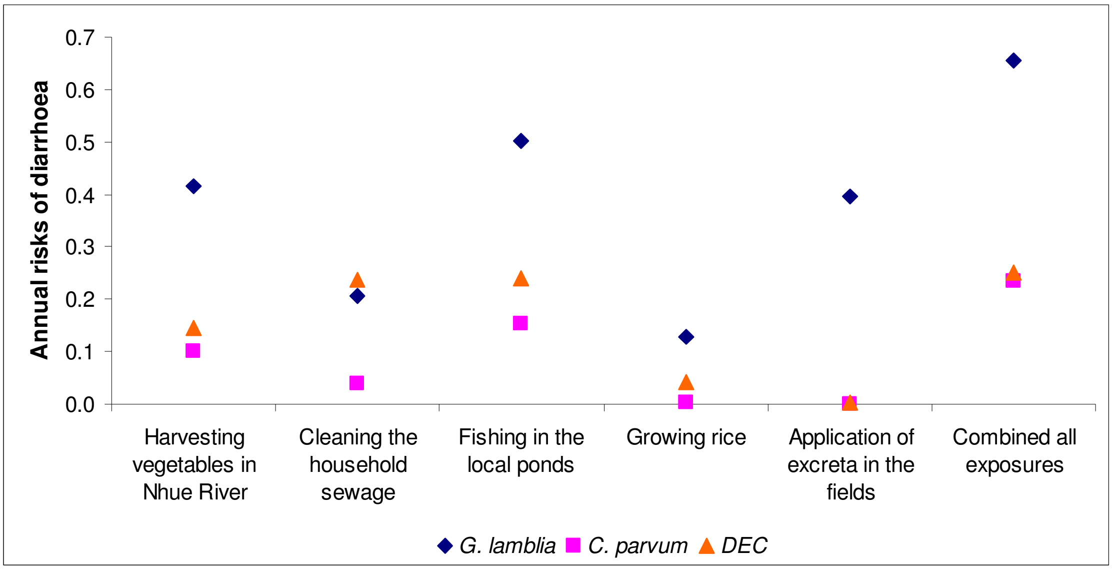

Figure 8.2 Annual risks of diarrhoeal diseases in the different exposure scenarios in Hoang Tay and Nhat Tan communes, Hanam province, Northern Vietnam

8.4.3 The burden of diarrhoeal disease

The DALY contribution per infection and exposure is presented in Figure 8.3. The burden of diarrhoeal disease contributed by DEC highest in the exposure scenario of growing rice (8,000 x 10^{-6}), followed by fishing in the local ponds (5,200 x 10^{-6}), cleaning household sewage (1,600 x 10^{-6}), and harvesting vegetables in Nhue River (1,400 x 10^{-6}). The diarrhoea burden for DEC was not associated with the scenario of application of excreta in the fields. For *G. lamblia*, the highest burden of diarrhoeal disease also in the growing rice exposure scenario (12,000 x 10^{-6}), application of excreta in the fields (10,200 x 10^{-6}), fishing in the local ponds (5,100 x 10^{-6}), harvesting vegetables in Nhue River (1,900 x 10^{-6}) and cleaning household sawage (660 x 10^{-6}). The scenarios of application of excreta in the fields and growing rice were not a burden of diarrhoeal disease for *C. parvum*, whereas the burden of diarrhoea for *C. parvum* in the scenarios of fishing in the local ponds (1,500 x 10^{-6}), cleaning househol sewage (126 x 10^{-6}), and harvesting vegetables in Nhue River (45 x 10^{-6}).

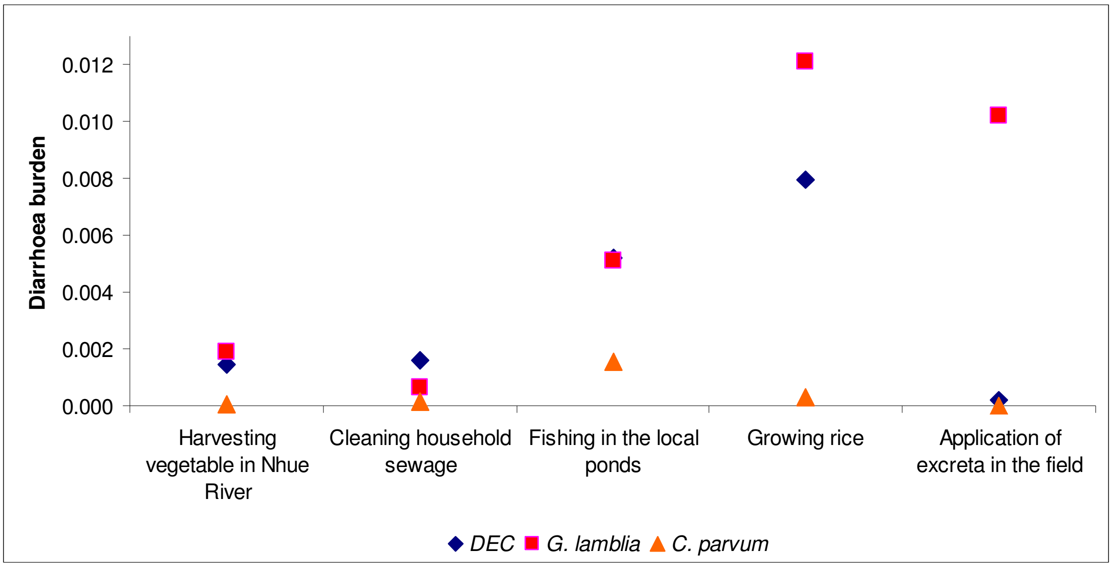

Figure 8.3 Annual burden of diarrhoeal diseases in the different exposure scenarios in Hoang Tay and Nhat Tan communes, Hanam province, Northern Vietnam

8.5 Discussion

Health risks related to the different practices of using wastewater and excreta were assessed in an agricultural community in Northern Vietnam. Based on the QMRA, the calculated annual infection risks in all the exposures in the present study were much higher than the commonly proposed thresholds of 10^{-4} (i.e., ≤ 1 infection per 10,000 individuals) [233]. The estimated annual risks of infection by DEC was at least 100-fold greater than the acceptable risk, with the maximum in the exposure scenarios of fishing in the local ponds (9,600-fold) and cleaning household sewage (9,500-fold). Infection risk calculated for *G. lamblia* was 1,900-fold higher in all the exposure scenarios in comparison with the acceptable risk, with a maximum in the scenario of fishing in the local ponds (7,500-fold). For *C. parvum,* with an exception of the exposure scenario of application of excreta in the fields that was inapplicable, the annual risk of infection estimated was at least 100-fold greater than the acceptable risk, and a greatest in the scenario of fishing in the local ponds (3,900-fold). In most cases, variation of the pathogen concentrations in the different water sources had a significant impact on the uncertainty of the estimated annual risk of infections. Overall, the combined annual risk of infection by *G. lamblia* and DEC from all the exposure scenarios were higher than the annual infection risk by *C. parvum* (Figure 3). In comparison with a study in Thailand, which assessed the risk of diarrhoea infection related to exposure to canal water, the infection risk for DEC, *C. parvum,* and *G. lamblia* were higher than the estimated risk of infection in our study [235]. Furthermore, a case of restricted irrigation with an exposure of 150 days per year in Mexico, the risk of *Cryptosporidium* infection is 1,000-fold higher than the acceptable risk [95].

The annual risk of diarrhoeal diseases were greater than threshold risks of waterborne disease from drinking water set by the WHO [83] at 10^{-3} pppy. For DEC, the risks of diarrhoeal diseases were greater than the tolerable risk, ranging between 3-fold and 240-fold, with the highest in the exposure scenario of fishing in the local ponds and the cleaning the household sewage (0.24 pppy), and lowest in the scenario of application of excreta in the fields (0.003 pppy). Whereas, the risk of diarrhoea due to *G. lamblia* was at least 130-fold higher than the tolerable risk, with the maximum in the scenario of fishing in the local ponds (0.50 pppy), and lowest in the scenario of growing rice (0.13 pppy). Our findings was lower than in comparison with the study result in Thailand which showed that the risk of diarrhoea for *G. lamblia*, fluctuated between 0.54 and 0.67 pppy [235]. The study shows the risk of diarrhoeal disease due to *C. parvum* was much lower than for *G. lamblia* and DEC. Indeed, the highest risk of diarrhoea for *C. parvum* (0.15 pppy) was found in the scenario of fishing in the local ponds, and lowest in the scenario of growing rice (0.004 pppy). For all the scenario of exposures to wastewater, the risk of diarrhoeal diseases for pathogens was highest in the scenario of fishing in the local ponds. This finding could be interpreted that we assumed quantity of water involuntarily ingested by people who are fishing in the local ponds was higher than in compared to other exposure scenarios.

In combination of all the exposure scenarios, we calculated the combined annual risks of diarrhoea for *G. lamblia* was 0.66 pppy, DEC (0.25 pppy) and *C. parvum* (0.23 pppy) (Figure 8.2), and the annual risks of diarrhoea due to all of three pathogens estimated was 0.80 pppy. This is in comparable with the overall incidence of diarrhoeal disease in the study area of 0.28 pppy (Phuc et al., 2011). The estimated risk of diarrhoeal disease due to *G. lamblia* appeared to be up to 2.4-fold greater as compared to the reported cases of diarrhoeal incidence from our epidemiological study. While the estimated risk for DEC and *C. parvum* were lower than those according to the reported cases of diarrhoea in the study area. It may be possible that DEC is the main etiological agent of diarrhoea in Vietnam. This is in close agreement with Trang and colleagues found more bacterial pathogens, especially DEC, in people exposed to watewater than in unexposed people [61]. Otherwise we simply put that assumptions in our study might have most accurately modeled DEC. In addition, the estimated annual risk of diarrhoea for *G. lamblia* was higher than reported cases of diarrhoea. It is important to note that the reported incidence cases of diarrhoea present only in adults with aged 16-65 years, who are mainly working related to agriculture. This could be explained that farmers working associated with wastewater and excreta, even carrying pathogens, may be protected because of the repeated exposure and their immunity levels were high to common enteropathogens [10]. Moreover, for diarrhoeal disease, local people perceived it as a private issue which was not to be shared to others, especially strangers. We also observed that people usually self-treatment by experience from family members or neighbours at home when they had diarrhoea (45%), implying a safe estimate of 55% of underestimated cases.

Overall, the burden of diarrhoeal diseases distributed by pathogens in all the exposure scenarios in our study was extremely exceeded compared to the reference level of health target of 10^{-6} DALYs loss pppy recommended by WHO [83]. Especially, in the scenario of growing rice, the burden of diarrhoeal disease was a greater than the reference level for DEC (7,900-fold), *G. lamblia* (12,000-fold) and *C. parvum* (280-fold). We observed that 62% people in the study sites involved agricultural activities as growing rice. This result suggests the important in reducing concentrations of pathogens in wastewater before use for irrigation to the fields as a means of mitigating public health. In the scenario of fishing in the local ponds, the burden of diarrhoeal diseases contributed by pathogens were higher than the WHO reference level of risk at 10^{-6}, for DEC (5,200-fold), *G. lamblia* (5,100-fold) and *C. parvum* (1,500-fold). DEC contribution to the diarrhoeal diseases burden was a higher than contributed by *G. lamblia* and *C. parvum* in the scenario of cleaning household sewage. In fact, we observed in the study areas animal manure and wastewater from on-site sanitation is directly dumped into ponds to feed fish. It is known that main source of pathogenic *E. coli* is from animals such as cattle, pigs, chickens, goats [83, 97]. In the scenario of application of excreta in the fields, the burden of diarrhoeal disease contributed by *G. lamblia* was over 47 orders of magnitude higher than for DEC. It should be noted that the handling excreta in the field was found to be associated with protozoa infections, because of the survival time of protozoan cysts is more longer than bacteria in the excreta composts and environment [30].

8.6 Conclusions

The assessment indicated exceeded risks for *G. lamblia, C. parvum* and diarrhegenic *E. coli* infections among people exposed to wastewater and excreta. In particular, the most hazardous exposures identified, included fishing in the local pond, harvesting vegetables in Nhue River and application of excreta in the field. These could be controlled by an improved proper composting process of human excreta and use of protective measures while doing field work. Our study revealed that the applied method can be used for comparison between the various routes of exposures and also between the different exposed populations. Study results are useful in developing an integrated strategy for pathogen management and public health control measured in the agricultural settings where wastewater and excreta are intensively used as irrigation water sources and fertilisers; and where household wastewater is freely discharged into irrigation systems.

8.7 Conflicts of interest

All authors declare that there are no conflicts of interest.

8.8 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 the technicians of the Division of Enteric Infections, Microbiological Department at the National Institute of Hygiene and Epidemiology, Hanoi who examined the wastewater and excreta samples for bacteria and protozoan parasite detection. This work was carried out with financial support from 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.

8.9 Authors´ contributions

PPD, HNV, JZ, CZ and PO planned the study and designed the protocols. PPD, HNV 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, PO, JZ and HNV carried out the data analysis and interpretation. PPD, HNV and JZ 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.

9. ASSESSING NUTRIENT FLUXES IN A VIETNAMESE RURAL AREA DESPITE LIMITED AND HIGHLY UNCERTAINTY DATA

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9.1 Abstract

Material Flow Analysis is a useful methodology to describe and quantify complex systems based on the law of mass conservation. It was further adapted to suit the specific conditions in developing countries where data scarcity and uncertainty. The "adapted MFA" methodology optimises the number of parameters, describes these parameters as probability distributions, and assesses the accuracy and uncertainty of model values by Monte Carlo simulation.

This study illustrates the first successful application of the "adapted MFA" methodology in a small and low-income area including two neighbouring communes in rural Northern Vietnam, where environmental sanitation and traditional agricultural practices are strongly interlinked and impact on the surrounding environment. Moreover, data on these two practices is typically scarce and uncertain. The obtained results reveal that the agricultural system was a significant source of nutrients (nitrogen [N] and phosphorus [P]), which affect the surrounding environment mainly due to the overuse of chemical fertilisers. Every year, there were 103 ± 39 tonnes N released into the atmosphere, 25 ± 3 tonnes of N leached to the surface water and 14 ± 2 tonnes of P accumulated in the soil, all originating from applied chemical fertilisers. In addition, the sanitation system was also a critical source of nutrients that enter the surface water. 69 ± 6 tonnes of N and 23 ± 4 tonnes of P came from households through effluents of on-site sanitation systems (such as latrines and septic tanks) and were directly discharged to surface water every year. Moreover, the whole system annually generated a large nutrient source (214 ± 56 tonnes of N; 58 ± 16 tonnes of P) in the form of wastewater, faecal sludge, animal manure and organic solid wastes.

The validated MFA was used to model different scenarios for the study site. The first scenario was demonstrated that if nutrient management is not improved, wastewater as well as faecal sludge and organic solid waste are expected to double in the year 2020 as compared to that in 2008. The second and third scenario revealed possible strategies to significantly reduce environmental pollution and reuse nutrient sources predicted to be available in the year 2010.

Keywords: Material Flow Analysis (MFA); Monte Carlo simulation; environmental sanitation; Vietnam; developing countries; data uncertainty and scarcity.

9.2 Introduction

The methodology of Material Flow Analysis (MFA) was first applied to quantify the industrial process in the 1990s [236, 237]. It has also been proved to be a suitable instrument for early recognition of environmental problems in developed countries by quantifying material flows in the system and then forecasting the impact of possible interventions on the environment [123, 238-241]. In recent years, this method has been modified to consider the uncertainty in input data sources [242]. As a result, the modified MFA method has also been successfully applied in developing countries that typically face considerable data scarcity and uncertainty problems.

The Pak Kret municipality, Nonthaburi province, Thailand applied MFA in order to assess mitigating measures to maximize nutrient recovery and minimize environmental pollution [243] . Results revealed that creating a wastewater treatment plant and composting solid wastes could reduce nitrogen loading to the environment by 45% and optimize nutrient recovery. Therefore, MFA might be effectively applied during environmental sanitation planning in developing countries.

The city of Kumasi, Ghana was another example of MFA application [244]. The data obtained revealed that private households were a key contributor to the organic material fluxes of nitrogen (N) and phosphorus (P). MFA results confirmed that measures taken at the household level such as appropriate household waste management greatly enhanced resource recovery and environmental protection in Kumasi.

Furthermore, MFA was also useful in quantifying N and P flows in urban areas of Hai Phong city, Vietnam, including five urban districts. The aim was to identify weaknesses related to nutrient management in this region [245].

MFA results demonstrated that appropriate management of human excreta and wastewater from households was needed to mitigate the environmental impacts of these nutrients.

In particular, this MFA methodology was profitably adapted once more by introducing innovative methods to fill data gaps and reduce data inaccuracy. The first test of this ‘adapted MFA’ was in Hanoi, the capital of Vietnam, studying the environmental sanitation system in terms of N and P [246]. Initially, the sensitivity analysis methodology identified parameters that had the biggest impact on model outcomes, which were then needed to be reassessed more accurately using the expert judgment method [247, 248]. The number of primary data required was reduced by using approximate values first (secondary data and assumptions).

