Human excreta use in agriculture in Vietnam: Health, economic and environmental aspects

INAUGURALDISSERTATION

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät der

Universität Basel

von

Vu Van Tu

aus Vietnam

Basel, 2018

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät der Universität Basel auf Antrag von

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Basel, 26. Juni, 2018

Prof. Dr. Martin Spiess

Dekan der Philosophisch-Naturwissenschaftlichen Fakultät

LIST OF PAPERS

This PhD thesis is written based on the following papers:

1. **Vu-Van, T**., P. Pham-Duc, M. S. Winkler, C. Zurbrügg, J. Zinsstag, H. Le Thi Thanh, T. H. Bich and H. Nguyen-Viet (2017). "Ascaris lumbricoides egg die-off in an experimental excreta storage system and public health implication in Vietnam." International Journal of Public Health **62**(1): 103-111.

2. **Van Vu, T.**, P. D. Pham, M. S. Winkler, C. Zurbrügg, J. Zinsstag, B. H. Tran and H. Nguyen-Viet (2018). "Estimation of involuntary excreta ingestion rates in farmers during agricultural practices in Vietnam." Human and Ecological Risk Assessment: An International Journal: 1-11.

3. **Vu-Van, Tu** et al. "Disease Burden of *Ascaris* infection in the context of human excreta use in agriculture in Vietnam". To be submitted to Human and Ecological Risk Assessment

4. Le-Thi, T., P. Pham-Duc, C. Zurbrügg, T. Luu-Quoc, H. Nguyen-Mai, **T. Vu-Van** and H. Nguyen-Viet (2017). "Diarrhea risks by exposure to livestock waste in Vietnam using quantitative microbial risk assessment." International Journal of Public Health **62**(1): 83-91.

5. Nga, D. T., T. T. Thao, **V. Van Tu**, P. D. Phuc and H. Nguyen-Viet (2016). "Development of nutrient cycle through agricultural activities of a rural area in the North of Vietnam." Journal of Material Cycles and Waste Management: 1-9.

6. Tran-Thi, N., R. J. Lowe, J. M. Schurer, **T. Vu-Van**, L. E. MacDonald and P. Pham-Duc (2017). "Turning poop into profit: Cost-effectiveness and soil transmitted helminth infection risk associated with human excreta reuse in Vietnam." PLOS Neglected Tropical Diseases 11(11): e0006088.

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ABBREVIATIONS

BOD	Biochemical Oxygen Demand
CDC	Centers for Disease Control and Prevention
DALYs	Disability Adjusted Life Years
DNRB	Day-Nhue River Basin
EAWAG	Swiss Federal Institute of Aquatic Science and Technology
HE	Human excreta
MFA	Material Flow Analysis
MOH	Vietnamese Ministry of Health
NCCR North-South	National Centers of Competence in Research North-South
NGOs	Non-governmental organizations
PDF	Probability Density Functions
PPPY	Per Person Per Year
RRD	Red River Delta
SANDEC	Department of Water and Sanitation in Developing Countries
SDC	Swiss Agency for Development and Cooperation
SLR	Systematic Literature Review
SNSF	Swiss National Science Foundation
STH	Soil Transmitted Helminths
Swiss TPH	Swiss Tropical and Public Health Institute
UNICEF	United Nations Children's Fund
VND	Vietnam Dong
WHO	World Health Organization

SUMMARY

The use of excreta in agriculture has a long tradition in Vietnam. While this practice has advantages in terms of environmental and economic impacts, it represents potential health risks if excreta are not properly managed. International and national organizations have established guidelines for safe use of excreta in agriculture to ensure the safety of health for users. However, information on excreta treatment and health risk is still missing to refine the guidelines and policies on excreta handling and health protection. The goal of this PhD work is to identify proper options for storing and using human excreta in agriculture in a perspective of minimizing the health safety and maximizing the environmental and economic benefit.

The objective of this study was to assess the health, economic and environmental impact of human excreta use in agriculture in Vietnam. The specific objectives were as follows: i) to define the main environmental factors that influence the die-off of *Ascaris lumbricoides* egg as well as nutrient values of excreta along the process of storage and use in agriculture, ii) to assess human exposure to excreta handling along the process of storage and use in agriculture focusing on the pathways, frequency, and intensity of exposure, and then to estimate the health risk by handling human and animal waste, iii) to assess the environmental and economic impact of excreta use.

The study used an integrated approach of experiments in the field and laboratory, and coupled with health, environmental and economic analyses. First, human excreta were collected and stored in an experimental station that is built under semi-field conditions. Main environmental factors chosen for the study were temperature and pH, which are controlled by the addition of rice husk, lime and ash – locally available materials – to study the influence of these factors on the die-off of *Ascaris lumbricoides* eggs and nutrient values of stored excreta. Human exposure to excreta, main pathways of exposure as well as intensity of exposure in agricultural activities and environmental sanitation systems were assessed by participatory observation, surveys and experiments. Quantitative data on the transmission of *Ascaris lumbricoides* eggs in excreta to humans through involuntary ingestion from “soil to hand” and “hand to mouth” was collected. Health risk related to waste reuse and waste handling from biogas systems

were evaluated by quantitative microbial risk assessment (QMRA) which includes the assessment of the risk of *Ascaris lumbricoides* infection during excreta reuse and biogas handling. Material Flow Analysis was used to assess the environmental impact of sanitation systems. Finally, a Systematic Literature Review (SLR) combined with field data were used to study the profitability of human excreta reuse in agriculture. The study was conducted in Kim Bang district, Ha Nam province, Vietnam.

The results showed that the number of *Ascaris lumbricoides* eggs in all vault options stored excreta at was less than 1 egg per gram of total solid excreta at the 181st storage day, and thus the stored excreta met the World Health Organization (WHO) safety limit for reuse in agriculture. A mixture of high pH and the addition of 10% lime already reached the WHO standard for safe reuse of excreta at 111 storage days. With regard to human exposure, we estimated that a farmer may ingest 91 mg of excreta per year (95% confidence interval**:** 73-110; range: 5-1082 mg**).** QMRA simulation models predicted annual risks of helminth infection for farmer used excreta stored from 3.7 months to 6 months and used excreta stored below 3 months were 1.69±5.88% and 9.67±16.37% respectively. The annual diarrhea risk caused by exposure to biogas effluent through irrigation activities ranged from 17.4 to 21.1% (*E. coli* O157:H7), 1.0 to 2.3% (*G. lamblia*), and 0.2 to 0.5% (*C. parvum*), while those caused through unblocking drains connected to biogas effluent tanks were 22.0% (*E. coli*), 0.7% (*G. lamblia*), and 0.5% (*C. parvum*). The Material Flow Analysis (MFA) of excreta utilization showed that half of the pig manure was collected for biogas production, and the remainders were freely discharged to the commune's drainage system (25%) or directly reused in the paddy fields (25%). While wastewater in the drainage system was the biggest source of nitrogen (contributed 46%), paddy fields were the biggest source of phosphorous (contributed 55%) discharged to the Nhue River, totaling 57 ± 9 ton N and 29 ± 6 ton P, annually. Consequently, mitigation measures for nutrient resource management were proposed. The most effective option was to to reuse all excreta and manure in the paddy fields which reduces the use of chemical fertilizers by 50%. The economic analysis identified average cost-savings were highest for farmers using fresh excreta (847'000 VND) followed by those who stored over 183 days (312'000 VND) and 153 days with 10% lime (37'000 VND/yr), without considering the health care costs of treating acute or chronic soil transmitted helminth

infection.
In conclusions this PhD study could generate new knowledge on the behaviour of pathogens in human excreta in Vietnam under different handing conditions, the health, environmental and economic impact of human excreta management and its use in agriculture in Vietnam. It showed when and how excreta are safe enough to use in agriculture and how much we save lives, environmental quality and economic cost be doing so. These results can help plan the management and intervention to minimize the human health risks and maximize the benefits of human excreta and animal manure in agricultural areas of Vietnam.