In addition, MFA was coupled with quantitative microbial risk assessment and stakeholder analysis to implement the Household-Centred Environmental Sanitation approach, and was used to assess an existing environmental sanitation system and evaluate potential future systems with regard to resource management, water pollution control and microbial health risks. These methods could also be used to identify and involve stakeholders in order to plan demand-responsive environmental sanitation systems. Relationships between the various tools and between the planning approach and the tools were discussed as a basis for their integration [249].

The above 'adapted MFA' was also successfully applied in a multi-provincial area like Thachin River Basin, Thailand, including six provinces and a part of Bangkok. This study provided an overview of the origins and flow paths of the various point- and non-point pollution sources of the entire area in terms of N and P [250-252]. The results showed that aquaculture (as a point source) and rice farming (as a non-point source) was the key nutrient (N and P) sources in this river basin. When simulated and measured nutrient concentrations were compared, retention in the river system appeared to be significant.

While the 'adapted MFA' methodology has been successfully applied in the urban context of Vietnam, its applicability in the rural context has not yet been demonstrated. In addition, environmental sanitation coverage in rural areas of Vietnam is far lower than in cities, resulting in an alarming increase in the level of environmental pollution [253]. The uniqueness of Vietnam rural areas is the close link between environmental sanitation systems and agricultural activities including rice production, husbandry and fish farming. Though the number of rural households with sanitary latrines increased from 52% in 2004 to 67% in 2010, there is still 8% open defecation [254]. In addition, because of the in situ recycling processes, manure waste from pigs, cows and buffaloes has not been estimated thus far. Runoff from paddy fields or vegetable and fruit gardens carries with it a great amount of chemical fertiliser rich in ammonium, nitrate and phosphate, causing the eutrophication of lakes and ponds [76]. This kind of runoff is one of the major pollution sources of the Nhue River [255]. From a reuse perspective, it would be interesting to reclaim nutrients from the above sources for agricultural activities. This would not only reduce river pollution but also improve the cost-benefit ratio for poor farmers.

As can be seen, MFA methodology could serve as a profitable assessment and planning tool for nutrient resource management. This paper is the first investigation of 'adapted MFA' applicability in a typical rural context of Hoang Tay and Nhat Tan communes, Hanam province, Vietnam where data sources are scarce and highly inaccurate though environmental and health risk status is alarming. This research focuses on assessing the suitability of the methodology for illustrating interconnections between the environmental sanitation system and the agricultural system, their impact on the surrounding environment in terms of nutrients N and P and identifying relevant nutrient sources. The current study is part of a large research project designed to assess the environmental and health risks related to wastewater and excreta reuse in the Hanam province in Northern Vietnam [77].

9.3 Methodology

9.3.1 Description of study area

The target site, covering around 8 km^2, includes two neighbouring communes of Hoang Tay and Nhat Tan in the Hanam province, and is located about 60 km south of Hanoi, Northern Vietnam (Figure 3.1). In the year 2008, the population of this site was 16,200, among a total of 4,100 households [256]. These two low-income communes represent typical land use pattern in Northern Vietnam, where residents' houses and water sources are very close to barns and within a limited space.

Residential areas are surrounded by aquaculture and agriculture. Households mostly rely on very rudimentary forms of on-site sanitation facilities, for instance, pit latrines, pour-flush latrines and septic tanks. In addition, human and animal faeces are used as fertiliser for paddy fields, and in some cases, are directly dumped into ponds to feed fish. Grey-water is released without treatment into drainage networks, and is then passed into canals or the Nhue River together with the onsite sanitation system effluent. It is important to note that the Nhue River is one of the three most polluted rivers in Vietnam because it receives wastewater from domestic activities, industries and hospitals in high density urban areas such as the Hanoi Capital [253]. However, the Nhue River is still the main water source for irrigation and fish ponds. As for solid waste, it is not totally uncontrolled but it is poorly controlled.

9.3.2 Methodology

Based on the 'adapted MFA' framework [98], a research flowchart for this study was simplified and represented in three main steps, as shown in Figure 9.1.

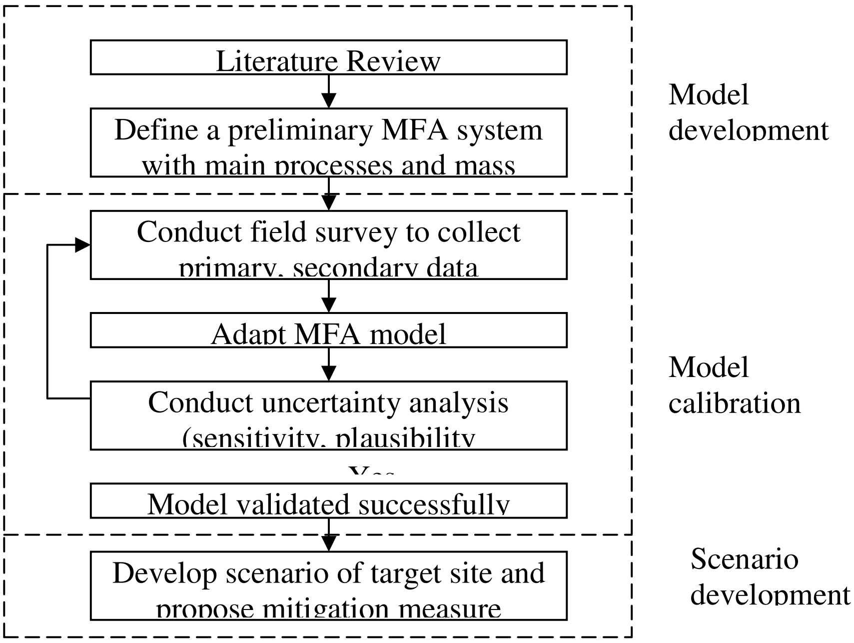


Figure 9.1 Simplified MFA framework (adapted from Montagero, 2006)

Model development

(a) MFA system development

Based on the literature review, information available on the internet, published reports and previous researches, the MFA system used in this study generally describes all local human activities and surrounding environments as processes and describes interlinks among these in terms of indicator (N and P) flows in 2008.

(b) MFA equation development

There are two types of equations in a MFA model [123]. One is the balance equation formulated for each process within the system border on the basis of the law of mass conservation.

$$dM_i^j / dt = \sum_r A_{i,r-j} - \sum_s A_{i,j-s} \qquad (1)$$

Where:

dM_i^j / dt : Stock change rate of substance *i* within process *j* through time *t*.

$\sum_r A_{i,r-j}$: Total of substance *i* from different processes r go into process *j*.

$\sum_s A_{i,j-s}$: Total of substance *i* go out of process *j* through different processes s.

The second is the model equation, which was developed using information from the literature review and short interviews with experts.

$$\sum_r A_{i,r-j} = f(p_1, p_2 \cdots p_n) \qquad (2)$$

Where p_1, p_2,…p_n: parameters represent the variables in the system. These parameters were described as probability distributions and could be replaced by other parameters that are easily estimated or measured (see list of parameters and MFA equations shown in Table 9.1).

Table 9.1 List of necessary data (Hoang Tay and Nhat Tan offices, 2000-2008; Montangero, 2006) and detailed equations in Household (1) process of MFA calibration

Symbol	Description of data	Unit	Statistical distribution	Mean	±	Standard Deviation
n	Number of inhabitants in target site	inhabitants	normal	16,293	±	1,600
rgrey_ST	Ratio greywater to septic tank	%	lognormal	0.1	±	0.10
rgrey_AC	Ratio greywater to aquaculture	%	lognormal	0.5	±	0.10
aHH_RW	Household rainwater consumption	l/cap x day	normal	50	±	10.00
aHH_GW	Household groundwater consumption	l/cap x day	normal	70	±	10.00
CN,RW	N content rainwater	mg/l	lognormal	2.5	±	0.50
CN,GW	N content groundwater	mg/l	lognormal	6.3	±	0.80
aN, kitc_wastes	N load in kitchen waste	gN/cap x day	lognormal	0.8	±	0.20
aN,food	N load food	g/cap x day	normal	6.5	±	0.70
aN_excreta	N load excreta	gN/cap x day	normal	6.1	±	0.60
aN_grey	N load greywater	gN/cap x day	normal	0.4	±	0.05
rN_body_loss	N losses from human body to the air	-	lognormal	0.04	±	0.01

	Description of flow (tonnes N/ year)	**Equation**
dMN(1)/dt	Amount of N storage in the system every year (Stock change rate of Household process)	(AN6-1 + AN16-1 + AN17-1) - (AN1-2 + AN1-3 + AN1-4 + AN1-13 + AN1-17)
AN6-1	N flow from Market process to household process	n x aN,food x 365 x 10^{-6}
AN16-1	N flow from groundwater to household	n x aHH_GW x CN,GW x 365 x 10^{-9}
AN17-1	N flow from rain to household	n x aHH_RW x CN,RW x 365 x 10^{-9}
AN1-2	N flow from household process to on-site sanitation	n x (aN_excreta x 10^{-6} + aN_grey x rgrey_ST x 10^{-9}) x 365
AN1-3	N flow from household process to drainage system	n x aN_grey x (1- rgrey_ST - rgrey_AC) x 365 x 10^{-9}
AN1-4	N flow from household process to “solid wastes collection”	n x aN,kitc_wastes x 365 x 10^{-6}
AN1-13	N flow from household process to aquaculture	n x aN_grey x rgrey_AC x 365 x 10^{-9}
AN1-17	N flow from household process to atmosphere	rN_body_loss x AN6-1

Model calibration

The MFA model was adapted on the basis of both primary and secondary data. Primary data included field observations, questionnaire surveys for local households, interviews with key informants and experts in local, provincial and national governmental agencies, and secondary data included statistics and reports from local offices collected during the field survey. This MFA model was calibrated consequently. Both collected data and estimated results from the model were used in subsequent uncertainty analyses, sensitivity analysis and plausibility assessment.

(a) Sensitivity analysis

Sensitivity analysis was performed to quantify the effect of a 10% increase in each parameter on the simulation result according to the Hanoi case study [247, 248]. This quantification identified the parameters that had a more significant influence than the others. The list of sensitive parameters was then taken into account when, if necessary, conducting further field surveys in designing effective scenarios.

(b) Plausibility assessment

Plausibility assessment was conducted to evaluate the accuracy of simulated MFA results using a list of plausibility criteria that were successfully utilized in many previous studies [257]. One thousand iterations were set to run Monte Carlo simulation using Visual Basic for Applications (VBA, Microsoft Excel, Microsoft Office®). A criterion would pass the assessment if at least 68% of the above generated values were in the corresponding plausibility range [247]. Moreover, for those plausibility criteria that did not pass, the respective sensitive parameters were reassessed by carrying out additional literature reviews or surveys. These steps were repeated until all plausibility criteria passed [247, 248].

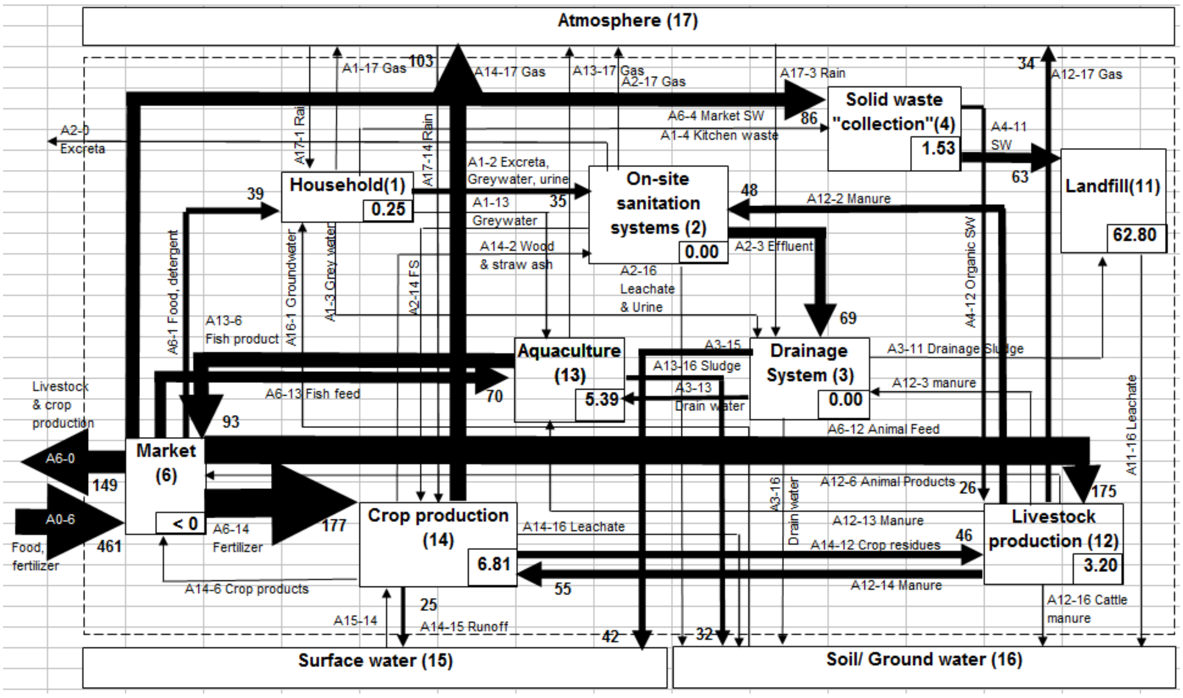
Scenario development

The validated MFA model was used to develop different scenarios for the target site. Since the development strategy for this area was under consideration, Scenario 1 was designed to visualize the local environment in the year 2020. The status of year 2020 was regressed on the basis of the previous nine years of statistical data (2000–2008) [256], i.e. population increase would be 1.16% per year; the number of pigs, cattle and poultry would triple and agriculture farming area and sanitation systems would remain the same as in 2008. Scenario 2 was created as a mitigation measure for 2020, reducing the quantity and improving the quality of wastewater from onsite sanitation systems discharging to drainage systems in these two poor communes. This scenario was based on the recommendation of environmental sanitation experts during the field survey, which included replacing pit latrines and pour-flush latrines with septic tanks; pre-treating grey-water using septic tanks and maintaining the same number of biogas latrines as in 2008. Scenario 3 was also developed as a solution for 2020, which included reusing huge available nutrient sources in drainage water instead of purchasing chemical fertilisers. Accordingly, an assumption of scenario 3 was reduction of the total chemical fertilisers used in 2008 by half, and then directly connecting drainage to paddy fields to reuse the drainage water in the paddy fields.

9.4 Results and discussion

9.4.1 Model development

There were twelve processes, divided into three focus groups: the environmental sanitation system (household, on-site sanitation system, drainage system, solid waste collection, landfill processes), agricultural activities (paddy field, aquaculture, livestock production) and the surrounding environment (Air, Surface water, Soil/groundwater). Moreover, the process ‘Market’ was also included in the MFA, acting as a ‘platform’ for the exchange of goods produced in the target area and distributed to the households in and outside communes, and where imported products such as food and agricultural input (fertilisers) were distributed to the households and agricultural processes [248]. Regarding target indicators N and P, interlinks among these processes were created as shown in Figure 9. 2 a & b.



(a)

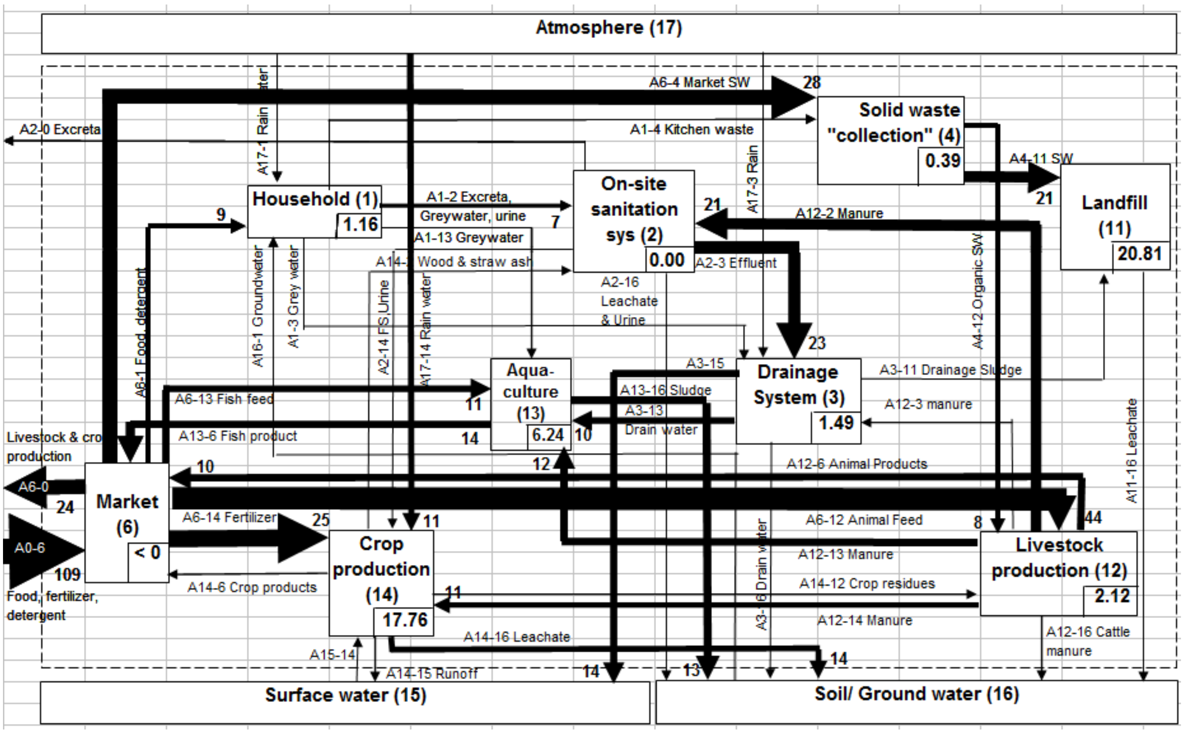


Figure 9.2 a & b. MFA result in 2008 for nitrogen (a) and phosphorus (b) (unit: tonnes/year)

In Figure 9.2 a & b, Boxes and arrows represent processes and nutrient flows, respectively. The wider arrows represent the bigger nutrient flows among processes. The small arrows indicate very little nutrient flows compared to the other. The number in each process box represents the amount of nutrients stocked in that process.

9.4.2 Model calibration

Table 9.1 provides the detailed data collected and model equations used for calibrating the Household (1) process, for instance. After all processes are calibrated, MFA calibration results are shown in Figure 9.2 a & b - the environmental status of the target site in 2008. Boxes and arrows represent processes and nutrient flows, respectively. The wider arrows represent the larger nutrient flows among processes. The black arrows indicate very small nutrient flows compared with the others. The number in each process box indicates the amount of nutrients stocked in that process.

Close interconnections between agricultural activities and the environmental sanitation system and their critical impact on the surrounding environment as well as on nutrient sources were quantified and visualized at this stage. As can be seen in Figure 9.2 a & b, nutrient sources of surface water (the Nhue River) were water runoff from the paddy field process and drainage system, including grey-water from the household process, wastewater after washing pig farms of the livestock process and black-water from the on-site sanitation system process. Moreover, the nutrient load on the atmosphere was estimated on the basis of nitrogen emissions from applied chemical fertilisers, commercial feed for fish or animal manure and urine coming from paddy field, aquaculture, livestock and on-site sanitation system processes, respectively. Nutrient sources to the soil/groundwater environment included leachate from chemical fertilisers used in paddy fields or water from aquaculture, drainage, on-site sanitation systems (pit latrines, pour-flush latrines and septic tanks) or landfills and uncollected cattle manure.

Figure 9.2 a & b illustrates the fact that the market process was the source as well as the destination of almost all large arrows in the system. The market process was considered to be the platform for nutrient supply of the target site as well as a nutrient exchange to other sites even outside the system. The main annual nutrient sources of the system through Market processes were chemical fertilisers for paddies and commercial food supplied for fish and animals (461±76 tonnes of N and 109±42 tonnes of P). In addition, products from aquaculture, livestock and paddy field process were nutrient sources which were distributed inside or exported outside the area annually (149±52 tonnes of N and 24±7 tonnes of P).

On the other hand, there were significant nutrient sources generated by the whole system every year (214±56 tonnes of N and 58±16 tonnes of P) in the form of wastewater, organic solid wastes or faecal sludge. Fifty-three percent of all N and 50% of all P came from pig manure (112±23 tonnes of N and 42±5 tonnes of P). In addition, Figure 9.2 a & b also indicates the significant connections between Livestock and Paddy field were supplying manure for paddy fields (55±12 tonnes of N, 11±4 tonnes of P per year) and consuming residues (straw, vegetables, etc.) to feed pigs and poultries (46±16 tonnes of N, 5±1 of P per year). The pig manure (48±6 tonnes of N and 21±2 tonnes of P every year) represented 58% of all N and 75% of all P sources for the on-site sanitation system.

The impact of agricultural activities and the environmental sanitation system on the surrounding environment in terms of nutrients is also shown in Figure 9.2 a & b.

Regarding the atmosphere, agricultural activities were the main emission source. Agriculture annually contributed 147±54 tonnes of N, equal to 85% of all N emissions from the entire system, of which 64% was from applied chemical fertiliser and 21% was from animal manure. Nitrogen produced as a result of burning solid wastes in the solid wastes collections process or evaporation of water in the drainage system process was not included in the calculations.

Concerning the Nhue River surface water environment, the environmental sanitation system was the main nutrient source. The on-site sanitation system discharges 69±6 tonnes of N and 23±4 tonnes of P to the drainage system every year. This figure accounted for 93% of all N and 85% of all P in drainage water. In addition, 42±7 tonnes of N and 14±5 tonnes of P in drainage effluence reached the Nhue River each year. The other huge nutrient source of surface water was in runoff from paddy fields, which annually contributed 25±3 tonnes of N originating from applied chemical fertiliser.

Finally, with regard to the soil/ground water environment, key factors were P accumulated in sludge from the Aquaculture system (32±3 tonnes of N and 13±2 tonnes of P) or P from applied chemical fertiliser accumulating in paddy fields (14±2 tonnes of P) every year. For that reason, soil/ground water received 32±3 tonnes of N and 27±4 tonnes of P yearly from agriculture systems alone. Thus, the impact of agricultural activities and the environmental sanitation system on the atmosphere and surface water were far greater than on soil/ground water.

9.4.3 Sensitivity analysis

Because of the considerable impact of agricultural activities and the environmental sanitation system on surface water and the complexity of the procedure used to quantify nutrient flows, sensitivity analysis was applied in surface water and drainage system simulation processes. Table 9.2 presents a list of sensitive parameters which have a significant impact on the quantity of nutrients entering the drainage system and surface water.

As can be seen in Table 9.2, the amount of chemical fertiliser applied and the area of paddy fields had the largest effect on nutrient flows to the surface water. With an increase of 10% in each parameter, the total quantities of N (or P) entering the surface water grew by 3.75% (or 8.95%) and 3.35% (or 7.10%), respectively.

Table 9.2 Effect of 10% parameter increase on nutrient flow to drainage system, surface water

Parameter	**% of total nutrient to *drainage system change***		**% of total nutrient to *surface water change***	
	N	P	N	P
Population	+ 5.92	+ 4.12	+ 0.48	+ 0.50
Area of paddy fields	*na**	*na*	+ 3.35	+ 7.10
Number of pigs	*na*	*na*	+ 1.31	+ 6.00
Amount of applied chemical fertiliser	*na*	*na*	+ 3.75	+ 8.95
Ratio of households equipped with				
septic tank	+ 0.30	+ 2.13	+ 0.83	+ 3.25
pour-flush latrine	+ 0.10	+ 0.98	+ 0.48	+ 3.20
pit latrine	+ 0.09	+ 1.00	+ 0.78	+ 3.00
biogas	+ 6.85	+ 2.12	+ 1.25	+ 3.50
Faecal sludge emptying frequency factor for				
septic tank	- 3.98	- 10.00	- 2.12	- 8.23
pour-flush latrine	- 4.25	- 10.25	- 1.75	- 7.15
pit latrine	- 7.00	- 10.00	- 4.30	- 7.00
biogas	- 8.00	- 11.49	- 5.98	- 9.14

*na** *: not available*

In Table 9.2, Positive (+) or negative (-) in front of value represents increase or decrease, respectively of total nutrients to the drainage system or surface water when increasing each parameter by 10% while keeping other parameters constant.

Furthermore, Table 9.2 indicates that the ratio of households equipped with different types of latrines was also a critical parameter. Among four types of on-site sanitation equipment, biogas latrines had the greatest impact on nutrient flows to surface water and drainage systems. If the number of biogas latrines increased by 10%, then the total amount of N or P released to the surface increased by 1.25% or 3.50%, respectively, and to the drainage system increased by 6.85% or 2.12%, respectively. On the other hand, the faecal sludge emptying frequency factor of latrines played an important role in reducing nutrient quantities to surface water and drainage systems. When the emptying frequency factor of biogas latrines increased by 10%, there was a decrease in total nutrients released into the drainage systems (8.00% of N and 11.49% of P) or the surface water (5.98% of N and 9.14% of P).

9.4.4 Plausibility assessment

Figure 9.3 shows the plausibility assessment results from Criterion 1, which was created on the basis of the assumption that there was no N stock within the Household process (1). However, the N stock change rate ($dM^{(1)}_N/dt$) should be in a range 0±15% of total N sources to this process [98]: $dM^{(1)}_N/dt = 0 \pm 15\%$ (Criterion 1).

Given the collected criterion, the N stock change rate of the Household process ($dM^{(1)}_N/dt$) was calculated on the basis of the law of mass conservation (Table 9.1). After running the Monte Carlo simulation, the obtained values of this stock change rate were demonstrated in Figure 9.3. Values in the marked range represent values in the range 0±6.45 tonnes of N (equals to 15% of total N input to Households). Therefore, 86% of obtained values were in range of Criterion 1. Hence, the MFA model passed this criterion. The other eleven criteria used to assess plausibility of MFA simulation outcome also passed. As a result, the MFA model was considered to be validated.

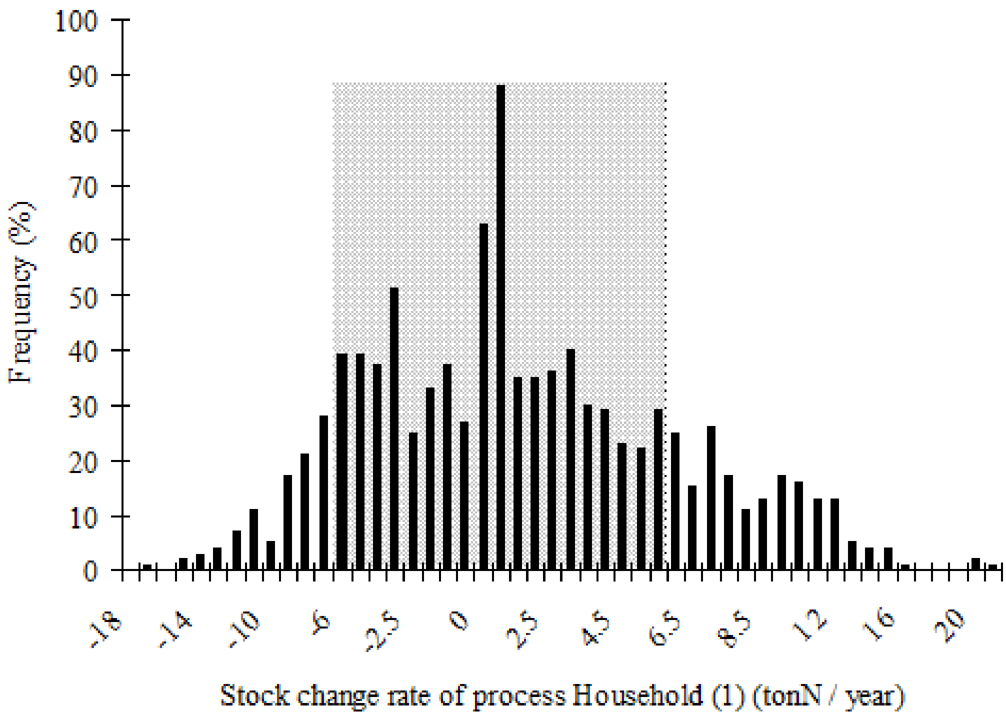


Figure 9.3 Plausibility assessment results from Criterion 1

In Figure 9.3, Model outcomes are illustrated as frequency histogram. Eighty-six percent of these generated values within the plausible range of stock change rate in Household (1) process.

9.4.5 Scenario development

Figure 9.4 shows the different scenarios for nutrient flows releasing to drainage systems, surface water or nutrient quantities in the sludge and solid waste for the simulated year (2020) compared with the current status of 2008.

Scenario 1 demonstrates that nutrient flows releasing to drainage systems or surface water, nutrients in sludge and organic solid waste would almost double in 2020. Furthermore, the total wastewater to drainage systems and surface water in 2020 was estimated to contain 374±45 tonnes of N and 118±15 tonnes of P. The amount of N was twofold and that of P was fivefold compared to amounts of applied chemical fertiliser for 2008. Therefore, in 2020, with the same paddy field area of 2008, this wastewater would contain enough nutrients to fertilize rich paddies without adding more chemical fertilisers. Moreover, other large nutrient sources from sludge and organic solid wastes (25±4 tonnes of N and 14±5 tonnes of P) should be considered as well.

Scenarios 2 and 3 are proposed solutions for the year 2020. Mitigation measures in scenario 2 would reduce the N and P discharged into drainage systems or surface water by nearly 50% and also decreased the N and P from sludge and organic solid waste by 32% and 43%, respectively. On the other hand, the load of N and P to drainage systems and surface water were still higher than the artificial fertiliser annually needed for paddy fertilisation. Therefore, even when the environmental sanitation system was improved as in scenario 2, nutrient sources would still be available for use in paddy fields. Based on assumptions in scenario 3, 50% of N and P in wastewater discharging to drainage systems would be reused as nutrient sources for paddy fields and chemical fertilisers applied would be reduced by half. Amount of nutrients releasing to surface water would accordingly decrease to 27% and 15% in terms of N and P, respectively.

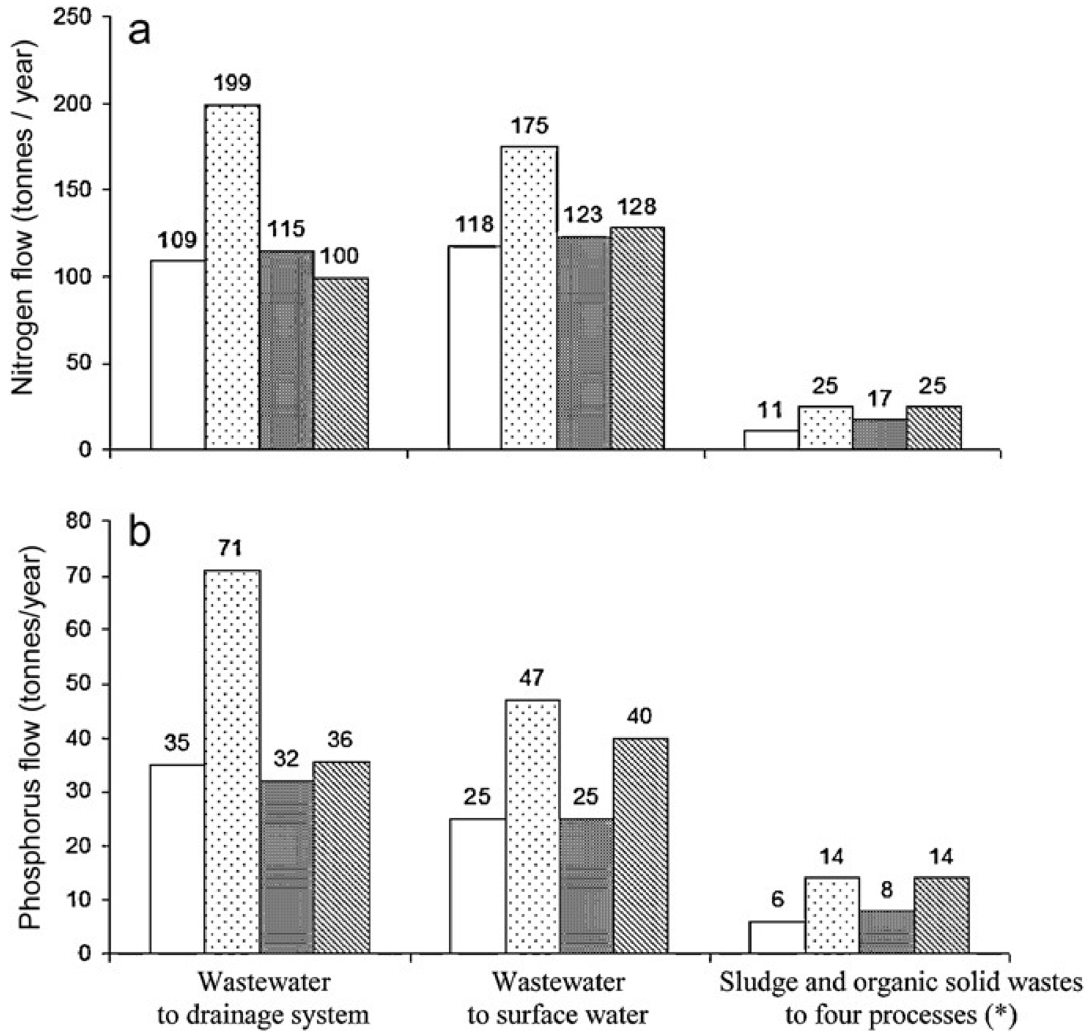


Figure 9.4 Nitrogen (a) and phosphorus (b) flows in wastewater to the drainage system and to surface water and in sludge and organic solid waste to four processes.