Keyword: Ascaris lumbricoides, excreta use, storage, exposure assessment, health risk, environmental and economic benefit.

ACKNOWLEDGEMENT

I would like to specially thank my principal supervisor Professor Jakob Zinsstag and associate supervisor Dr. Nguyen Viet Hung for their kind supports, guidance and encouragement throughout my long candidature journey at University of Basel. Their support and specific advices helped me to clearly set the objectives and develop methods for tackling a crucial public health challenge in Vietnam – "Human Excreta Use in Agriculture: Health, Economic and Environmental aspects". I sincerely thank Dr. Nguyen Viet Hung and Dr. Pham Duc Phuc for creating opportunities for me to be involved in the sanitation and health research field in Vietnam since 2009 during the implementation of my Master of Public Health thesis, which formed a good foundation for my present study.

Being an international post graduate student from Hoa Binh, a Northern province of Vietnam, with English not being my first language and having been facing several personal challenges during my study, the support, understanding and encouragement from my supervisors and staff at Department of Epidemiology and Public Health, Swiss Tropical & Public Health Institute gave me strength to complete my research. I also would like to thank to the staff at Sandec/Eawag for their support and many inspirational discussions while I stayed there for four months with funding from the Eawag Partnership Program (EPP), especially Dr. Christian Zurbrügg – former Director of Sandec/Eawag and now member of Eawag directorate for his enthusiastic assistance in gathering and providing very useful information related to the NCCR North-South project activities and other fields is highly appreciated.

My study was conducted within the framework of the Swiss National Centre of Competence in Research (NCCR) North–South: Research Partnerships for Mitigating Syndromes of Global Change. The funding was provided from the project RP8 Productive Sanitation co-led by Dr. Hung Nguyen-Viet and Dr Ives Kengnes, PAMS1 project on Development of a training module on health risk assessment related to water, sanitation and food in Vietnam - SEA-3_01 led by Dr. Tran Thi Tuyet Hanh (HUPH) and PAMS 2 led by Dr. Nguyen Ngoc Bich (VPHA). So, my sincere appreciation and special thanks go to the support from these projects, which helped me to complete my study.

Special thank staff of the Center for Public Health and Ecosystem Research (CENPHER) and

of the Environmental Health Department at HUPH, of the International Livestock Research Institute (ILRI) Vietnam Office, Ha Nam Provincial Department of Preventive Medicine (Dr. Nguyen Cong Khuong), related local departments at Ha Nam province, the commune health centers of Hoang Tay (Mr. Le Van Tuan), The family Mr. Le Van Bao – Mrs. Vu Thi Kim Vinh, Mrs. Vu Thi Lien, Nhat Tan, Chuyen Ngoai communes for their kind support throughout the study's implementation and during the data collection phase. Great appreciation goes to my colleagues, Prof. Le Vu Anh, Dr. Pham Duc Phuc, Dr. Tran Huu Bich, Associate Prof. Hoang Van Minh, Associate Prof. Nguyen Thanh Huong, Dr. Le Thi Thanh Huong, Dr. Tran Thi Tuyet Hanh (HUPH), Prof. Phung Dac Cam and Dr. Nguyen Thuy Tram (National Institute of Hygiene and Epidemiology), Dr. Mirko S. Winkler (Swiss Tropical and Public Health Institute), Dr. Samuel Fuhrimann, Dr. Esther Schelling (Swiss TPH), Dr. Chris Zurbrügg, Dr. Christoph Lüthi (Sandec/Eawag, Switzerland) for their comments on the draft of my publications and thesis. I also wish to express my deep appreciation to Ms. Vi Nguyen, Mrs. Julie Hood, Ms. Elizabeth Lartey and Ms Talia Glickman, Ms. Kylie Cuthbertson, Dr Janna Schurer, Ms Lauren MacDonald and Ms Racher Lowe (Canadian VWB/VSF volunteers) for their kind supports in providing comments and editing the English content of the papers, which form the main components of this thesis. We would like to thank Dr. Do Trung Dung and the staff of the Parasitology and Entomology Department at the National Institute of Malariology for performing the stool examina-tions for the helminth eggs, as well as Mr. Nguyen Xuan Huan and the staff of the Department of Pedology and Soil Environment at Hanoi National University for performing humidity analysis of excreta samples. I thank Ms Tran Thi Ngan (CENPHER) for her support to edit the thesis. I thank all the people that I should thank but forgot.

Most of all my thanks go to my father Vu Dinh Tach, mother Phung Thi Hanh, mother in law Hoang Thi Kim Que, my wife Dinh Thi Dieu, older brother Vu Manh Tuan and older sister Vu Thi Thu Hang who have been encouraging and supporting me during this special and challenging journey. Sweet thanks to my lovely children, Trang and Trong for being such great and strong children while daddy was away. You two gave me strength and courage to complete my PhD journey!

Hanoi 25 May 2018

1. INTRODUCTION AND LITERATURE REVIEW

1.1. The overview of human excreta used in agriculture

Applying human excreta to agricultural fields has for long been part of the agricultural tradition in Vietnam and other countries like China (AgriCultures Network 1994, Phuc et al. 2006). Every day, each healthy human being produces on average 128g excreta (ranging from 51 to 796 g/person/day), which contains nutritional elements to plants (Rose et al. 2015). Excreta contain nutrients essential to plants such as phosphorus, nitrogen and potassium. According a review of the literature, in excreta total phosphorus ranges from 0.35 to 2.7g/person/day, total potassium ranges from 0.2 to 2.52 g/person/day and total nitrogen ranges from 0.9 to 4.9 g/person/day (Rose et al. 2015). So, it can be used as fertilizer for plants to produce human food, animal feeds, natural fibers, medical herbs, ornamentals, timber and shadow vegetation (Heinonen-Tanski and van Wijk-Sijbesma 2005).

The use of excreta in agriculture can help communities to grow more food and make use of nutrient resources. The composted human excreta, used frequently as night soils, could also improve soil structure. Excreta use can help to reduce the mining of finite phosphorus reserves and energy expended to produce chemical fertilizers. The supply of phosphorus from mined phosphate rock could peak as soon as 2033 after which it will become increasingly scarce and expensive (Soil Association 2010). Historically in Europe, phosphorus was returned to agricultural land through the application of animal manure and human excreta, but from the mid nineteenth century it was replaced by phosphate mined in distant places (Cordell et al. 2009). To date, human excreta could play a key role in securing future food security, help to prevent a sharp drop in yields of crop such as wheat due to a shortage of phosphorus inputs (Nigel Hunt 2010, Soil Association 2010). Nowadays, human excreta and urine might be considered as an energy resource. Globally, biogas generation made from human waste could be valued annually at up to US$ 9.5 billion worth of natural gas as well as producing electricity enough to supply over 138 million homes (Schuster-Wallace C.J. et al. 2015). In the northern provinces of Vietnam, 85% of the households use excreta as fertilizer in agriculture (Phuc et al. 2006). If human excreta use replaces imported chemical fertilizers, Vietnam would save money at least US$ 83 million per year (Jensen et al. 2010). However,

from a health risk perspective, using inadequately treated human excreta is considered as unsafe, due to the presence of pathogens in human excreta. Enteric infections can be transmitted by enteric bacterial pathogens, enteric viruses, the parasitic protozoa and geohelminth (World Health Organization 2006a), and health burden due to this is important in poor population. While in rural households pathogens are likely the sole risk for humans, waste water from cities may also be mixed with chemical pollutants which are a further risk for their use in agriculture.