In Figure 9.4, white bar represents status quo (2008); spotted bar represents forecasting situation of study site in year 2020 (Scenario 1); grey bar represents environmental sanitation solution for environmental status of year 2020 (Scenario 2); striped bar represents reduce and reuse solution for year 2020 (Scenario 3). Arterisk (*) indicates that four process referred are on-site sanitation system, drainage system, solid waste collection and aquaculture.

9.5 Conclusions and recommendations

This research proves that the adapted MFA method is suitable to quantify nutrient flows in an area clearly faced with data uncertainty and scarcity. Because of the data specificity of developing countries, secondary data was used mainly as input data. By describing parameters as probability distributions instead of discrete data, the uncertainty of both parameters and outcome data could be assessed by its potential variation. Moreover, the developed mathematical model could be used to successfully quantify nutrient flows among environmental sanitation and agriculture systems and to assess the impact of these processes on the surrounding environment by conducting plausibility and sensitivity analyses.

This study is also the first fruitful investigation of 'adapted MFA' in a representative rural area in Vietnam. MFA simulation results pointed out critical control sources of nutrients in Hoang Tay and Nhat Tan communes, Hanam province, with overuse of chemical fertilisers in paddy fields, uncontrolled solid waste such as faecal and fish pond sludge, organic solid wastes and on-site sanitation system effluents. Consequently, options for nutrient resource management could be proposed, such as waste materials could be reused as fertilisers in agriculture and on-site sanitation technologies could be further developed and greatly improved. On the other hand, sustainable sanitation must consider the potential health impact of applying wastes, particularly human wastes. Untreated sewage sludge in manually worked rice paddies is potentially serious health hazard. Therefore, pre-treating organic solid wastes, like composting, should be done carefully at the household level.

In short, applying MFA as a part of environmental sanitation planning allows decision makers to identify potential problems and simulate the impact of remediation measures on resource consumption and environmental pollution in an integrated way. Suitable environmental sanitation options may thus be chosen by taking into account nutrient supply on the one hand and nutrient demand for food production on the other.

9.6 Acknowledgements

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10. DISCUSSION

The use of waste water and animal and human excreta has a benefit in agricultural production in developing countries. However, it is well known that there are at the same time clear health and environmental risk associated with these practices. With the research conducted in this PhD thesis we wanted to assess in detail today's health and environmental impacts associated with these cultivation practices in order to develop and target intervention to mitigate these negative effects.

We used epidemiological approaches to quantify the extend of intestinal parasitic infections and incidence of diarrhoeal episodes and identify risk factors in particular those related to waster and excreta reuse in an agricultural community in Northern Vietnam, where wastewater and excreta are commonly used (Chapter 5, 6 & 7). Second, we applied QMRA methodology to quantify the infection risks of diarrhoea related to pathogens in wastewater and excreta use in agriculture (Chapter 8). Third, we employed a MFA approach to establish interconnections between the environmental sanitation and agricultural systems and by quantifying the discharge of nutrients N and P to the environment (Chapter 9). In this chapter the main findings of our investigations are summarised and discussed and we will draw links between methods used in this study to propose future integrative assessment of epidemiology, microbial risk assessment and material flow analysis.

10.1 Epidemiological studies

10.1.1 Intestinal parasitic infections associated with wastewater and excreta reuse

The risk of transmission of helminth infection with wastewater and excreta reuse in agriculture is well known and has been described in many parts of the world. The practice, however, is particularly frequent in Asia in general and rural and peri-urban Vietnam in particular. The concrete transmission risk and routes of intestinal parasitic infection in today's high risk areas are insufficiently known.

The results from two cross-sectional surveys indicated that helminth infections were highly prevalent: almost half of the studied individuals had a helminth infection (47%), 24% had *A. lumbricoides* and 40% *T. trichiura* infection. This result was higher than a finding of 39% in another study in peri-urban Hanoi [55], but lower than the estimates for the whole country, as well as the Red River Delta region [71] and other studies conducted in different areas of Northern Vietnam at 53-82% [131, 132, 135-137, 139, 147]. It is noted that our 2% hookworm infection rate is much lower than in other places in Vietnam, where it varied between 11 and 26% [55, 71, 139]. Thus, the prevalence of helminth infections varied widely in different study areas in Vietnam, perhaps due to the differences in geographical areas, environmental sanitation conditions or demographic differences in participants (i.e. sex, age groups, occupation, and education). Furthermore, climatic conditions, type of soil and crop, SES and human hygiene behaviour are important factors including the ant-helminthic treatment practices [148].

Our study demonstrated that increased risk for STHs, in particular *A. lumbricoides* and *T. trichiura* infections, was associated with use of human excreta as fertiliser in the fields and direct contact with Nhue River water. We confirm results from earlier studies on excreta [130] and waste water [34, 56, 153] use and infection risks. Widespread use of human excreta in the fields [21, 179], create favourable conditions for helminth transmission. However, wastewater use was not associated with increased helminth transmission in peri-urban Hanoi and Nam Dinh province [55, 139]. The concentration of helminth eggs in irrigation water, and the intensity and period of direct contact can explain the observed differences [152]. Furthermore, frequent contacts with irrigation water might increase the risk of helminth infection. Indeed, a study in Hanoi indicated that people who frequently had contact with irrigation water throughout the year rather than seasonally had a higher risk of infection with *T. trichiura* [55].

As mentioned above, STHs was associated with agricultural field practices involving contact with Nhue River or use of human excreta. However, these practices are not relevant for the transmission of *E. histolytica* (Chapter 6). Although *E. histolytica* cysts are resistant, they become nonviable in human excreta within a short time period of composting. Protozoan cysts, including those of *G. lamblia* and *E. histolytica*, are likely to survive more than 10 days in soil as they are susceptible to desiccation [30]. It is noteworthy that in the study area, all animal and human excreta are stored or composted more or less before being brought to the fields, but the time period and conditions of storage and composting often do not fully conform with safety indications and regulations [179]. However, they are sufficient to eliminate an important portion of the infectious agents, including *E. histolytica* cysts [20, 30]. Moreover, people who handle excreta are more likely to use personal protective measures and wash their hands with soap after work. In our study area, the Nhue River provides a good opportunity being situated next to the agricultural land. Indeed, 96% of those handling animal excreta washed their hands after work, compared to 61% of those who did not handle animal excreta.

10.1.2 Diarrhoeal episodes associated with wastewater and excreta reuse

We assessed diarrhoeal diseases episodes in adult farmers and associated them with exposures to different risky agricultural practices. We identified an incidence rate of diarrhoea in adults of 0.28 episodes pppy, which is a similar magnitude incidence observed in Hanoi (0.28 episodes pppy) [61]. Our incidence was higher than what was reported for children in Northern Ghana (0.10 episodes pppy) and urban and suburban Malaysia (0.24 episodes pppy) [258, 259] however much lower than the global estimated incidence of diarrhoea for developing regions, ranging from 0.40 to 0.60 diarrhoea episodes pppy [216].

Our results may have been affected by under-reporting of diarrhoeal episodes because of the unwillingness and attitudes of some subjects to participate in the study. We also observed that people often self-treated for diarrhoea using traditional medicine at home (45%). Self-medication is very common in Vietnam. People avoid time and costs of health services visits [260]. Moreover, diarrhoeal disease episodes are perceived as a personal issue which is not easily shared with others, even in a study setting. Therefore, it is most likely that we underestimated the true incidence rate of diarrhoeal episodes in this setting.

The incidence rate of diarrhoea was lower in the dry season than in the rainy season (peak in August). This is in contrast to the other studies, which found that the diarrhoeal incidence did not differ much between two seasons, although the diarrhoeal episodes were more frequent in the dry and cool season [61]. We can explain our findings with the fact that in the rainy season in the study sites people usually handle human excreta and are therefore more potentially exposed to diarrhoeal causing pathogens.

Among the examined exposures we identified the direct contact with wastewater (Nhue River and local pond) and exposure to human and animal excreta (Chapter 7) as important risk factors for diarrhoeal episodes. Our results are in line with previous studies, which found that people who were exposed to wastewater had a higher risk of diarrhoeal disease [48, 50, 51, 61]. However, our study is one of the few studies to report a significant association between direct contact with wastewater and the risk of diarrhoea in adult farmers [48, 61]. In addition, we could attribute 27% and 14% of the episodes to exposure to Nhue River and local pond water, respectively. In a study in Hanoi 35% of diarrhoeal episodes were attributed to wastewater exposures [61]. The difference could be explained by the quality of the wastewater. It must be noted that our study area is at a considerable distance from Hanoi and important agglomerations (60 km) where substantial contamination takes place. Hence, the concentration of infectious agents is much lower to diluting effects [180].

An important result of our study was that adequate treatment of human excreta before use as fertiliser (i.e., composting time longer than 3 months) could decrease diarrhoeal episodes by 51%. This finding indicated that safe composting of human excreta should be intensively promoted in agricultural settings. As indicated by Jensen and colleagues [158], approximate compost duration of 3-4 months under the conditions of high pH and temperature and low moisture could provide a safe compost product for application in the fields. Such composting practices destroy enteric pathogens. Furthermore, direct contact with composted human excreta during application in the fields accounted for 7% of diarrhoeal episodes, whereas 36% were attributable to animal excreta handling. In our study area, pigs play an important role in the agricultural system, especially in integrated pig-fish farms [224]. Pig manure is discharged without treatment into the drainage system, which interconnects to the irrigation channels [192]. It is known that animals such as cattle and pigs are the major reservoir of pathogenic *Escherichia coli* [83, 97]. Therefore, the farmers with a higher exposure to animal excreta are at higher risk of infection. On-site sanitation systems, including facilities for treatment of human and animal excreta, could be an adequate and low cost intervention to reduce diarrhoeal risks associated with excreta use.

10.1.3 Other risk factors for intestinal parasitic infections and diarrhoea

Besides exposures to wastewater and excreta, other risk factors for intestinal parasitic infections and diarrhoeal diseases were identified (Chapter 5, 6 &7).

Firstly, the use of a tap water source in the household was an important protective factor against helminth infections. Other studies, e.g. from Ethiopia have also documented the protective importance of tap water [159]. We observed that boiled water was used for drinking in almost all households. Nevertheless, the use of rainwater as drinking water was significantly associated with increased diarrhoeal risk, and accounted for 77% of diarrhoeal episodes. Franklin and colleagues [261] made the same observation and noted that rainwater tanks may increase the risk of water-borne disease outbreaks, unless they are appropriate maintained. We observed during household visits that the roofs and gutters where rainwater was collected were covered with a sludge layer, which may be a favourable condition for the growth of mico-organisms. Furthermore, most rainwater tanks did not have lids or were frequently not covered, and dust, leaves, and faeces from domestic animals contaminate the tank water. The association between the quality of rainwater and the risk of diarrhoea should further be investigated.

Secondly, the consumption of raw vegetables was also associated with increased risk for diarrhoea. It may be interpreted that the vegetables were grown in fields irrigated with contaminated wastewater, as indicated by high concentrations of thermotolerant coliform and the presence of protozoan parasites [211]. Also in our study consumption leftover of food from the precedent day was not associated with diarrhoeal episodes which have been seen in some previous studies [112, 197]. In our households leftover food was typically heated before consumption, and thereby reduced health risks.

Thirdly, not using protective measures (e.g. gloves, boots and face mask) during field work was common in our study area. It was indeed associated with an increased diarrhoeal risk. The farmers felt that use of protective measures was uncomfortable and constrained work [133]. When working with wastewater, women used protective measures more often than men. Typically women spend more time in the fields than men and paid more attention to their health [106]. Our results suggest that wearing protective equipment (i.e., gloves, etc) while doing field work reduces 78% the risk of diarrhoea.

Finally, contaminated hands play a central major role in pathogen transmission. We could show a more than three-fold increase for an *E. histolytica* infection when hands were not washed properly. Personal hygiene practices, including washing hands with soap, has proven to be an important factor in reducing infectious disease transmission and can reduce diarrhoea by 42-47% [61, 160]. Our study indicated a reduction of 51% of diarrhoeal episodes in adults and once more provides sound evidence of the major importance of proper hand washing. Earlier studies have documented its benefits [209] [262-265]. Therefore, sound hand-washing promotion programs must be an essential public health activity in agricultural communities where wastewater and excreta are intensively used.

10.1.4 Methodological considerations

We conducted observational studies and assessed outcomes, i.e., intestinal parasitic infections and diarrhoeal episodes, and various exposures at the individual, household and the field level. We employed a most rigorous diagnosis of intestinal parasitic infections and used a multiple stool samples (two stool samples per person) per person and multiple diagnostic tests (e.g. Kato-Katz, and FECT) per examined stool. This approach showed a high diagnostic sensitivity [266] taking into account variability of eggs and cyst shedding pattern between days and within a stool samples.

Interviews with questionnaires were used to measure exposures to wastewater, human and animal excreta and other potential risk factors. It is known that questionnaire assessments are associated with considerable recall and reporting bias. Therefore, there is a considerable uncertainty associated with these measures. We address these challenges by thoroughly validating the questionnaire prior to field use. Furthermore, we have extensively trained field workers to adhere to a standardized questioning procedure and monitored the interviews in order to keep the inter-observer variations as little as possible. Despite these efforts we can not exclude uncertainties associated with these measures.

A further challenge was the amount of exposures assessed during questionnaires. In order to keep the questionnaire on enrolled participants at an acceptable time limit, we used a household questionnaire to measure general characteristics. Clearly not every household member experiences exactly the same exposures. However, we restricted the household questionnaire to general characteristics which most likely possess a rather limited variation within a household. Nonetheless, we can not rule out some misclassifications of exposures, in particular for exposure to excreta. Therefore, the results of dose-response associations must be interpreted with caution. E.g. it is known that STHs tend to cluster in certain individuals within households and certain households within communities [209]. In our study, for addressing this issue, the GEE method was used in both uni- and multivariable models to adjust for intra-correlation within a household [143].

As a start we used cross-sectional surveys. They have a clear advantage of a relative quick and cheap study design [267]. However, in cross-sectional study the temporal sequence between exposure and disease can not be assessed which is a cause of frequent difficulties in the interpretation of the results [110]. We have addressed this issue by conducting a cohort study. Incident diarrhoeal episodes were our outcomes of interest. Therefore, all assessed exposures were prior disease onset [110].

The nested case-control study design has also other major advantages. Exposures, in particular those related to agricultural activities were highly seasonal. It is only this nested case-control on diarrhoeal disease which was able to address the changing exposures.

Nevertheless, for household information such as household SES, water source, latrine use and sanitary condition, general human and animal excreta use, it was not convenient to collect the information repetitively during the household visits, which was considered as a nuisance to a number of participants. Therefore the household information was only collected once during the baseline cross-sectional surveys and applied to all individuals living in the same families for the analysis of risk factors for the disease outcomes with the individual as the analysis unit. The household variables were used under the assumption that they remained unchanged throughout the cohort study period.

In the cohort study, a considerable number of participants were lost to follow-up or suspended their participations. Toward the end of the follow-up a number of participants were tired of weekly reporting and/or refused to report their health status. This might have resulted in an overall lower number of diarrhoeal episodes reported towards the end of the cohort period and might have also contributed to marked seasonal diarrhoeal peak in the rainy season, in which the study was started. In general did farmers not consider enteric diseases as a serious ill-health. Auto-medication is a common practice in this farmer community which might have additionally led to an underreporting of diarrhoeal episodes.

10.2 Quantitative Microbial Risk Assessment

The QMRA provided complementary risk estimation for selected pathogen infection for specific exposure points in the environmental sanitation and agricultural systems (Chapter 8). The examined hazardous exposure points were harvesting vegetables in Nhue River, growing rice, fishing in the local ponds, cleaning the household sewage and application of excreta in the field. All exposure scenarios estimated annual infection risks at a much higher than the allowed thresholds of 10^{-4} (< 1 infection per 10,000 individuals) [233]. The highest annual risks of infection by DEC, *G. lamblia* and *C. parvum* were observed for fishing in the local ponds exposures. The lowest annual risks of infection by DEC and *C. parvum* were at the scenario of application of excreta in the fields and for *G. lamblia* at the scenario of growing rice. In general, the annual risks of infection by DEC and *G. lamblia* were higher than those by *C. parvum* (Chapter 8). In most cases, variation of the concentrations of pathogens in the different exposure points had a significant impact on the uncertainty of the estimated annual risk of infections.

One of the original points of this study is that we integrated risk of considered exposure infection risk into the risk of disease. Thus QMRA annual risks of diarrhoea due to pathogens are higher than the allowed threshold of 0.001 pppy of waterborne disease set by the WHO [83]. The annual risks of diarrhoea fluctuated for DEC between 0.003 and 0.24 pppy, for *G. lamblia* between 0.13 - 0.50 pppy and for *C. parvum* between 0.004 - 0.15 pppy. For all the exposures to wastewater, the risk of diarrhoeal diseases from pathogens was highest in the scenario of fishing in the local ponds. It seems that people exposed while harvesting fish in the local ponds had a greater risk of diarrhoea than the other exposure scenarios.