1.2. Treatment of human excreta

In order to maximize public health protection and the beneficial use of important resources, the excreta should be treated before they are used as fertilizer and the treatment methods should be validated. We present here some key common methods of treating human excreta that are practiced in Vietnam and the product is used in agriculture as fertilizer.

Composting

The composting of faeces in pits can significantly reduce the number of helminth eggs and pathogens. Composting may be divided into two categories, which are anaerobic and aerobic composting. Anaerobic composting is the decomposition of organic wastes in the absence of oxygen, with the end-products being methane (CH_4), CO_2, NH_3 and trace amounts of other gases, and other low molecular-weight organic acids. Aerobic composting is the decomposition of organic wastes in the presence of oxygen, with the end-products being CO_2, NH_3, water and heat (Chongrak Polprasert 2007). Aerobic composting can release more heat energy resulting in a rapid decomposition rate by aerobic microbes, so this category has been a preferred technology for large quantities of organic wastes. Because of its simplicity, anaerobic composting has found some application in many rural areas of developing countries in the stabilization of wastes generated from household and farms (Chongrak Polprasert 2007).

The effectiveness of a composting process is dependent upon the groups of organisms that inhabit and stabilize the organic wastes (Chongrak Polprasert 2007). The unbalanced chemical and physical conditions in the compost piles can be unfavorable in microbial growth. The nutrient balance, particle size and structural support of compost pile, moisture, aeration, temperature and pH are the major environmental parameters, which we need to control properly in the aerobic composting. The most important nutrient parameter is the carbon/

nitrogen (C/N) ratio. C/N ratio ranging between 20/1-40/1 can be considered as an optimum for biological processes (Chongrak Polprasert 2007). A moisture content of between 50-70% (average 60%) is most suitable for composting and should be maintained during the periods of active bacterial reactions (Chongrak Polprasert 2007). Sufficient oxygen is needed to provide properly for the aerobic microbes. If aeration cannot supply sufficient oxygen, aerobic conditions prevail only at the outer surface of the compost, while anaerobic conditions exit inside. Therefore, the composting rate is slow and requires a longer composting period. The temperature is approximately 55^0C, which is optimal for both the breakdown of organic material and pathogen inactivation (Chongrak Polprasert 2007). Temperature can be controlled by the adjustment of aeration and moisture content. In regard to pH, aerobic composting normally proceeds at a neutral pH and rarely encounters an extreme pH drop or rise (Chongrak Polprasert 2007).

Due to the prevailing design of the latrine and the three annual cropping seasons, it was found that for a minimum of one cultivation season per year, 74% of the households have only 3-4 months for composting before the input is needed in production, which is shorter than the 6 months composting time stipulated in the national guidelines. The community associated great benefits from using human excreta in agriculture, especially if composted, and did not associate risks with the use of composted excreta if it was dry and lacked bad odor. It is recommended to revises the guidelines will be revised, and attempts are made to identify ways of reducing the time needed to ensure the die-off of helminth eggs, including the use of pH regulators, such as an increased use of lime in the latrine (Peter Kjaer Mackie Jensen et al. 2008).

There are guidelines for excreta treatment depending on the municipal levels. The recommendation for storage treatment of dry excreta and fecal sludge before use at household and municipal levels corresponding to the WHO guideline, excreta should be composted during time > 6 months with pH > 9, temperature > 35ºC and moisture < 25% (World Health Organization 2006a). In Vietnam, the Ministry of Health promulgated the decision No. 08/2005/QĐ-BYT, regarding issuing the sector standards: hygiene standards for various types of latrines (Ministry of Health 2005b). The decision stipulated the construction of latrine and methods of using and storing excreta. Human excreta are often managed in composting stacks placed in surrounding fields. To facilitate composting, the excreta is typically mixed with

different types of organic material, e.g. urea, lime and occasionally probiotic products. Mud or sheets of plastic may be used to cover the manure to protect the stack from rain and animals. According to the MOH decision, the stacks are usually stored at least 6 months depending on different crop requirement for fertilizer (Phuc et al. 2006).

Figure 1.1 Composting excreta in Ha Nam, Vietnam

Biogas

Treating human waste through anaerobic digestion system is an incredibly effective sanitation technology. Simple biogas technology has gained popularity among rural pig farmers in Vietnam, as the anaerobic bio-digestion process generates gas, reduces odour and provides an effluent with a high fertilizer value that can be used to fertilize field and garden crops and fish ponds (An 2002). Anaerobic digestion occurs in a bio-digester and reduces *Biochemical Oxygen Demand (BOD)* value in sewage, conserves nutrients (especially nitrogen compounds) and most importantly reduces pathogens.

Various attempts have been made to implement biogas digesters at a wider scale in Vietnam, but none has achieved long-term success to date because each biogas digester design have different advantages and disadvantages and the lifetime of each biogas digester depends on many factors such as location, cost, and required skills (Nguyen et al. 2012). Biogas technology has been used in Vietnam since 1960, but since 1990 biogas technology and

research on biogas have strongly developed in both quality and quantity. The technology is supported by the Vietnamese government and by non-government organizations (NGOs) (Khai and Luong 2010). There are several biogas models in operation for livestock management (Nguyen, Phan, and Vo 2012) and the fixed-cover spherical dome- shaped digester build with bricks is popular among farmers in northern Vietnam. This biogas unit consists of three components: the inlet tank where pig slurry enters the biogas digester; the anaerobic digestion tank where the manure is digested and CH_4 is produced, and the compensation (collection) tank, where the effluent is collected. Some farms have also built an effluent tank to collect excess effluent from the digester.

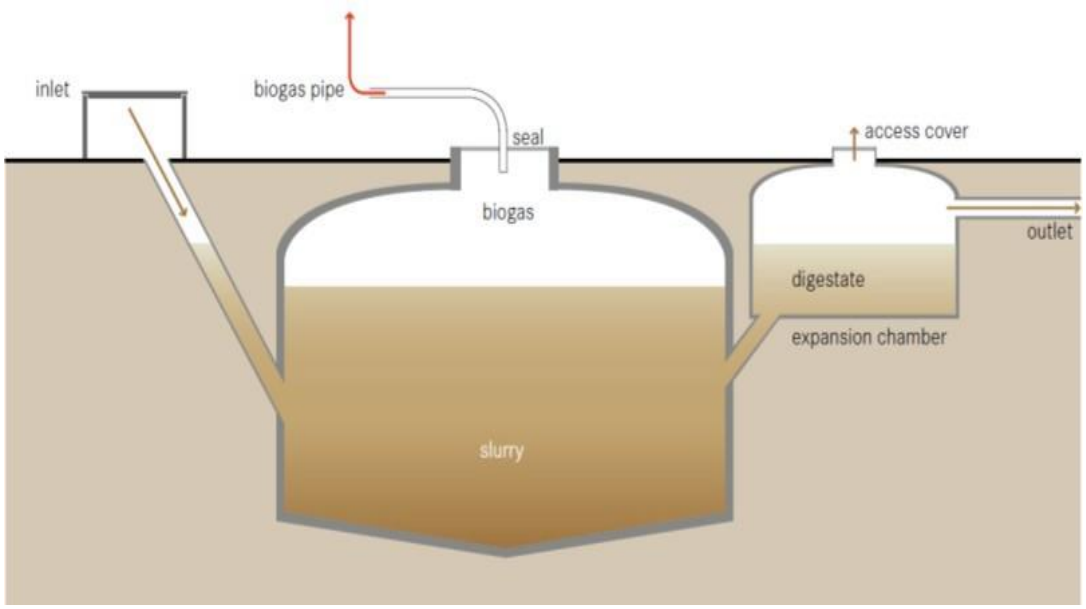


Figure 1.2. A scheme of a Biogas system [source adapted from (Tilley et al. 2014)]

Septic tank

A septic tank is the most common small-scale decentralized treatment unit and is a form of on-site sanitation that provides the convenience of a sewerage system. It is usually linked to flush toilets and can receive domestic wastewater. Since flush toilets tend to use large amounts of water, septic tanks are usually appropriate only for households with water piped into the home. The tank is offset from the house and linked to the toilet and domestic wastewater by a short drain. It is designed to hold solids and is linked to a soak away to dispose of liquid waste (effluent) (Tilley et al. 2014).

Septic tanks generally require relatively large amounts of land and periodic emptying by

vacuum tankers. This is often expensive, and the trucks will need easy access to the tank. Septic tanks thus tend to be high-cost solutions for improving sanitation. They are commonly used only by communities whose members have access to water supply within the home, have land available and who can afford the cost of emptying the tanks. Communal septic tanks may be feasible if a large number of households close to the tank can be connected with very short lengths of sewer pipe. For such a system to work, however, each household needs sufficient water to flush faeces into the septic tank effectively. This approach will probably be effective only when water is supplied to at least one tap on each plot (Tilley et al. 2014).

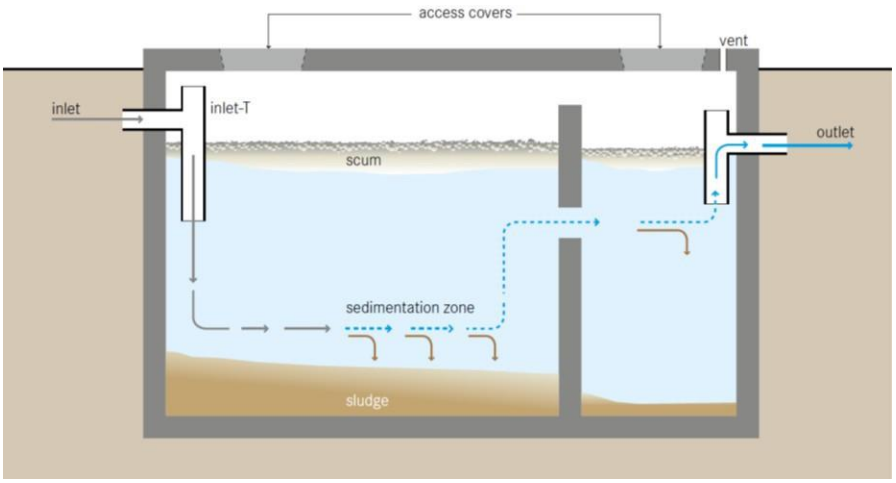


Figure 1.3. Septic tank [source adapted from (Tilley et al. 2014)]

1.3. Factors affecting human excreta composting and survival of feacal organisms

According to the literature, the main factor influences the die-off rate of microorganisms in process of composting excreta was pH, especially values above 10. The combined effect of high pH and low moisture content seemed to have contributed to the quick die-off (Anneli Carlander and Westrell 1999). In a laboratory experiment, lime treatment and storage of sewage sludge at pH>12 for at least three months completely destructed the ability of *A. suum* eggs to embryonate (Lis Eriksen et al. 1995). In contrast, in a laboratory, the study on the inactivation of fecal coliforms and *Ascaris* ova in faeces by lime, based on the results obtained of this study it is suggested that lime (pH>12) or OH^- possesses ovicidal effects upon *Ascaris*, through at a low efficiency, is encouraging, and research is currently underway to improve the vicinal efficiency of lime through combined treatment with other physic-chemical methods (Polprasert and Valencia 1981). On the other hand, regardless of the

starting pH, which varied from 9.4 to 11.6, by inactivating > 99% of all *A. suum* eggs in human excreta during a storage period of only three months in the commonly used double vault composting latrine, in which urine is not separated, could therefore potentially provide a hygienic acceptable fertilizer (Peter KM Jensen et al. 2009).

Regarding incineration of fences that offers a treatment method that is useful in reducing the final quantities of faeces and it is also useful as a sanitation method for fences. However, Nitrogen loss of 90-94% and loss of available phosphorus of 70-94% mean that incineration is not a good method where the use of nutrients is a high priority (Niwagaba Charles, Nalubega M. , et al. 2006). Charles Niwagaba investigated that successfully thermally compost source separated faeces/ash and attain sanitizing temperatures to disinfect with *Escherichia coli* and total coliforms were reduced below detection in composts that maintained above 50^0C at least six days (Niwagaba Charles, Nalubega M., et al. 2006). The mixture of faeces/ash was higher alkaline mineral content (giving high pH) and lower moisture of ash compared to mixtures of faeces/sawdust. The die-off of *E. coli* in the faeces/ash mixture was faster initially (first seven days) compared to that achieved in the faeces/sawdust mixture (Niwagaba et al. 2009).

On the other hand, liming seems as an advanced treatment for sludge sanitization with helminth eggs elimination. Liming must produce a homogeneous mixture at a pH of 12 or more and must maintain this mixture in a time/temperature regime leading to a negligible level of viable *Ascaris* eggs. The result has demonstrated in the four investigated situations, either 75 min at 55^0C or eight min at 60^0C will lead to a negligible level of viable *Ascaris* eggs (Capizzi-Banas et al. 2004). During alkaline sludge stabilization, the reported inactivation of *Ascaris* eggs is highly variable. At 30^0C and 40^0C, raising pH from 7 to 12 decreased t_{99} (the time for 99% inactivation), but at 20^0C no pH effect was seen over 80 days (Pecson et al. 2007). Nevertheless, high *Ascaris* removal efficiency (90- 100%) was reached after 80 days due to heat generation during the composting process, thus exposing the helminth eggs for more than 1 month to temperature over 45^0C (Kone et al. 2007).

Because temperature and pH all contributed to *Ascaris* egg inactivation, it is essential that these parameters are measured and accounted for when assessing the effectiveness of alkaline stabilization. In fact, as mentioned earlier, limited research has been done on *Ascaris lumbricoides* eggs survival in faecal material with real conditions. A current project led by London School of Hygiene and Tropical Medicine and funded by Gates foundation is looking

for bio-additives in order to facilitate composting process of pit latrines. The project proposes new concepts for on-site sanitation based on bio-additives and pit design the decomposition processes occurring in the pit what is carrying out in Asia and Africa (Bill and Melinda 2011).

1.4. Health risks related to the use of excreta

There is available evidence showing that possible health risks of wastewater and excreta use include diarrhea, skin infection, parasitic infection, bacterial infection, and epilepsy for people who use it and their community members who consumed contaminated fish, vegetables, or fruits (Lam et al. 2015). Globally, 842,000 people die every year from diarrheal diseases, that is attributed to unsafe water supply, inadequate sanitation and hygiene. Among children under 5 years old, 361,000 deaths could be prevented, mostly in low middle-income countries (Prüss-Ustün et al. 2014). Concurrently, 1.7 billion people suffer from low to high intensity intestinal helminths infections, which often lead to severe consequences such as cognitive impairment, massive dysentery, or anemia (Pullan et al. 2014). These diseases cause around 4.98 million years lived with disability (YLDs) (Pullan et al. 2014). According to WHO/UNICEF (2015), 2.4 billion people lacked access to improved sanitation (UNICEF and World Health Organization 2015). On the other hand, approximately 90% of the sewage in cities in developing countries is discharged untreated, polluting rivers, lakes and coastal areas and seriously affecting the environment (Steven A. Esrey 2000). Pooled estimates from all meta-analyses indicated that at least a 33% reduction in odds of infection associated with long-term solutions require improvements in water, sanitation, and hygiene practices or access (Strunz et al. 2014).