In combining all the exposure scenarios, the estimated annual risks of diarrhoea from *G. lamblia* was 0.66 pppy, DEC was 0.25 pppy, *C. parvum* was 0.23 pppy. The combined annual risk of diarrhoea due to all three pathogens was 0.80 pppy (Chapter 8). This finding is similar to the diarrhoeal disease incidence in developing regions (for all ages, 0.8 - 1.3 pppy) suggested by WHO [31]. The result is also comparable with the reported cases of diarrhoea in our study area of 0.28 pppy (Chapter 7). The estimated risk of diarrhoea for all three pathogens was approximately 3-fold, and due to *G. lamblia* 2.4-fold, greater than the reported cases of diarrhoea. Whereas, the estimated risk for DEC and *C. parvum* were slightly lower than those of reported cases of diarrhoea. It may be possible that DEC is the main etiological agent of diarrhoea in Vietnam. As indicated by Trang and colleagues, more bacterial pathogens, especially DEC, caused diarrhoea in people exposed to wastewater than in unexposed people [61]. Alternatively, it may be that assumptions in our study have more accurately modelled DEC. The estimated annual risks of diarrhoea for *G. lamblia* and all pathogens were higher than reported cases of diarrhoea. Several points are important regarding this result. First, it is important to note that the reported incidence cases of diarrhoea present only in adults aged 16-65 years, who are mainly working in the area of agriculture. It could be that the farmers working with wastewater and excreta, possibly even carrying pathogens, may be protected because of the repeated exposure resulting in high immunity levels against common enteropathogens [10]. Second, as we mentioned in the above section, the epidemiological studies may under-report cases of diarrhoea. Third, the QMRA may overestimate the risks of infection. It is noted that although QMRA is being applied widely in different fields to assess health risk, this method still has several limitations. For instance the dose-response models (e.g., Exponential and Beta-Poisson model) for almost all pathogens were developed and validated in developed countries, which may not be accurate when applying it in developing countries [268, 269]. Typically the QMRA model has been developed using data from healthy adults in developed countries who have been exposed to various doses of micro-organisms [268]. Moreover, the current status of the art of QMRA is that this risk assessment method does not yet account for other factors that could influence the infection such as degree of immunity of targeted people, or considering the distribution of infection over time due to the initial exposure [270]. Other issues with QMRA are related to the data availability on exposure assessment in developing countries. For instance, the assumptions on the volume of wastewater ingestions when exposed to water or quantity of food consumption are usually used from studies conducted in developed countries. For further application of QMRA in the developing world, local data on exposure need to be generated.

We observe a lack of agreement between the QMRA estimated diarrhoea incidence and reported episodes of diarrhoea in the cohort study. We conclude that the QMRA model needs to be adjusted developing countries. In particular research is needed to produce modified factors to incorporate the immunological differences between healthy adults in developed and undeveloped countries. In addition there is often a wide range of population groups with different immunity levels and contacts with the wastewater and excreta. This makes it impractical to integrate the varied health risks to produce an overall community level risk [219].

The first task in any health risk assessment is to establish the maximum tolerable additional disease burden (i.e. DALY loss pppy) [271]. In the 2006 WHO guidelines for the safe use of wastewater, grey-water and excreta in agriculture and aquaculture (third edition), the recommended reference level of health target was 10^{-6} DALY loss (≤ 1 DALY/million persons) [83]. Based on the QMRA results of this study, the estimated burden of diarrhoea fluctuated between 45 x 10^{-6} and 12,000 x 10^{-6} DALY loss from different exposure routes, and it was much greater than the tolerable level set by the WHO. The highest burden of diarrhoea was in the scenario of growing rice, followed by fishing in the local pond, application of excreta in the fields, harvesting vegetables in Nhue River and cleaning household sewage. As proposed by Mara 2011, the level of tolerable additional burden of disease is ≤ 10^{-4} DALY loss pppy [271]. The burden of diarrhoea due to pathogens at different exposure scenarios in our study was still higher at least 0.45-fold than the acceptable level proposed by Mara. This result suggests the importance of reducing concentrations of pathogens in wastewater and excreta before use in agriculture as a means of reducing the risk of diarrhoea.

10.3 Material flow analysis

In our study we applied an adapted MFA methodology to the environmental sanitation and agricultural systems with the emphasis on nutrient flows of N and P. After understanding and describing the N and P nutrients fluxes in study area, the CCPs related to environmental pollution caused by nutrients were identified. The system was divided into nine processes including: agriculture, aquaculture, livestock, market, solid waste, landfill, on-site sanitation system, drainage system and household (Chapter 9). The main source of nutrient N and P inputs into the system are chemical fertiliser and commercial fodder for fish and animals. In addition, the major nutrient N and P source affecting the surface water, soil and groundwater originates from households through the discharge of effluents of on-site sanitation systems, faecal sludge, animal manure, solid waste and N and P leaching or accumulating into soil, ground and surface water.

Analysis of simulation results revealed that the critical control sources of nutrients in an agricultural community were identified as follow: First, nutrients N and P in fertiliser supplied to agriculture were high and the use of chemical fertiliser was predominant over organic fertiliser. The extended use of artificial fertiliser in agriculture causes nutrients' accumulation in soil and water, pollution of surface and ground water and others. Second, with poor household sanitation facilities and drainage systems in the study area, a large amount of nutrients delivered to the households in the form of food is ultimately discharged with the excreta, wastewater and solid waste into water bodies or directly on the soil, resulting in pollution of water and soil.

Agriculture is a key component of the system, representing a main income source for the farmers, but as we could show also an important nutrient discharge source. It is important to develop a strategy to control and mitigate environmental pollution caused by agricultural activities. The agricultural process can significantly contribute to environmental protection though its sustainable development by reducing N and P discharge into the environment and through reusing of excreta, sludge and organic solid waste as fertilisers.

Related to on-site sanitation facilities, the replacement of poorly designed and managed pour-flush latrines and pit latrines by septic tanks in households could considerably reduce the amount of nutrients discharged to water bodies and soil. Another alternative would be to introduce urine diversion latrines in households and to promote the reuse of urine and faeces as fertiliser. On-site sanitation systems to reduce the discharge into the drainage system, and subsequently the water bodies, can also be considered. And improvement of the drainage system can also help to optimize nutrient management. Indeed, by redirecting drainage channels to agricultural areas rather the water bodies, nutrient loads to surface water could be significantly reduced, while chemical fertiliser needs for agricultural production could be substantially reduced.

The mitigation measure by 2020, for example, replacing pit latrines and pour flush latrines with septic tanks, pre-treating grey-water using septic tanks and reusing the drainage water in the paddy fields. This mitigation measure would reduce nearly 50% of the N and P discharged into drainage systems or surface water and also decrease 32% of the N and 43% of the P from sludge and organic solid wastes. The load of N and P to drainage systems and surface water were still higher than the artificial fertiliser annually needed for paddy fertilisation. Therefore, nutrient sources would still be available for use in paddy fields. Moreover, 50% N and P in wastewater discharging into drainage systems would be reused as nutrient source for paddy field and chemical fertilisers applied would be reduced by half.

The options for nutrient management could be proposed, such as waste materials could be reused as fertilisers in agriculture and on-site sanitation technologies could be further developed and improved. Treatment of organic solid wastes, like composting of human excreta should be done carefully at the household level. It is recommended to discuss adaptation in environmental sanitation and agricultural systems, contributing to a better balance between nutrient demand and supply and thus helping to close the nutrient cycle as well as mitigate public health risks.

10.4 Summary of the key findings of the work

- Prevalence rates of helminth infections among people living and working in the agricultural communities were high (i.e., any helminth infections 47%, *A. lumbricoides* 24%, and *T. trichiura* 40%), and were within the range of rates reported in previous studies in Vietnam.
- Helminth infections, especially *A. lumbricoides* and *T. trichiura* were significantly associated with exposure to wastewater (Nhue River water) and use of human excreta as fertiliser in agricultural fields. These factors were not associated with *E. histolytica* infection in the study area. *E. histolytica* infection was associated with close contact with domestic animals.
- Incidence rate of diarrhoeal disease in adults was 0.28 pppy, which is lower than the general estimates for age over five years in developing countries (0.4-0.6 pppy).
- The risks of diarrhoeal diseases were associated with wastewater and excreta use in agriculture, in particular direct contact with wastewater (Nhue River and local ponds) and human and animal excreta while doing work in the fields. The associated attributable fractions were considerable and worthy targets for public heath interventions.
- Personal hygiene practices such as use of protective measures (gloves, boots, face mask) during fieldwork and washing of hands with soap, as well as consumption of clean water for drinking and cleaning of vegetables were important protective factors against diarrhoeal diseases and intestinal protozoa infections. They should be intensively promoted in mitigating public health programs.
- In the environmental sanitation and agricultural systems the hazardous exposures identified were exposures to Nhue River water while harvesting vegetables and growing rice in the fields, fishing in the local ponds, cleaning household sewage and application of excreta in the fields.

- QMRA simulation models predicted annual risks of infection for DEC, *G. lamblia* and *C. parvum* which were greater than the commonly proposed thresholds of 10^{-4} (< 1 infection per 10,000 individuals).
- The predicted annual risks of diarrhoeal diseases from all the exposure scenarios for *G. lamblia* was 0.66 pppy, DEC (0.25 pppy), *C. parvum* (0.23 pppy) and all three pathogens combined (0.80 pppy).
- MFA simulation results indicated critical control nutrients sources in the study area, with uncontrolled on-site sanitation system effluents and faecal and sludge and chemical fertiliser overuse in paddy fields. If nutrient management is not improved, levels of nutrients due to wastewater, faecal sludge and organic solid waste will double by 2020.

11. PERSPECTIVE AND IDENTIFIED RESEARCH NEEDS

11.1 Epidemiology vs. QMRA, and combination of Epidemiology, QMRA, and MFA

Whenever a risk model has been set up in enough detail to have some uncertainty estimates, it would be highly interesting to try and confront the estimated risks with epidemiological data. Although this involves many additional uncertainties that cannot always be easily quantified, it is the closest we can get to a validation with real data. By choosing a scenario and calculating incidences (or prevalence in a transmission model) by means of Monte Carlo procedures, the agreement between the risk model and observed outbreak data could be quantified [83]. QMRA is a method to estimate the health risk due to the exposure to specific pathogenic hazards and in specific scenarios. The health outcomes of QMRA are the infection or diseases risk and remain predictive with uncertainty. In our study the risk of diarrhea caused by pathogens were estimated along agricultural activities for a period of one year. Epidemiology provides actual incidence and the important risk factors for diseases. In this study, the aim of epidemiological studies was to identify cases of diarrhoeal diseases, the duration of diarrhoeal episodes among people engaged with wastewater and excreta reuse in agriculture. Epidemiology also identified risk factors associated with diarrhoeal cases, including direct exposure to wastewater and human and animal excreta.

As QMRA and epidemiological studies provide complementary information (health outcomes) then there is a potential combination of these two methods to be used together or in alternative way to provide better overall estimates of risk. Indeed QMRA is used to estimate the probability of becoming infected by a specific pathogen after an exposure. QMRA uses densities of particular pathogens, assumed rate of ingestion and appropriate dose-response models for the exposed population to estimate the level of risk [97]. The QMRA results could help to understand what pathogens cause diarrhoeal diseases in the epidemiological studies and with what attribution of diseases. Epidemiology in its turn can provide with information on the real risk of disease to improve the dose-response of the model and thus reduce the uncertainly of risk calculated by QMRA. Combined epidemiology and QMRA results provide the current status of health of the local population, especially diarrhoea-related pathogens in wastewater and excreta reuse in agriculture. Finally, QMRA can be used to detect very low levels of infection or disease risk. In this case, epidemiology will need a large sample size which might imply important resource needed to be used and can complement epidemiological studies. Currently the concept of health based target of WHO to manage water quality relies very much on QMRA approach to set and ensure the quality of drinking water but also for the safe use of wastewater reuse [31, 83]. Some other discussion on the link between QMRA and Epidemiology attracted attention from litterature to show more complementary or alternative way of their use in assessing health risk [95]. From a developing country perspective, QMRA needs to be promoted because it does not require less resource than epidemiology althouht it might not provide the same level of precision on health risk.

The MFA result identifies the CCPs in terms of nutrient discharge in the environmental sanitation and agricultural systems. The health risks identified by epidemiology and in particular by QMRA showing also CCPs in terms of health risk in the same sytems. Thus we would see both MFA and QMRA could use the same platform, which is an environmental and agriculture system, to introduce a combined environmental and health risk. In this sense the MFA applied for environmental impact assessment will remain the same whereas the pathogens circulated the systems will be integrated into the MFA system so that we can examine "pathogen flows". The pathogen flows could provide data at CCPs (in terms of pathogen load) to apply QMRA which give health risks at CCPs. By combining environmental and health risks, we will be able to identify the combined CCPs for environmental and health risk then could propose more targeted intervention. Indeed this concept is discussed on the large framework proposed for combined health, environmental and socioeconomic assessment [77]. The first attempt of Narong Surinkul (2009) to use the pathogen flows of *E. coli* in an environmental system in Thailand showed that *E. coli* could be identified at different points of the system and consequencely health risk posed by *E. coli* was estimated by QMRA [272, 273]. Further development with integration of predictive microbiology in the pathogen flows and QMRA will be needed to estimate more accurately the health impacts in such an environmental sanitation system.

11.2 Wastewater and excreta treatment needs

It has been shown in the discussion chapter that the use of wastewater and excreta in agriculture in Hanam province can carry potential health risks, in particular diarrhoeal diseases and intestinal parasitic infections, for the exposed people. Furthermore, there are large nutrient N and P flows released to drainage systems or surface water. In this setting, Nhue River is a unique source of water for irrigation to the fields for large period of the year. On-site sanitation facilities in the household are rudimentary with single or double vault latrine, pour-flushed and semi-septic tanks. Greywater, together with the on-site sanitation system effluent, is discharged untreated into drainage networks and then passed into the irrigation system or the Nhue River. Additionally, human and animal excreta are still commonly used for agriculture.

It is important to find a relevant intervention to integrate the benefits of wastewater and excreta use and the protection of human health. The intervention option is to ensure that the wastewater and excreta are safe for use as nutrient and water sources for agricultural production, so that there is mitigation of public health risks for farmers, their families, nearby communities and consumers. Here we introduce some main proposals tailored to the local context to treat effectively wastewater and excreta. This is part of our recommendation from our policy brief [274].

As it is known that wastewater contains many pathogens and toxic chemicals, the hazard to public health can be reduced by limiting industrial effluents or toxic chemicals discharged into pond systems or removing the pathogenic agents. Moreover, aquatic food production systems can significantly reduce the level of pathogens in wastewater while reusing the nutrients it carries.

It is important to treat wastewater to reduce the health risks. Authorities need to monitor water quality from industrial zones regularly and to strengthen the regulation of wastewater treatment in factories and industrial zones that discharge wastewater into the sewage system or the environment. Monitoring contamination levels of pathogens and chemicals from wastewater needs to be done. The natural wastewater treatment systems should be recognized by policymakers and urban planners and should be sustainable when urbanisation is rapidly increased in Vietnam.

Wastewater treatment in designed plants or pond systems has long been considered the ultimate solution for reducing risks in wastewater-irrigated agriculture, and it is also widely studied and documented in both developed and developing countries [275-278]. Many European countries, the United States and several countries in North Africa and the Middle-East have recognised the importance of wastewater recycling through irrigation, developing national wastewater reuse programmes as part of their water resources management policy [62]. Most conventional systems (mechanical-biological wastewater treatment plants) have multiple treatment systems: primary treatment where suspended solids and organic matter are removed, secondary treatment for removing biodegradable organics and tertiary treatment for the removal of nutrients and toxic compounds [278]. The processes involved in several conventional treatment systems are difficult and costly to operate in developing countries like Vietnam, as they have high energy requirements, need skilled labour and have high installation, operation and maintenance costs [29]. At present, the use of partially treated wastewater in waste stabilisation ponds (a series of ponds to separate liquid and solid) for agriculture and aquaculture should be contemplated since this is considered a low-cost treatment facility and a feasible option for developing countries, especially those with warm climates [31, 63, 276]. The stabilization ponds are much as 80% lower than conventional activated sludge or trickling filtration systems, although they require a larger area. The third edition of WHO guidelines on the safe use of wastewater, excreta and grey water in agriculture and aquaculture [14, 20, 31], provide an excellent example of wastewater and excreta quality standards for use in agriculture and aquaculture that Vietnam can follow or adapt to incorporate into the existing national standards.

Wastewater treatment is an important measure to ensure the safe use of wastewater in agriculture and aquaculture. Moreover, at the household level, improved on-site sanitation systems, including treatment of wastewater using septic tanks and pre-treatment of organic solid wastes like composting of excreta, are also important measures to reduce nutrients and pathogens released into drainage systems and surface water, thereby mitigating the health risks for exposed population. In rural communities, farmers use human excreta as fertiliser and are likely to continue to apply this on their fields since this is seen as an important input to production and income generation. The guidelines for safe composting of human excreta should be developed and promoted in accordance with prevailing farmer perception and practices in order to be effective. The guidelines could be developed through further experimental study to decrease the pathogen viability in the latrine vault and composting heap, where pH, temperature and moisture are among the most important determining parameters. In the Vietnamese rural context, kitchen ash and lime are available and cheap materials which could be used in the latrine daily and in the composting process for absorbing moisture and increasing pH, to create a safe product for use in fertilising the fields.

11.3 Improvement of hygiene behaviour needs

It is important to recognize that in many situations where wastewater and excreta are used in agriculture, the treatment of wastewater and excreta are not feasible or appropriate or effective and may not be available for many years to come. To achieve the greatest benefits to health, the third edition of the WHO guidelines provides tools, methods and procedures to set health-based targets that can be achieved with different pathogen barriers from the wastewater and excreta sources to the consumption of wastewater-irrigated food. The guidelines highlight the importance of personal hygiene and use of protective measures in the protection of human health as and overall reduction of microbial targets. In Vietnam, while appropriate treatment of wastewater is not yet attained and composting of excreta is irrelevant, the improvement of personal hygiene, including washing of hands with soap and use of protective measures (e.g., gloves, boots and face mask), would be suitable alternatives to protect the health of the exposed population.

In agricultural communities, awareness is lacking at all levels for the role and risks of wastewater in food production. Farmers fail to recognize the health risks to themselves and to consumers, and they do not know how to reduce such risks. Consumers, traders and other actors in the value chain also have limited information about the health risks and how to prevent them. Policymakers lack information about the important role of rural and peri-urban agriculture and the value of nutrients in wastewater and excreta, although to some extent they are aware of health problems related to wastewater and excreta use. A study on farmers' perceptions and awareness of wastewater use in the study area showed that people perceive wastewater as smelly and black, and thought that contact with the wastewater could cause skin problems (e.g. itching). Farmers felt that fish from the Nhue River and vegetables irrigated with wastewater were potential causes of diarrhoea [106]. While issues related to health promotion and specific intervention strategies were not part of the conceptual framework of the research presented in this thesis, some of the findings may serve as inspiration for future health promotional activities. Future programs should be directed primarily towards responsibilities of authorities and farmers and include a discussion of the potential health risks and nutrient values related to wastewater and excreta use for agricultural production.

Producers, traders and authorities should be aware of the dangers of contamination of vegetables and fish after harvesting, and clean water and sanitation facilities should be provided in markets. Consumers should be made more aware of the importance of safe food and ways to disinfect food through washing and cooking. Product quality should be monitored and product certification schemes should be implemented. Sampling and laboratory testing of products should be carried out regularly by regulators. Public health concerns about unsafe products must be announced in public media.

This multiple barrier approach should be implemented with other health measures such as health education, hygiene promotion and the provision of access to safe drinking water and adequate sanitation. Successful implementation of the above measures requires active participation and collaboration from different stakeholders, especially local authorities and community members. The mass communication facilities available in Vietnam and the outreach organized by the Department of Preventive Medicine, Ministry of Health, provide many opportunities for more targeted communication. Similarly, the Ministry of Agriculture and Rural Development could play an important role in promoting sound practices from both an agricultural and hygienic perspective. Furthermore, the local drainage systems, appropriate waste disposal facilities and safe public water supply plants should further be invested in and improved in communities.

11.4 Identified research needs

Most reuse of wastewater and excreta in developing countries like Vietnam has been informal. These reuse practises should not be abandoned or ignored, as they most likely represent culturally acceptable and financially viable options. However, to have a safe reuse and reduce its health impact, a better understanding of the fate of pathogens with current methods of informal reuse, and how the current practises could be modified to achieve increased pathogen reduction, needs to be developed. This information could then be used in risk assessment to determine the relevant barriers to achieve health-based targets. Therefore, a study could be investigated to understand removal of pathogens and cycling of nutrients with different on-site treatment technologies so that treatment outcomes can be reliably predicted and appropriate solutions implemented. Further research on human exposure to wastewater and excreta need to be done so that QMRA can refine the local context.

As demonstrated in the introduction chapter, the concept towards an integrated framework combines different approaches to assess health status, physical environment and social, cultural and economic impacts in the areas with reuse of wastewater and excreta in agriculture is developed and tested in this study [77]. However, in the present study did not deal with socio- economic and cultural assessment in relation to the use of wastewater and excreta in agriculture. The benefits and health risks associated with the use of wastewater and excreta in agriculture have been underestimated or inadequately dealt with. Therefore a social study that investigates the perception of local people and stakeholders and their willingness to modify behaviour related to excreta and wastewater management is recommended. Health risks posed by using wastewater and excreta in agriculture would be prevented if wastewater and excreta are properly treated and used and hygienic practices and risk perception and prevention are well implemented. In addition, an economic assessment aimed at analysing economic benefits of wastewater and excreta reuse in agriculture and existing and improved on-site sanitation facilities is suggested. Suitable wastewater and excreta reuse in agriculture may be chosen by the economic benefits of improved environmental sanitation. We are aware that several analyses on cost-benefit of sanitation have been done at global or nationa level [279-281].

However this kind of study at local level will be needed to have convincing empirical data.

The present study showed that consumption of rainwater for drinking accounted for 77% of diarrhoeal cases. Rainwater is a major drinking water source in the study area and also in many rural areas of Northern Vietnam. Therefore, a study could be conducted for assessing the infection risks of diarrhoea-related pathogens in rainwater. The study results may be useful for improving quality of rainwater, thereby improving human health.

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ANNEX 1. INFORMED CONSENT FOR THE STUDY PARTICIPANTS

Health risks related with the use of wastewater and excreta in agriculture and aquaculture in Northern Vietnam

This study is carried out by Dr. Pham Duc Phuc from National Institute of Hygiene and Epidemiology (NIHE), Hanoi, Vietnam and the Swiss Tropical Institute, Switzerland. The study is funded by both the NCCR North-South (Switzerland) and NIHE.

This informed consent form has two parts:

- **Part I is the information sheet to share information about the research study with you, and**
- **Part II is the certificate for your consent for signature if you agree to take part.**

You will be given a copy of the full Informed Consent Form

PART I: INFORMATION SHEET

Introduction
In Vietnam the use of wastewater and excreta in agriculture or aquaculture have a long tradition since centuries. While this practice has advantages it may have potential health risks if excreta and wastewater are not properly managed.

Purpose
The purpose of this project is to evaluate the health risks related to the use of wastewater and excreta among different groups of people in Hanam province, Vietnam. We are particularly interested to study a relationship with diarrhoeal diseases and worm infections.

Choice of participation: Why ask me and my household?
You and your household have been selected randomly from the households in the village.

Participation is voluntary: Do I have participate?
Your participation is entirely voluntary. You are entirely free to decide to participate or not, and you can withdraw at any moment.

Procedures: What is going to happen to you?
A health worker will ask you to provide two stool specimens. They will be examined in the laboratory for parasitic infections. In addition will be contacted and a questionnaire filled. The questions will relate your wastewater and excreta use, your exposure to them and your health condition.

Risk: With this be bad or dangerous for me?
All the working procedures and examinations during this study are routinely conducted by a health worker. They do not bear any risks.

Discomfort: Will it hurt?
None of the procedures will hurt as none of the procedures is entering the body. However, you might be annoyed that we want to have 2 stool specimens; you might need to wait until it is your turn to be asked questions might take some time.

Benefit: is there anything good that will happen to me?
We will inform you on all infections which we diagnose in your stool samples. In addition you will receive a free drug treatment for the infections. The medicine corresponds to the recommended treatment in Vietnam.

Incentives: Do I get anything for participation in the research?
Apart from the treatment of the infections you will not receive any additional compensation.

Confidentiality: Is everybody going to know about this?
Your study records will be kept strictly confidential. Your records will have a code. Your name in not available for the scientific analysis or reports. Only authorized people are allowed to view or inspect the record of this study and have access to your name.

Sharing findings: Will you tell me the results?
You will receive the results of all your study information. Nobody except you will obtain a feedback on your personal examination. We will also inform you and your village about the results of the study.

Right to refuse or withdraw: Do I have the right not to participate? Can I change my mind?
You are absolutely free to participate and you are also free to change your mind any time.

Contact: Who can I contact to ask questions?
Dr. Pham Duc Phuc 84 4 38219074, National Institute of Hygiene and Epidemiology or local health worker knows to contact him.

PART II: CERTIFICATE OF CONSENT

Before signing the consent form, a member of the study team has explained the study to me in detail. I fully understand the nature and the main purpose of this study. The collected information will be kept confidential and will only be used for the study. The results of the stool examination will be given to me and I shall receive free medicine for the treatment of any parasitic infection with is diagnosed in the study. I understand that I will not receive any further compensation for participation in this study.

I have been given the opportunity to ask questions about this study. These questions were answered to my completed satisfaction. I am also aware that I can withdraw from the study at any time without giving any explanation and that I will not be victimized or disadvantaged as a result of my withdrawal. I have received a copy of the consent explanation or the explanation has been read to me.

I willingly participate in this study

Name, first name:……………………………………………Location:…………………...

Signature or thumb prints of informant.. .. ..date -------------2009

Signature of witness. ....date ----- -------2009

Signature of research interviewer:………………………………..date…………..2009

ANNEX 2. QUESTIONNAIRES

2.1 Questionnaire for household survey

(Interviewer will ask a head of household or adult people, who clearly know the information related with questions in this questionnaire)

I. General information

Date of interview: ___ ___/___ ___/___ ___ ___ ___ *(dd/mm/yyyy)*
Name of interviewer: ____
Province: District:
Commune: _________________ Village: ___________________________

Household ID: ______ (*it is following a category number in list of household which provided by the communal people's committee*)

Interview participation: ☐ Yes ☐ No
If no, what are reasons?
☐ Absence (after 3 times visited) ☐ Do not agreed
☐ Other reason (specify) ______________________________
Name of head of household: ___________________________________
Name of respondent: ____
Respondent's position in household:_____________________________

II. Social - Demographic information
Household Characteristics (HC)
HC1 How many people are in your household?......................................

##	**2. Household member (name)**	**3. Age**	**4. Sex**	**5. Living in household for whole year** 0. No 1. Yes	**6. Education** 0. never attended school 1. kindergarten 2. primary school 3. secondary school 4. tertiary school 5. college	**7. Relation with head of household** 1. spouse 2. son 3. daughter 4. son-in law 5. daughter in law 6. nephew 7. other (specify)	**8. major occupation** 1. rice 2. vegetable 3. breeding fish 4. commerce 5. service 6. officer 7.homework 8. none	**9. Minor occupation** 1. rice 2. vegetable 3. breeding fish 4. commerce 5. service 6. officer 7.homework 8. none	**10. Estimated income per month (VND)**	
									Major	Minor
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

HC11. How much land does your family cultivate?

1. Garden land:………m^2 2. Fish pond:………...m^2 3. Vegetable land:….. m^2
4. Rice land:…………m^2 5. Other:…….m^2

HC12. How many animals do your family has?

1. Chicken………..2. Ducks………..3. Pig…………..4. Cow…………5. Buffaloes…….. 6. Dogs/cats………7. Goats……… ..8. Others………….

HC13. What is kind of house's material?

1. Roof's material
   1. Thatches 2. Iron sheets 3. Tiles 4. Cements 5. Other…
2. Wall's material
   1. Bricks 2. Woods 3. Mud 4. Other…
3. Floor's material
   1. Earth 2. Tiles 3. Cements 4. Woods 5. Other…

HC14. What is the commodity your family has?

Items	1.Yes 0.No	Quantities
1. Bicycles		
2. Motorcycle		
3. Telephone		
4. Television		
5. Radio		
6. Beds		
7. Refrigerator		
8. Other (specify)		

III. Information about the water source and use in the household (W)

W1. What water sources are used by the household in the rainy season and dry season?

	Water source	Season (1 yes; 0 No)	
		Rainy	Dry
1	Tape water/pipe water/water plant		
2	Dug well		
3	Drilled well		
4	Rain water		
5	Lake/pond		
6	River		
7	Other (specify)		

W2. What is the water source usage for vary purposes in your family? (0 No; 1 Yes)

	Tape water		Dug well		Drilled well		Rain water		Lake/pond		River		Other	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
1.Drinking														
2.Cooking														
3.Handwashing														
4.Bathing														
5.Washing cloth														
6.Food prepare														

W3. How is treatment of water before drinking in your family?

0. No 1. Filtering 2. Added-chlorine 3. Boiling
4. Added-Alum 4. Other…

W4. In your family, do you have containers for drinking water storage?

0. No 1. Yes

W5. If yes, how many liters?

1. < 1 m^3 2. 1-2 m^3 3. > 2 m^3

W6. In your family, do you have a container for washing, bathing, cleaning water storage?

0. No 1. Yes

W7. If yes, how many liters?

1. < 1 m^3 2. 1-2 m^3 3. > 2 m^3

IV. Information about the toilet and excreta (S)

S1. In your household, do you have a toilet?

0. No 1. Yes

S2. If no, where do you defecate?

1. in the garden 2. Neighbor's toilet 3. Public toilet 4. Other (specify)…

S3. If yes, what type of toilet?

1. Temporary pit 2. Single vault 3. Double vault 4. Septic tank
5. VIP toilet 6. Biogas 7. Pour flush 8. Other….

S4. Distance between toilet and drinking water source?

1. < 5 m 2. 5-10 m 3. 11-20 m 4. > 20 m

S5. What do you do with the human excreta?

1. Poured into the septic tank 2. Poured into the local sewerage system
3. Buried into the ground 4. Storage in a pile outside the toilet
5. Poured into the animal faces 6. Storage inside the vault of toilet
7. Not treatment 7. Other (specify) ....

S6. If storage, do you add any matters?

0. No 1. Yes

S7. If yes, what are the added-matters?

1. Ash 2. Lime 3. Ash + Lime 3. Other (specify)…

S8. If storage, how long time?

1. < 1 month 2. 1-3 months 3. > 3-6 months 4. > 6 months

S9. Have your family use of excreta in agriculture and aquaculture?

0. No 1. Yes

S10. If yes, how is your family using fresh human excreta?

1. Used of fresh human excreta to apply in the rice fields
2. Used of fresh human excreta to apply for vegetables
3. Used of fresh human excreta to apply for vegetables and flowers
4. Used of fresh human excreta for fish breeding

S11. If yes, how is your family using composted human excreta?

1. Used of composted human excreta to apply in the rice fields
2. Used of composted human excreta to apply for vegetables
3. Used of composted human excreta to apply for vegetables and flowers
4. Used of composted human excreta for fish breeding

S12. How often does your family use of excreta for the rice, vegetable, flower, fish pond?

1. Rice….........time/year 2. Vegetable……………...time/year
3. Flower……...time/year 4. Fish pond………………time/year

V. Information about the wastewater (WW)

WW1. Where is domestic wastewater discharged?

1. Discharge to the garden 2. Discharge to the pond
3. Discharge to the local drainage 4. Discharge to anywhere

WW2. Have your family use of wastewater in agriculture and aquaculture?

0. No 1. Yes

WW3. If yes, how is your family using it?

1. Used of wastewater to irrigate for the rice field
2. Used of wastewater to irrigate for the vegetable
3. Used of wastewater to irrigate for the vegetable and flower
4. Used of wastewater for fish breeding
5. Other……………

WW4. If yes, how often does your family use of wastewater for rice, vegetable, flower, fish?

1. Rice…………time/year 2. Vegetable……….time/year
3. Flower……….time/year 4. Fish pond………..time/year

WW5. What are the seasons your family often uses of wastewater for the field and fish pond?

1. Dry season 2. Rainy season 3. Both

VI. Information about the animal husbandry (AH)

AH1. In your family has animal keeping place?

0. No 1. Yes

AH2. If yes, distance between animal keeping place and drinking water source?

1. < 5 m 2. 5-10 m 3. 11-20 m 4. > 20 m

AH3. How is treatment of animal wastes in your family?

1. Poured into the septic tank 2. Poured into the local sewerage system
3. Buried into the ground 4. Storage in a pile in the garden
5. Mixed with the human excreta 6. Not treatment 7. Other (specify) ....