Most pathogenic or potentially intestinal pathogenic micro-organisms enter a new host by ingestion (water, food, fingers, dirt on lips, aerosols caught in the nose and swallowed) or through the lungs (after inhalation of aerosol particles) or through the eye (when eyes are rubbed with contaminated fingers) (Feachem et al. 1983) while others may also enter through the skin or wounds. After infecting the host, large numbers of pathogens may be excreted. Depending on the health of the population, several species of pathogenic bacteria, viruses, parasitic protozoa and helminths may be found in the faeces from the population and thus also in its mixed wastewater. From a hygiene point of view, any exposure to faeces constitutes a risk (Feachem et al. 1983, Holmqvist and Stenstrom 2001, World Health Organization 2006a). The selected pathogen that may be excreted in faeces and diseases and symptoms that

they cause (Antony Daisy and S.Kamaraj 2011) are given in Table 1.1. It should not be forgotten than in addition to infectious pathogens, waste water is also polluted with heavy metals and chemical pollutants, requiring solutions at the source to avoid jeopardizing agricultural production in the long term.

Table 1.1. Pathogens and its harmful effects (Source: Antony Daisy and S. Kamaraj 2011)

Group	**Pathogens**	**Diseases/symptoms**
Bacteria	*Aeromonas spp.,* *Camplyobacter jejumi/coli* *Escherichia coli* (EIEC, EPEC, ETEC, EHEC) *Salmonella typhi/paratyphi* *Salmonella spp. Shigella spp.* *Vibrio cholerae*	Enteritis Campylobacteriosis-diarrhoea, cramping, abdominal pain, fever, nausea, arthritis, Guillain-Barre syndrome Enteritis. For EHEC there are also internal haemorrhages that are sometimes lethal Typhoid/ paratyphoid fever- headache, fever, malaise, anorexia, brady cardia, splenomegaly, cough Salmonellosis- diarrhoea, fever- abdominal

		cramps Shigellosis-dysentery (bloody diarrhoea), vomiting, cramps, fever, reiter's syndrome Cholera-watery diarrhoea, lethal if severe and untreated
Virus	Adenovirus Enteric adenovirus 40 & 41 Enterovirus types 68-71 Hepatitits A Hepatitits E Poliovirus Rotavirus	Various; respiratory illness, here added due to enteric types (see below) Enteritis Meningitis; encephalitis; paralysis Hepatitits-fever, malaise, anorexia, nausea, abdominal discomfort, jaundice Hepatitits Poliomyelitis- often asymptomatic, fever, nausea, vomiting, headache, paralysis enteritis
Parastitic Protozoa	*Cryptosporidium parvum* *Cyclospora cayetanensis* *Entamoeba histolytica Giardia intestinalis*	Cryptosporidiosis-watery diarrhoea, abdominal cramps and pain often asymptomatic; diarrhoea, abdominal pain Amoebiasis-often asymptomatic, dysentery, abdominal discomfort, fever, chills Giardiasis- diarrhoea, abdominal cramps, malaise, weight loss

Helminths	*Ascaris lumbricoides* *Taenia solium/ saginata* *Trichuris trichura* Hookworm *Schistosoma spp.* (blood fluke)	Generally no or few symptoms; wheezing; coughing; fever; enteritis; pulmonary eosinophilia Taeniasis Trichuriasis-unapparent through vague digestive tract distress to emaciation with dry skin and diarrhoea Itch; rash; cough; anaemia; protein deficiency Schistosomiasis, bilharzias

In Vietnam, the use of excreta in agriculture and aquaculture is still a common practice in some areas and the health risk related to this practice is a public health issue, in particular in the North and Centre regions of the country. Health risks associated with excreta using are linked mainly to occupational exposure of those who handle the excreta and consumption of potentially contaminated products. The results from a previous study showed that the prevalence of people infected with *Ascaris* is 44.4%, with *Trichuris* is 23.1%, and with hookworm is 28.6% (Wim van der Hoek et al. 2003). Infection with roundworm (*Ascaris*), whipworm (*Trichuris*), hookworm (*Necator* and *Ancylostoma*), and *Clonorchis sinensis* are widespread in Vietnam and with a declining trend in the prevalence of *Ascaris* and *Trichuris* from the North to the South of the country (Wim van der Hoek et al. 2003). A previous study in Vietnam showed that the prevalence of *Clonorchis sinensis* varied considerably, from a low of 0.2% in Thai Binh province to 26% in Nam Dinh province (De et al. 2003). The protozoan parasites such as *Cryptosporidium* and *Giardia* infect the gastrointestinal tract of humans and animals and cause severe diarrheal illness. Common to these organisms are the formation of environmentally resistant cysts, that are shed in faeces and contaminate soil, water, and the food chain (Fayer et al. 2000, Slifko et al. 2000). In Vietnam, the contact with wastewater is an important risk factor for dermatitis, helminth infection and diarrheal among farmers engaged in wastewater-fed peri- urban food production (Vuong et al. 2007, Lam et al. 2015). Although

the burden of helminth infection is high, general helminth infections are often given low priority by authorities as they are not associated with high mortality. However, there is strong evidence that helminth infections result in high morbidity, reduced growth among children, increase the risk of malnutrition and has negative impacts on the learning capabilities of children (Phiri et al. 2000, Stephenson et al. 2000). In this thesis, *E. coli*, *Ascaris lumbricoides*, *Cryptosporidium* sp. and *Giardia* sp. were enteric pathogens of interest for the study.

E. coli

E. coli is subset of the group comprising total coliforms. Since the development of bacterial culture media that are highly specific and indicative, most recent studies report *E. coli* rather than the thermotolerant coliforms. *E. coli* is a part of the normal mammalian intestinal flora and originate from excreta of human and warm-blooded animals. Some bacteria included in the group of total coliforms may be of environmental origin (Fuhrimann, Pham-Duc, et al. 2016). Therefore, their present and concentration in the environment, food and drinking water is an useful indication of the level of faecal contamination (World Health Organization 2004). In Vietnam, thermotolerant coliforms and/or *E. coli* are measured as faecal contamination indicators as part of the Vietnamese standards for water quality guidelines using for drinking and other domestic purposes (QCVN 01: 2009/BYT and QCVN 02: 2009/BYT).

Cryptosporidium sp.

Cryptosporidium is a parasite found in food and water that has been contaminated by feaces from humans or animals. Cryptosporidiosis is a disease that causes watery diarrhea and can infect any person, but it is likely to develop more serious illness in immune-compromised individuals, especially those with AIDS (CDC, 2016). *Cryptosporidium* has a complex life cycle with both sexual and asexual stages, which can complete within a single host (Keusch et al. 1992). *Cryptosporidium* begins its life cycle as sporulated, mature oocysts which enter the environment through the feaces of the infected host. The infective oocysts reside in food, water bodies and drinking water sources. Once ingested oocysts encyst in the gastrointestinal tract and release the infective sporozoites (Current and Garcia, 1991). Following several cycles of asexual multiplication (sponrogony, merogony and gametogony), merozoites then develop into thick-walled oocysts that will exit the host or thin-walled oocysts, which autoinfect the host. Only thick-walled oocysts are robust and can survive outside the host and

very resistant to chlorine disinfection, while thin- walled oocysts quickly die off after excretion. Once being excreted into the environment, the *Cryptosporidium* oocysts can survive for an extended period of time until a new host is encountered. *Cryptosporidium* can be spread in several different ways where contaminated food or water is the most common modes of transmission. Propagation of *Cryptosporidium* may take place in the environment without a host (Centers for Disease Control and Prevention 2016).

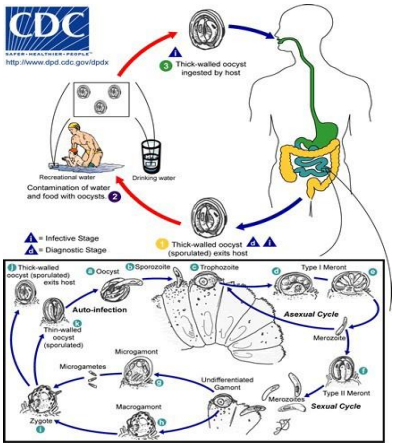


Figure 1.4. Life cycle of *Cryptosporidium* (Source: www.cdc.gov)

Giardia sp.

Giardia is a genus of anaerobic flagellated protozoan that colonize and reproduce in the small intestines of several vertebrates, causing giardiasis. The parasites have been found to affect humans and a range of domestic and wild animals throughout the world. During the life cycle of *Giardia*, the parasite is shed with the feaces as an environmentally robust cyst. Cysts are resistant forms and responsible for the transmission of giardiasis. Infection occurs via the faecal-oral route either directly via hand or fomites or indirectly by the ingestion of *Giardia* cysts in contaminated drinking water and food. In the small intestine, excystation releases trophozoites and they can be free or attached to the mucosa by a ventral sucking disk. The cyst is the stage found most commonly in non-diarrheal feaces. In addition, since the cysts are infectious when passed in the stool or shortly afterwards, person-to-person transmission is also possible. Giardiasis occur in different parts of the world. However, the disease is more common in overcrowded developing countries that lack adequate sanitary conditions and water quality control is still limited (Centers for Disease Control and Prevention 2016).

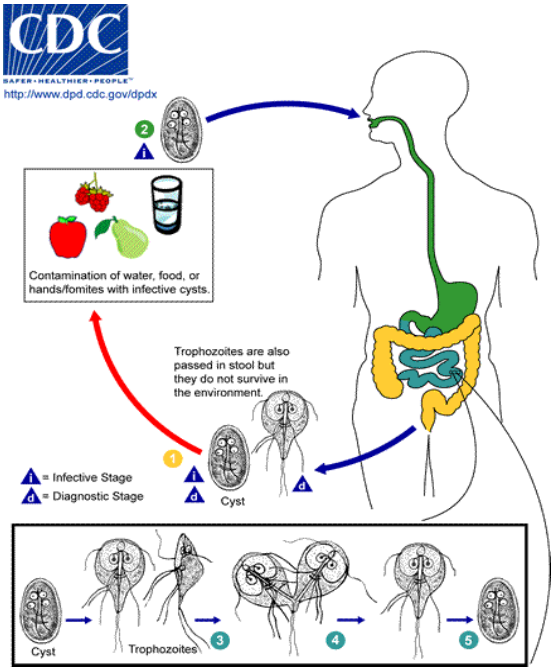


Figure 1.5. Life cycle of *Giardia* (Source: www.cdc.gov).

Ascaris lumbricoides

Ascaris lumbricoides is the largest nematode parasitizing the human intestine, which is approximately 15-35 cm in length in adulthood. *Ascaris* lives in the intestine and the eggs are passed in the feaces of infected persons. If the infected person practices open defecation or if the feaces of an infected person are used as fertilizer, eggs are deposited on soil, where they can then mature into a form that is infective. Ascariasis is caused by ingesting eggs, which can happen when dirty hands or fingers are put in the mouth or by consuming contaminated vegetables or fruits that have not been carefully cooked, washed or peeled. During the time frame of pulmonary symptoms, eggs are not being shed, and thus diagnosis is not possible. Eggs are not shed in stool until roughly 40 days after the development of pulmonary symptoms. After migrating up the respiratorytract and being swallowed, they mature, copulate, and lay eggs in the intestines. Adult worms may live in the gut for 6-24 months, where they can cause partial or complete bowel obstruction, or they can migrate into the appendix, hepatobiliary system, or pancreatic ducts and rarely other organs such as kidneys or brain (Centers for Disease Control and Prevention 2016).

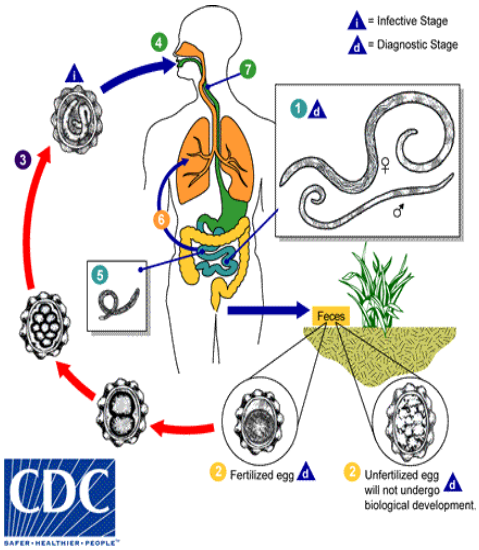


Figure 1.6. Life cycle of *Ascaris lumbricoides* (Source: www.cdc.gov)

1.5. Quantitative microbial risk assessment due to exposure to human excreta

The Quantitative Microbial Risk Assessment (QMRA) framework has been developed to evaluate the risks of transmission of infectious diseases related to the local use of faeces as fertilizer(Schonning et al. 2007). On the other hand, QMRA also was used to estimate the risk of treated sludge regarding helminth ova. QMRA data suggested addition protection measures, such as bio-solid application rates, crop restriction, and produce better washing practices (Navarro et al. 2009). *Ascaris* eggs need to reduce by wastewater treatment and peeling prior to consumption rely on the result of quantitative microbial risk analyses (Mara and Sleigh 2010). Other study using QMRA indicated very high water-related infection risk levels compared to the actual locally recorded disease occurrences (Yajima and Koottatep 2010). From a global perspective, QMRA is also recommended as a tool to assess the health risk of wastewater and excreta reuse in agriculture (World Health Organization 2006a). In Vietnam, QMRA was used to assess the risk related to wastewater in agriculture and flooding condition (Yen-Phi 2010, Pham Duc Phuc 2012). In addition, QMRA was also used to estimate the health risk of human exposure to biogas effluent in Ha Nam province (Le-Thi et al. 2017) and wastewater reuse in Hanoi (Fuhrimann et al. 2017).

1.6. Nutrient flow and economic value of human excreta

Adapted Material Flow Analysis (MFA) is widely known as a useful tool for identifying environmental problems by quantifying mass flows in an environmental sanitation system and

for forecasting the impact of possible interventions on the environment (Misselbrook et al. 2000, Montangero and Belevi 2007, Montangero et al. 2007). The adapted MFA methodology has been successfully applied in Vietnam, in the whole Day–Nhue River Basin(DNRB) (Do TN et al. 2013, Do et al. 2014, Do and Nishida 2014) in Hanoi, an urban area in the DNRB (Montangero and Belevi 2007, Montangero et al. 2007), and in Ha Nam, a rural area belongs to DNRB where environmental sanitation and agricultural activities are closely interlinked (Do-Thu et al. 2011). Connections of the environmental sanitation and agricultural system in terms of nitrogen and phosphorous were quantified in the case study of Hoang Tay and Nhat Tan communes in Kim Bang district, Ha Nam province, Vietnam (Do and Nishida 2014). Recognizing the potential nutrient sources from manure and human excreta, we originally analyzed the close interlinks of livestock (pig and poultry) with on-site sanitation system and agricultural system (paddy field and fishpond) in a commune located along the bank of the polluted Nhue River.