AH4. If storage, what are the added-matters?
1. Ash 2. Lime 3. Ash + Lime 3. Other………
AH5. If storage, how long time?
1. < 1 month 2. 1-3 months 3. > 3-6 months 4. > 6 months
AH6. Have your family use of animal wastes in agriculture and aquaculture?
0. No 1. Yes
AH7. If yes, how is your family using fresh animal excreta?
1. Used of fresh animal excreta to apply in the rice fields
2. Used of fresh animal excreta to apply for vegetables
3. Used of fresh animal excreta to apply for vegetables and flowers
4. Used of fresh animal excreta for fish breeding
AH8. If yes, how is your family using composted animal excreta?
1. Used of composted animal excreta to apply in the rice fields
2. Used of composted animal excreta to apply for vegetables
3. Used of composted animal excreta to apply for vegetables and flowers
4. Used of composted animal excreta for fish breeding
AH9. How often does your family use of animal excreta for the rice, vegetable, flower, fish pond?
1. Rice…...........time/year 2. Vegetable……………...time/year
3. Flower……...time/year 4. Fish pond………………time/year

VII. Information about the hygiene behavior (HB)
HB1. Do you eat outside the household in last 3 days?
0. No 1. Yes
HB2. If yes, which are places do you eat?
1. market 2. field 3. roadside inn 4. restaurant
HB3. When are you hand-washing?
1. After working in the field 2. After defecation
3. After disposal children faces 4. Before eating
5. Before food preparing 6. Before breast feeding
7. After handling animals 8. After handling excreta
HB4. How do you hand-washing?
1. Water only 2. Water with soap 3. Other…………
HB5. How often do you hand-washing with soap
1. Regularly 2. Sometime 3. Rarely 4. When is hand-dirty

VIII. Food hygiene (FH)
FH1. Where do you store it's left over food?
1. Refrigerator 2. Kitchen 3. Sleeping room 4. Other:…
FH2. Is the left over food covered when left in the room/kitchen?
0. No 1. Yes
FH3. How do you handle your left over food?
1. heat all 2. heat only soup 3. heat only rice
4. boil only soup 5. boil rice 6. other……………
FH4. When does the household clean its utensils after cooking and eating?
1. immediately 2. next day's morning 3. any day before cooking 4. other……
IX. Information about the health problem (HP)
HP1. What is the main health problem are you getting it?
1. Headache 2. Sore throat 3. Flu 4. Diarrhea 5. Vomiting 6. Abdominal pain
7. Nausea 8. Itching 9. Back pain 10. Sore eyes 11. Other…
HP2. How often are you getting a sick?
1. Regularly 2. Sometime 3. Rarely 4. Never
HP3. When you were sick, what did you do?
0. Not treatment 1. Go to the local doctor 2. Self-treatment 3. Go to the hospital
4. Traditional treatment 5. Other ………
HP4. Who in your family often are getting sick?
1. children < 5 2. children 5-15 3. women 4. men 5. all
HP5. Do you know, what are the diseases related with use of excreta and wastewater?

X. Observational checklist

##	Observational	0 No; 1 Yes 9 not available	Comments
1	Household has a toilet facility		
2	HT1. the toilet appear to be used by the family		
3	HT2. other outside toilet appear to be in use		
4	HT3. the toilet door is broken or missing		
5	HT4. the toilet door is closed with key		
6	HT5. the toilet wall are clean		
7	HT6. the toilet floor is clean		
8	HT7. the toilet smells badly		
9	HT8. there are flies around the toilet		
10	HT9. the ground around the toilet is muddy		
11	HT10. there are hand washing facilities near the toilet		
12	HT11. there is soap provide for hand washing after toilet use		
13	WS1. there is a store of drinking water		
14	WS2. the stored water is covered		
15	WS3. the ground around the water storage facility is muddy		
16	WS4. Water storage facility is easily accessible to children		
17	WS5. the water storage facility looks dirty from outside		
18	YA1. there is grey/stagnant water in the yard		
19	YA2. there is domestic waste and litter in the yard		
20	YA3. there is animal/human faeces in the yard		
21	YA4. there is garbage in the yard		
22	FH1. household has a refrigerator for food storage		
23	FH2. some cooking utensils are left unwashed		
24	FH3. there are uncovered left over meals at the cooking area		
25	FH4. there are houseflies in the cooking area		
26	FH5. cooking area/kitchen is clean with no food debris		

Thank you very much for your time and information!

2.2 Laboratory forms

Kato-Katz thick smear results - Stool number................ Date:___/____/_____

Village name:_____________________________ Commune:___________________ Province: Hanam

			Slide 1 (eggs per gram)				Slide 2 (eggs per gram)			
##	PersonID	Name	*Ascaris*	Hookworm	*Trichuris*	Other	*Ascaris*	Hookworm	*Trichuris*	Other
1										
2										
3										
4										

Laboratory form SAF method **Date: / /**

Village name:_____________________________ Commune:___________________ Province: Hanam

			Stool 1				Stool 2			
##	PersonID	Name	*Giardia*	*Entamoeba*	*Crypto*	*E. coli*	*Giardia*	*Entamoeba*	*Crypto*	*E. coli*
1										
2										
3										
4										

2.3 Questionnaires for diarrhoeal disease case and control study

Study ID: ___ ___ ___ ___ ___ ___ Case (1)/Control (0): ___ ___ ___ ___ ___

CASE DEFINITION

- *at least three or more loose (or watery) stools within 24 hours, regardless of other gastrointestinal symptoms;*
- *or two or more loose stools associated with at least one other symptom of gastrointestinal infection (abdominal pain, cramping, nausea, vomiting, and fever);*
- *or passage of a single loose stool with grossly evident blood/mucous.*

Two independent diarrhea episodes will be separated by at least three days without diarrhea, and an episode of diarrhea with duration of 14 days or more will be regarded as an episode of persistent diarrhea.

PART A. GENERAL INFORMATION

Date of interview: ___ ___/___ ___/___ ___ ___ ___*(dd/mm/yyyy)*

Name of field worker: ____

Name of study subject: ____

Study ID: ___ ___ ___ ___ ___ ___

Sex *(circle one)*: 0 = Female 1 = Male

Year of birth: ___ ___ ___ ___

PART B. CASE CHARACTERIZATION

1. On what date did your diarrhea begin?
1 ___ ___/___ ___/___ ___ ___ ___
9 Do not remember

2. How many days did your diarrhea last? ___ ___ days

3. What symptoms did you have with your diarrhea? *(check all that apply)*

		Yes	No	DN/NS
1	Fever	☐	☐	☐
	Temperature: ___ ___ °C (if measured)			
2	Abdominal pain	☐	☐	☐
3	Nausea	☐	☐	☐
4	Vomiting	☐	☐	☐
5	Fatigue	☐	☐	☐
6	Drinking more than usual	☐	☐	☐
7	Other	☐	☐	☐
	(specify) ______________________________			

4. Were your stools: *(circle all that apply)*

1 Bloody 2 Watery 3 Mucous

5. Frequency of diarrhea (highest per day): ___ ___ stools/day

Treatment

6. Have you consulted a physician or health care for this illness?
0 No 1 Yes

6.1 If yes, what type of facility have you visited?
1. Health center ☐ 2. Local pharmacist ☐ 3. Hospital ☐
4. Other physician ☐ *(specify)* ________________________

7. Have you taken any treatment for your diarrhea?

		Yes	No	DN/NS
1	ORS	☐	☐	☐
2	Antibiotic treatment *(please specify the names of the antibiotics that you have taken)* ____	☐	☐	☐
3	Herbal or traditional medicines *(please specify the names of the antibiotics that you have taken)* ____	☐	☐	☐
4	Other treatment *(specify)* ____________________________________	☐	☐	☐

PART C EXPOSURE HISTORY

I. <u>Exposure with wastewater from Nhue River</u>

Exposure to wastewater is defined as direct contact with wastewater, or getting wet by the wastewater. Definition of wastewater must be explained to interviewee.

8. Did you have contact with wastewater from Nhue River when doing your agricultural work during the last week?

0 No 1 Yes 9 Don't know/Not sure

9. What type of work were you doing that involved contact with wastewater from Nhue River?

Items	Yes (1)	No (0)	Do not know/not sure (9)
1. Fish harvest			
2. Fish breeding			
3. Watering gardens/fields			
4. Rice sowing			
5. Vegetable/rice harvest			
6. Weeding/manuring			
7. Spraying pesticides			
8. Other *(specify)*____________			

10. How long were you in contact with wastewater from Nhue River during the last week?

Days before the onset of diarrhea	Estimated contact hours with wastewater from Nhue river per day					
	No contact (0)	Less than 1 hour (1)	1 - 2 hours (2)	3 - 4 hours (3)	More than 4 hours (4)	Do not remember (9)
Onset of diarrhea (0)						
1						
2						
3						
4						
5						
6						
7						

11. Which parts of your body were mainly in contact with wastewater from Nhue river?

Items	Yes (1)	No (0)	Do not remember (9)
1. Hands			
2. Arms			
3. Feet (up to ankle)			
4. Legs (up to ankle)			
5. Legs (up to ankle)			
6. Whole body (up to the chest)			

12. Did you have contact with soil/land irrigated by wastewater from Nhue River during the last week?
0 No 1 Yes 9 Don't know/Not sure

13. Were you on bare feet while working in the field during the last week?
0 No 1 Yes 9 Don't know/Not sure

14. Did you use any of the following protective measures when you were working in wastewater from Nhue River during the last week?

Items	Yes (1)	No (0)	Do not remember (0)
1. Shoes			
2. Rubber boots			
3. Cloth gloves			
4. Rubber gloves			
5. Plastic clothing			
6. Face mask			
7. Hat/cap			
8. Glasses			

II. <u>**Exposure with wastewater from domestic pond**</u>

15. Did you have contact with wastewater from domestic pond during the last week?
0. No 1. Yes 9. Do not know/not sure

16. What type of work were you doing that involved contact with wastewater from domestic pond?

Items	Yes (1)	No (0)	Do not know/not sure (0)
1. Fish harvest			
2. Fish breeding			
3. Watering gardens			
4. Vegetable sowing			
5. Vegetable harvest			
6. Vegetable cleaning			
7. Pig pen/animal shed cleaning			
8. Wash your hand/foot			
9. Working equipments washing			
10. Washing/flushing toilet			
11. Washing clothes			
12. Other *(specify)*___________			

17. How long were you in contact with wastewater from domestic pond during the last week?

Days before the onset of diarrhea	Estimated contact hours with wastewater from domestic pond per day					
	No contact (0)	Less than 1 hour (1)	1 - 2 hours (2)	3 - 4 hours (3)	More than 4 hours (4)	Do not remember (9)
Onset of diarrhea (0)						
1						
2						
3						
4						
5						
6						
7						

18. Which parts of your body were mainly in contact with wastewater from domestic pond?

Items	Yes (1)	No (0)	Do not remember (9)
1. Hands			
2. Arms			
3. Feet (up to ankle)			
4. Legs (up to ankle)			
5. Legs (up to ankle)			
6. Whole body (up to the chest)			

19. Did you use any of the following protective measures when you were working in wastewater from domestic pond during the last week?

Items	Yes (1)	No (0)	Do not remember (9)
1. Shoes			
2. Rubber boots			
3. Cloth gloves			
4. Rubber gloves			
5. Plastic clothing			
6. Face mask			
7. Hat/cap			
8. Glasses			

III. <u>Human excreta (HE) Exposure</u>

Exposure to HE as a contact with HE (includes collecting, transporting, applying, and touching).

20. Did you have contact with HE when doing your work during the last week?
0. No 1. Yes 9. Do not know/not sure

21. What type of work /activity were you doing that involved contact with HE during the last week?

Items	Yes (1)	No (0)	Do not remember (9)
1. Removing out HE from the toilet vault			
2. Preparing composting HE			
3. Collecting HE and bring it to the field/pond			
4. Applying HE in the field/pond			
5. Preparing the soil mixed with HE			
6. Planting rice and/or vegetable in the soil had mixed HE			
7. Fish breeding			
8. Fish harvest			
9. Rice/vegetable harvest			
10. Weeding			
11. Spraying pesticides			
12. Other *(specify)*______________________________			

22. How long were in you contact with HE during the last week?

Days before the onset of diarrhea	Estimated contact hours with HE per day					
	No contact (0)	Less than 1 hour (1)	1 - 2 hours (2)	3 - 4 hours (3)	More than 4 hours (4)	Do not remember (9)
Onset of diarrhea (0)						
1						
2						
3						
4						
5						
6						
7						

23. What parts of your body were in contact with HE?

Items	Yes (1)	No (0)	Do not remember (9)
1. Hands			
2. Arms			
3. Feet (up to ankle)			
4. Legs (up to ankle)			
5. Legs (up to ankle)			
6. Whole body (up to the chest)			

24. Did you use any of the following protective measures when you were contact with HE during the last week?

Items	Yes (1)	No (0)	Do not remember (9)
1. Shoes			
2. Rubber boots			
3. Cloth gloves			
4. Rubber gloves			
5. Plastic clothing			
6. Face mask			
7. Hat/cap			
8. Glasses			

IV. **<u>Animal excreta (AE) exposure</u>**

Exposure to AE as a contact with AE (includes collecting, transporting, applying, cleaning, and touching).

25. Did you have contact with AE when doing your work during the last week?

0. No 1. Yes 9. Do not know/not sure

26. What type of work / activity were you doing that involved contact with AE during the last week?

Items	Yes (1)	No (0)	Do not remember (9)
1. Removing out AE from the animal sheds			
2. Preparing composting AE			
3. Collecting AE and bring it to the field/pond			
4. Applying AE in the field/pond			
5. Preparing the soil mixed with AE			
6. Planting rice and/or vegetable in the soil had mixed AE			
7. Fish breeding			
8. Fish harvest			
9. Rice/vegetable harvest			
10. Weeding			
11. Spraying pesticides			
12. Other *(specify)*______________________________			

27. How long were in you contact with AE during the last week?

Days before the onset of diarrhea	Estimated contact hours with AE per day					
	No contact (0)	Less than 1 hour (1)	1 - 2 hours (2)	3 - 4 hours (3)	More than 4 hours (4)	Do not remember (9)
Onset of diarrhea (0)						
1						
2						
3						
4						
5						
6						
7						

28. What parts of your body were in contact with AE?

Items	Yes (1)	No (0)	Do not remember (9)
1. Hands			
2. Arms			
3. Feet (up to ankle)			
4. Legs (up to ankle)			
5. Legs (up to ankle)			
6. Whole body (up to the chest)			

29. Did you use any of the following protective measures when you were contact with AE during the last week?

Items	Yes (1)	No (0)	Do not remember (9)
1. Shoes			
2. Rubber boots			
3. Cloth gloves			
4. Rubber gloves			
5. Plastic clothing			
6. Face mask			
7. Hat/cap			
8. Glasses			

V. <u>Personal hygiene and habits</u>

30. Do you wash your body right after finishing your work?
1 Always 2 Sometimes 3 Rarely 4 Never

31. Did you wash your body with soap?
1 Always 2 Sometimes 3 Rarely 4 Never

32. Did you wash your hands/feet after finishing your agricultural work engaged in wastewater/excreta?
0. No 1. Yes 9. Do not know/not sure

33. How often did you wash your hands/feet right after finishing your work?
1 Always 2 Sometimes 3 Rarely 4 Never

34. Did you wash your hands after defecation?
0 No 1 Yes 9 Don't know/Not sure

35. Did you wash your hands with soap?
1 Always 2 Sometimes 3 Rarely 4 Never

VI. <u>Drinking water</u>

36. During the last week, what type of water did you mainly drink:
1 Rain water 2 Water from a supply plant 3 Tube well water
4 Dug well water 5 Others *(specify)*______ 9 Don't know/ Not sure

37. Yesterday, what type of water did you mainly drink:

1	Rain water	2	Water from a supply plant	3	Tube well water
4	Dug well water	5	Others *(specify)*____	9	Don't know/ Not sure

38. During the last week, did you boil the water before you drank it?
1 Always 2 Sometimes 3 Rarely 4 Never 9 Don't know/Not sure

39. Was the water container from which you drank covered?
0 No 1 Yes 9 Don't know/Not sure

40. Was the water that you drank used for washing fruits or vegetables to be eaten as raw during the last week?
0 No 1 Yes 9 Don't know/Not sure

VII. Food consumption

41. Did you eat any leftovers in the week before the onset of diarrhea?
0 No 1 Yes 9 Don't know/Not sure

42. Was the food stored in the refrigerator?
0. No 1. Yes 9. Do not know/not sure

43. Was the food reheated before you ate it?
0. No 1. Yes 9. Do not know/not sure

44. Did you eat any food that was raw or undercooked in the week before the onset of diarrhea?
0. No 1. Yes 9. Do not know/not sure

45. Which of the following food items did you eat as raw or undercooked?

		Yes	No	DN/NS
1	Fish	☐	☐	☐
2	Other aquatic animals *(shrimp/snails/mussels)*	☐	☐	☐
3	Pork	☐	☐	☐
4	Beef	☐	☐	☐
5	Poultry *(chicken/duck/other birds)*	☐	☐	☐
6	Egg	☐	☐	☐
7	Animal internal organs *(liver/heart/intestines/gizzards/blood)*	☐	☐	☐
8	Water morning glory	☐	☐	☐
9	Coriander	☐	☐	☐
10	Salad	☐	☐	☐

VIII. Ill contacts and health problems

46. In the last 3 days, had a member of your family diarrhea?
0. No 1. Yes 9. Do not know/not sure

47. Did the ill person cook meals for the family during the last week?
0. No 1. Yes 9. Do not know/not sure

48. Did you get any gastrointestinal problems during the last week?

		Yes	No	DN/NS
1	fever	☐	☐	☐
2	nausea	☐	☐	☐
3	vomiting	☐	☐	☐
4	abdominal pains	☐	☐	☐
5	watery stools	☐	☐	☐
6	blood/mucus stools	☐	☐	☐
7	loose stools	☐	☐	☐
8	others *(specify)* _________	☐	☐	☐

IX. **Contact with animals**

49. Does your family have animals in the household?

0 No -- *STOP THE INTERVIEW*

1 Yes

50. During the last week, how did you come into contact with the animals?

Activities	Cattle (1)	Pigs (2)	Chicken (3)	Ducks (4)	Dogs/Cats (5)
0. No contact					
1. Washing animals					
2. Cleaning animal sheds					
3. Handling animal feces					
4. Feeding animals					
5. Other *(specify)*___________					
6. Don't know/ Not sure					

51. How long were in you contact with the animals during the last week?

Days before the onset of diarrhea	Estimated contact hours with animal per day					
	No contact (0)	Less than 1 hour (1)	1 – 2 hours (2)	3 - 4 hours (3)	More than 4 hours (4)	Do not remember (9)
Onset of diarrhea (0)						
1						
2						
3						
4						
5						
6						
7						

52. What parts of your body were mainly in contact with the animals?

Items	Yes (1)	No (0)	Do not remember (9)
1. Hands			
2. Arms			
3. Feet (up to ankle)			
4. Legs (up to ankle)			
5. Legs (up to ankle)			
6. Whole body (up to the chest)			

53. Did you use any of the following protective measures when you were contact with the animals during the last week?

Items	Yes (1)	No (0)	Do not remember (9)
1. Shoes			
2. Rubber boots			
3. Cloth gloves			
4. Rubber gloves			
5. Plastic clothing			
6. Face mask			
7. Hat/cap			
8. Glasses			

THANK YOU VERY MUCH FOR YOUR TIME AND INFORMATION

ANNEX 3. TERMS AND DEFINITIONS FOR MFA

- *Substance:* A substance is any (chemical) element and compound composed of uniform units. All substances are characterized by unique and identical constitution and are thus homogeneous. Example of elements and compounds are carbon (C), nitrogen (N), phosphorus (P), and ammonium (NH3).
- *Goods*: this term is defined as economic entitlements of matter with a positive or negative economic value. Goods are made up of one or several substances. Examples of goods are drinking water, garbage, sewage, sludge, and others.
- *Material*: the term of material has already been explained by substance and goods. In MFA, material serves as an umbrella term for both substance and goods. So, carbon as well as wood can be addressed as a material.
- *Process*: a process is defined as the transformation, transport, or storage of material. Examples of transformation processes are: (i) the human body, where food, water, and air are transformed mainly into urine and faeces, (ii) the entirety of private households in a defined region, where countless inputs are converted to faecal sludge, sewage, waste, emissions, and some useful outputs.
- *Flow and flux*: for MFA, the term flows and fluxes have been often used in a random way. A flow is defined as a mass flow rate which is ratio of mass per time while, a flux is defined as a flow per cross section or is called specific flow. In MFA, commonly used cross sections are a person, a habitant and a region.
- *Transfer coefficient*: this term describes the partitioning of substance in a process which are defined for each output good or process. It is a substance-specific value that stands for the characteristics of a process.
- *System and system boundaries*: a system is defined by a group of elements, the interaction between these elements, and the boundaries between these and other elements in space and time. Temporal boundaries are commonly 1 h, 1 day or 1 year and spatial system boundary is usually fixed by the geographical area in which the processes are located.

- *Activities*: this term is defined as comprising all relevant processes flow, and stocks of goods and substances that are necessary to carry out and maintain a certain human need. The most important activities can be defined as to treat, clean, reside and work, transport and communicate and also adding with health and sports.
- *Material accounting*: the art of materials accounting is to find those key parameters that yield maximum information at minimum cost. Materials accounting can be applied to all sizes of systems, form single companies to national economies.

ANNEX 4. CURRICULUM VITAE

PROFILE	
	Phuc Pham-Duc, MD, MIH Born February 13, 1971; Married since 2001; Address in Switzerland : Friedensgasse 69, CH-4056 Basel Tel: ++84 438 33 64 74 Mobile: ++84 904 04 99 69 Email: phucnihe@gmail.com; phuc.pham-duc@unibas.ch
EDUCATION	
1989-1995	Medical Doctor, Hanoi Medical University, Vietnam.
1996-1999	Bachelor of economic, National Economic University, Hanoi, Vietnam
2002-2003	Master of International Health, University of Copenhagen, Denmark
2008-2011	PhD student, University of Basel, Switzerland. Field works in Vietnam
PRESENT APPOINTMENTS	
2008-2011	Work at the National Institute of Hygiene and Epidemiology, Hanoi, Vietnam. PhD student belong the NCCR North-South project on “Health risks associated with excreta and wastewater reuse in agriculture in Vietnam”. Participated the courses on “Epidemiological concepts and methods; Biostatistics; Health Systems; Ecosystem and infectious diseases”. Swiss Tropical and Public Health Institute and University of Basel. Contributions to develop a training module on the quantitative microbial risk assessment related with water, sanitation and food in Vietnam. PAMS project - NCCR program; Hanoi School of Public Health. Contributions to develop a book on “Quantitative Microbial Risk Assessment in Foods in Vietnam”, supported by WHO and Vietnam Food Association. Researcher “Health risks associated with the use of wastewater and excreta in agriculture in Hanam province, Vietnam: Epidemiology and Quantitative Microbial Risk Assessment”. Funded by NCCR North-South and KFPE. Obtained the Eawag Partnership Program (EPP) Scholarship, at the Department of Water and Sanitation in Developing Countries (Sandec), Eawag. 5-8/2011.
PROJECTS	
2000-2007	Researcher “Human excreta use in agriculture in Vietnam (HEAV) - a study from the field to the latrine”, funded by Council for Development Research (RUF), Denmark. Project leader “The use of composted human excreta in agriculture in Nghean, Vietnam”, funded by International Foundation for Sciences (IFS), Sweden.

	Researcher "Practices and perceptions of hygiene and health risks associated with the use of human excreta for agricultural production in Vietnam", funded by IWMI, Colombo, Srilanka. Researcher "Sanitary aspects of drinking water and wastewater reuse in Vietnam", funded by DANIDA, Denmark. Researcher "Production Aquatic Peri-urban Systems in Southeast Asian - PAPUSSA", funded by EU.
CONSULTANCIES	
2005-2008	Situation analysis and project design- water, sanitation and hygiene (WASH) improvement for Northern and central Vietnam, funded by Plan International Organization. Adequacy of water, sanitation and hygiene in relation to home-based care strategies for people living with HIV/AIDS, funded by WHO. Vietnam Tracking study: Safe Water Project in High-Risk Communities, funded by Population Services International (PSI), US. Testing of Chlorine dosing for drinking water in Mekong Delta River, Vietnam, funded by Population Services International (PSI), US.
ADDITIONAL POSTGRADUATE EDUCATION	
2004-2011	Data analysis using STATA. SANIVAT project, DANIDA, Denmark, NIHE, Hanoi, 4/2011. Method for impact evaluation of health project. Hanoi Medical University - Centre for health system research. 14-15/3/2011. Assessing impacts of climate changes on health - Longitudinal analyses of mortality against background factors including weather. Epidemiology and Global Health, Umea University; Hanoi Medical University. 3-4/3/2011 Environmental behaviour and human health risk assessment associated with the use of agricultural pesticides in Vietnam; Health Environment Management Agency, Ministry of Health and Griffith University School of Public Health; 7/2010. Fundamentals on Ecohealth Southeast Asia Region Workshop; IDRC Canada, 5/2010. Intervention research methodology, the Netherlands project, Hanoi Medical University, Hanoi. 2/2010. The fundamentals of data management workshop. International Clinical Sciences Support Centre: Durham, North Carolina, USA. 6/2009. Data management using MS Access. SANIVAT project - DANIDA, Denmark, NIHE, Hanoi, 4/2009. NCCR North-South Integrated training and capitalization of experience. Costa Rica, 9/2008.

	Research Ethics Training Workshop, NIHE and National Institute of Health (NIH), US. Hanoi, 6/2008. Good Clinical Research Practices, Organized by Quintiles company, Singapore and Vietnam Ministry of Health, 8/2006. HIV/STIs Research and Prevention, Organized by The University of Texas School of Public Health, US - Hanoi School of Public Health, Vietnam (7/2006). Water supply and sanitation in Emergencies, in Denmark (8-9/2005). Ecological Alternatives in Sanitation, in Sweden (8-9/2004) and India (3/2005).
CONFERENCES & WORKSHOPS	
2007-2011	Poster “Assessment of infection risks of diarrhoea-related with pathogens in wastewater and excreta reuse in agriculture in Vietnam”. Bio-Valley Life Sciences Week 2011 - Infectious Diseases of Global Impact, Basel, Switzerland, 9/2011. Poster “Risk factors for *Entamoeba histolytica* infection associated with wastewater and excreta use in agriculture in Hanam province, Vietnam”. The 7th National Scientific Conference - Vietnamese Association of Public Health: Towards a Sustainable Development of Vietnamese Public Health in the Future, 4/2011. Oral presentation “Health risks related to excreta and wastewater reuse in agriculture using quantitative microbial risk assessment (QMRA) in Hanam, Vietnam”. The first International Conference on Environmental Pollution, Restoration and Management, Ho Chi Minh, 3/2010. Risk Analysis/Risk management for Scientific Advisors Microbiological Hazards, Food and Agriculture Organization, Vietnam Food Association, 10/2008. Oral presentation “Human excreta reuse in agriculture in Nghe An, Vietnam”, Sustainable sanitation - Eco-sanitation Conference, Dongseng, Inner-Mongolia, 8/2007. Ecosanres, SEI, Sweden.
LANGUAGES	
	Vietnamese - native languages English - good
COMPUTER SKILLS	
	Word, Excel, PowerPoint, Epi-Info, Access, STATA, SPSS, @Risk, Internet
MEMBERSHIPS	
	Member of the Vietnamese Association of Public Health
ARTICLES PUBLISHED IN PEER-REFERRED JOURNALS	
2005-2011	**Phuc P.D.**, Nguyen-Viet H, Hattendorf J, Zinsstag J, Cam PD, Odermatt P: Risk factors for *Entamoeba histolytica* infection in an agricultural community in Hanam province, Vietnam. *Parasites & Vectors* 2011, **4:**102. Nga DT, Morel A, Nguyen-Viet H, **Phuc P.D**., Nishida K, Kootattep T: Assessing nutrient fluxes in a Vietnamese rural area despite limited and highly uncertain data.

	Resour Conserv Recy 2011
	Jensen, P.K., **Phuc P.D**., Line Kram Knudsen., (2010). How do we sell the hygiene message, with dollars, dong or excreta? Environmental Health. 2010.
	Jensen, P. K., **Phuc, P. D.**, Konradsen, F., Klank, L. T., & Dalsgaard, A. (2009). Survival of *Ascaris* eggs and hygienic quality of human excreta in Vietnamese composting latrines. *Environ.Health, 8,* 57.
	Cole, B., **Phuc, P. D.**, & Collett, J. (2009). A qualitative and physical investigation of a double-vault composting latrine programme in Northern Vietnam. *Waterlines, 28,* 333-342.
	Knudsen, L. G., **Phuc, P. D.**, Hiep, N. T., Samuelsen, H., Jensen, P. K., Dalsgaard, A. et al. (2008). The fear of awful smell: Risk perceptions among farmers in Vietnam using wastewate and human excreta in agriculture. *S.E.Asia J.Trop.Med.Pub.Health, 39 (2).*
	Jensen, P. K., **Phuc, P. D.**, Knudsen, L. G., Dalsgaard, A., & Konradsen, F. (2008). Hygiene versus fertiliser: the use of human excreta in agriculture--a Vietnamese example. *Int.J.Hyg.Environ.Health, 211,* 432-439.
	Phuc, P. D., Konradsen, F., Phuong, P. T., Cam, P. D., & Dalsgaard, A. (2006). Practice of using human excreta as fertiliser and implications for health in Nghean Province, Vietnam. *Southeast Asian J.Trop Med Public Health, 37,* 222-229.
	Jensen, P. K., **Phuc, P. D.,** Dalsgaard, A., & and Konradsen, F., (2005). Successful sanitation promotion must recognize the use of latrine wastes in agriculture - the example of Vietnam. *Bull.World Health Org., 83,* 273-274.
OTHER ARTICLES	
2009-2011	Nguyen-Viet, H., Anh, V. T., **Phuc, P. D.**, Tu, V. V., (2011) safe use of wastewater in agriculture and aquaculture in Vietnam. Evidence for Policy series, Regional edition Southeast Asia, No. 2, ed. Thammarat Koottatep. Pathumthani, Thailand: NCCR North-South.
	Tu, V. V., Huong, N. T., **Phuc, P.D.**, Nguyen-Viet, H., Zurbrügg, C., (2011). Developing a questionnaire to measure peoples' awareness and behaviours related to wastewater use in agriculture in Hoang Tay and Nhat Tan communes, Hanam province following Protection Motivation Theory. *Vietnam Journal of Public Health,* 22, 66-72 (Vietnamese).
	Khuong, N. C., Bich, T. H., **Phuc, P.D.**, Nguyen-Viet, H., (2011, in press). Assessment of diarrhoea risks by microorganisms in wastewater used in agriculture in Hanam. *Vietnam Journal of Public Health*, 22, 14-20 ((Vietnamese)
	Tu, V. V., Huong, L. T. T., **Phuc, P. D.**, Thao, N. B., Nguyen-Viet, H., (2011, in press). Human excreta: management, reuse and public health in Vietnam. *Vietnam Journal of Public Health*, 22, 4-13 (Vietnamese)
	Nguyen-Viet H., Hanh T. T. T., **Phuc, P. D.**, Bich, N. N., Huong, B. T. M., (2011). Microbial Risk Assessment in Vietnam: Water, Sanitation, and Food safety - from

	training to policy. Outcome Highlights of the NCCR North-South. No. 7, 10/2011. **Phuc, P.D.**, Nguyen-Viet H., Zinsstag J., Odermatt P., (2010). Transmission dynamics of parasitic infections from wastewater and excreta use among risk groups in North Vietnam. Sandec News, August 2010. **Phuc, P.D.**, Nguyen-Viet, H., Zinsstag J., Tanner M., Cam P.D., Odermatt P.,. Transmission of parasitic infections by wastewater and excreta re-use in agriculture in Vietnam.– Highlights of the NCCR Research Partnerships for sustainable development in South East Asia, period 205-2009 Khuong, N. C., **Phuc, P.D.**, Bich, T. H., Nguyen-Viet, H., (2010). Assessment of microbial infection risks related to excreta and wastewater use. Sandec News, August 2010 Do-Thu Nga, Morel, A., **Phuc, P.D.**, Nguyen-Viet, H., Koontattep, T., (2010). The Material Flow Analysis (MFA) method is applied to nutrient resource management and recycling in Hanam, Vietnam. Sandec News, August 2010 Tu, V. V., **Phuc, P.D.**, Huong, N. T., Tamas, A., Zurbrugg, C., (2010). Awareness and agricultural wastewater reuse practices are assessed to improve interventions of safe reuse practices. Sandec News, August 2010. **Phuc, P.D.**, Nguyen-Viet, H., Zinsstag, J., Cam, P. D., Odermatt, P., (2009). Parasitological infections related to re-use of wastewater and excreta in agriculture and aquaculture in Northern Vietnam In: Morel A. (Ed.) Research Partnerships for Sustainable Development in Southeast Asia, NCCR North-South, JACS SEA, Thailand.
CONTRIBUTIONS TO BOOK CHAPTERS	
2011	Khan, N. C., Huong, B. T. M., Long, N. H., Hung, L. Q., **Phuc, P.D.**, Nguyen-Viet, H., (2011). Chapter 1: Introduction of Microbial Risk Assessment for Food Safety - Microbial Risk Assessment for Food Safety (Chief editors Khan, N. C., Nguyen-Viet, H.,) (Vietnamese). Medical Publishing House. Hanoi. 2011. **Phuc, P.D.**, (2011). Chapter 2: Hazard Identification - Microbial Risk Assessment for Food Safety (Chief editors Khan, N. C., Nguyen-Viet, H.,) (Vietnamese). Medical Publishing House. Hanoi. 2011. **Phuc, P.D.**, (2011). Chapter 3: Risk Characteristics - Microbial Risk Assessment for Food Safety (Chief editors Khan, N. C., Nguyen-Viet, H.,) (Vietnamese). Medical Publishing House. Hanoi. 2011. Nguyen-Viet, H., Minh, H. V., Anh, V. T., Hanh, T. T. T., Tu, V. V., **Phuc, P. D.**, Nhung, N. H., Thao, N. B., (2011). NCCR North-South dialogue: Glossary of terms in water supply and sanitation (Vietnamese). Medical Publishing House. Hanoi. 2011.