Farmers in some agricultural regions of Vietnam have used their sources of fertilizer from human excreta to commercial inorganic products, either wholly or in part. It is unclear whether this trend will become universal as not all farmers are able to afford or access commercial fertilizer, and others consider human excreta a superior source of long-term nutrition for plants and soil (Jensen et al. 2008). Inorganic fertilizers are primarily imported, and their costs are influenced by a wide range of factors, including energy prices (Food and Agriculture Organization of the United Nations 2015). Using human waste to fertilize crops is recognized as a way to decrease household expenditures; however, it is unclear how costs and health risks associated with STH infection interact. We have conducted the study to compare the costs and STH risk associated with fertilizing rice paddies in the Red River Delta (RRD) and there is a need to study ways to minimize the human health risks and maximize the benefits of human excreta and animal manure in agricultural areas of Vietnam.

2. AIM AND OBJECTIVES

Aim

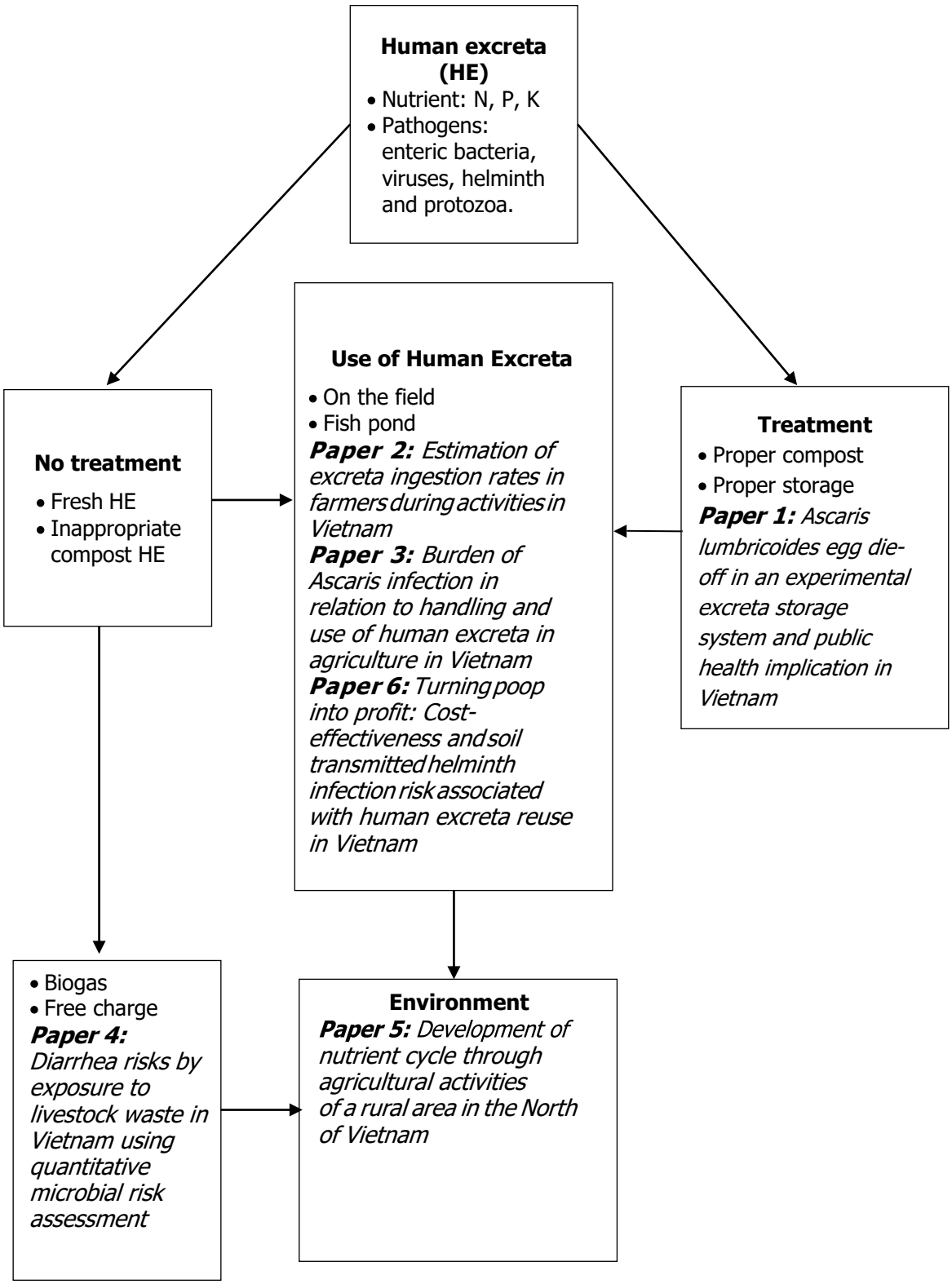
This PhD work aimed to minimize the human health risks and maximize the benefits of human excreta and animal manure in agricultural areas of Vietnam.

Objectives

This PhD has the following objectives:

1. To determine the *Ascaris lumbricoides* eggs die-off in different storage of human excreta options.
2. To estimate the amount of human excreta accidentally ingested by farmer during use of human excreta in agriculture.
3. To assess human helminth infection and diarrheal risks in relation to use of stored human excreta and biogas wastewater in agriculture.
4. To analyse the economic value of human excreta used as fertilizer in agriculture.
5. To analyse the nitrogen and phosphorus flow in the environment from human excreta and animal manure

Figure 2.1. Presents the conceptual framework



3. METHODOLOGY

3.1 Description of the study sites

Hoang Tay commune, a peri-urban agricultural area, located in Kim Bang district, Ha Nam province, in Northern Vietnam, was chosen as the study site for the intervention phase (Figure 3.1). Approximately 5,761 people reside in this area (1,762 households), and some parts of their income source were derived from agricultural production, such as rice cultivation. Application of human excreta as fertilizer was 13.73% of households and the presence of unimproved latrines (World Health Organization and Unicef 2017) was 37% of households (Hoang Tay People's Committee 2016). The economic basis of the commune relies on both livestock and crops. The total swine population commune was 6500 in 2015 and almost all of them were raised at small household scale. Household biogas was used to treat manure and human excreta. While the treatment technology looked promising and clean, the treatment quality and health risk posed by biogas effluent are questionable.

The residents of the study area were found to experience STH infections with hookworms (2%), A. lumbricoides (24%) and T. trichiura (40%) being the pre-dominant species (Pham-Duc et al. 2013).

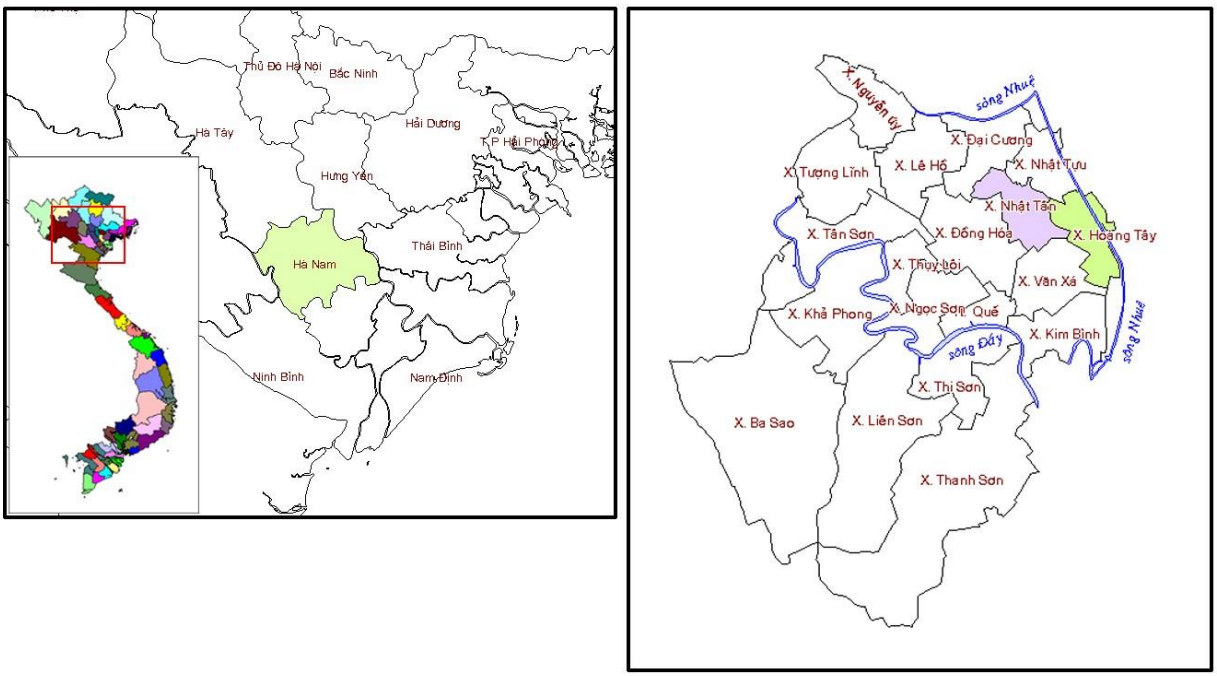


Figure 3.1. Study site of Hoang Tay Commune, Kim Bang, Ha Nam Province

3.2 Experiment in the field for determining the existence of Ascaris lumbricoides eggs in different human storage options and providing data for assessing health risk by using stored human excreta as fertilizer in agriculture

We constructed 24 identical vaults with a size of 0.4 m (width) × 0.4 m (length) × 0.7m (height), with bricks and cement for the experiment in Hoang Tay commune (Figure 3.2). Details of experimental design, excreta storage vaults, excreta sample collection and experimental data collection were described in Chapter 5 *(Paper 1: Ascaris lumbricoides egg die-off in an experimental excreta storage system and public health implication in Vietnam*).

Figure 3.2. The vaults built for the study.

3.3 Exposure to excreta handling

We estimated the amount of human excreta accidental ingested by farmer during use of human excreta in agriculture by quantifying farmer ingestion of human excreta during normal agricultural practices in three steps. 1) we quantified the weight of excreta that remained on farmers' hands after handling waste in the field and in the laboratory; 2) we conducted a laboratory simulation to estimate the weight of excreta that would remain on a farmer's mouth after contact with a soiled glove; 3) we quantified the frequency of contact between soiled gloves and mouth by observing farmers while they took excreta from their latrine for storing, composting, and applying on the field. All steps were described in detail in Chapter 5 *(Paper 2: Estimation of excreta ingestion rates in farmers during activities in Vietnam).*

3.4 Assess health risks related to excreta and wastewater handing

We used quantitative microbial risk assessment (QMRA) for assessing the health risk of

farmers. This included 2 contexts i) assessment of human helminth infection and diarrheal risks in relation to use of stored human excreta and biogas wastewater in agriculture. The data used for QMRA was combination of the data on experiment in the field in Chapter 6 *(Paper 3: Burden of Ascaris infection in relation to handling and use of human excreta in agriculture in Vietnam*)**.** ii) QMRA for biogas wastewater in Hanam Province, Vietnam. A total of 451 representatives from households that use biogas were interviewed about their practices of handling biogas plant and reuse of biogas effluent for irrigation. In addition, 150 samples of biogas wastewater were analyzed for Escherichia- coli, Cryptosporidium parvum, and Giardia lamblia. We used Monte Carlo simulation for calculating risk characterization. All steps of QMRA in this study were described in Chapter 7 (*Paper 4**:** Diarrhea risks by exposure to livestock waste in Vietnam using quantitative microbial risk assessment).*

3.5 Analysis of the nitrogen and phosphorus flow in the environment from human excreta and animal manure

We applied material flow analysis (MFA) to assess the environmental impact of human activities on nutrient flows at the commune scale. Human excreta and animal manure were considered as a nutrient source for paddy fields and fishponds in Hoang Tay commune, Ha Nam province, Vietnam. Development of the MFA model, uncertainty analysis, development of scenarios and sensitivity analysis were described in Chapter 8 (*Paper 5**:** Development of nutrient cycle through agricultural activities of a rural area in the North of Vietnam*).

3.6 Analysis of the economic value of human excreta used as fertilizer in agriculture.

We investigated the cost-savings and infection risk of soil transmitted helminths (STHs) in four scenarios where farmers used either inorganic fertilizer or fresh/composted human excreta (153 days storage with 10% lime and 181 days storage without lime) supplemented by inorganic fertilizer to meet the nutrient requirements of rice paddies in the Red River Delta, Vietnam. Details of the method were described in Chapter 9 (*Paper 6: Turning poop into profit: Cost-effectiveness and soil transmitted helminth infection risk associated with human excreta reuse in Vietnam*)**.**

3.7 Data analyses

We use Microsoft Excel version 2016 for data entry and management. Statistical data analysis using R 3.1.0 (The R Foundation 2016) was conducted. To conduct QMRA, both @Risk and the R first package of “fitdistrplus” were used to choose and fit parametric univariable

distributions to our given data. @Risk and the second package of "mc2d" were used to build Monte-Carlo simulations in which the estimation of variability and uncertainty in the risk estimates is separated (Pouillot and Delignette-Muller 2010).

3.8 Ethical consideration

This study was approved by the Ethics Review Board of the Hanoi University of Public Health (020/2012/YTCC-HĐ3). Before the field work, permission was obtained from the Provincial and District Health Offices of Ha Nam that was informed about the study and had partnership with the research team. Detailed information on study objectives and procedures were provided and working procedures were explained to farmers. Written informed consent was obtained from each individual prior to study enrolment.

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4. *ASCARIS LUMBRICOIDES* EGG DIE-OFF IN AN EXPERIMENTAL EXCRETA STORAGE SYSTEM AND PUBLIC HEALTH IMPLICATION IN VIETNAM

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This paper was published as:

Vu-Van, T., P. Pham-Duc, M. S. Winkler, C. Zurbrügg, J. Zinsstag, H. Le Thi Thanh, T. H. Bich and H. Nguyen-Viet (2017). "Ascaris lumbricoides egg die-off in an experimental excreta storage system and public health implication in Vietnam. International Journal of Public Health 62(1): 103-111.

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Int J Public Health
DOI 10.1007/s00038-016-0920-y

ORIGINAL ARTICLE

Ascaris lumbricoides egg die-off in an experimental excreta storage system and public health implication in Vietnam

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Received: 15 May 2016 / Revised: 29 October 2016 / Accepted: 31 October 2016
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Abstract
Objectives We studied the influence of different additive materials (lime, and rice husk) and aeration conditions on *Ascaris lumbricoides* egg die-off in 24 vaults of an experimental excreta storage unit.
Methods Excreta samples were collected once every two weeks over a 181-day period. Temperature, pH, and moisture content were recorded. *A. lumbricoides* eggs were quantitatively analyzed by the Romanenko method, which identified and counted live and dead eggs.
Results From the first sampling (0 storage day) to the final sampling (181 storage days) the average percentage of viable *A. lumbricoides* eggs decreased gradually from 76.72 ± 11.23% (mean ± SD) to 8.26 ± 5.20%. The storage time and the high pH value significantly increased the die-off of helminth eggs. Over 181 storage days, all vaults option effectively reduced *A. lumbricoides* eggs die-off.
Conclusions The best vault option, with aeration and 10% lime per total weight, met the WHO standard for excreta treatment on the 111th storage day.

Keywords *Ascaris lumbricoides* · Helminth · Human excreta · Waste reuse · Vietnam

Introduction

Globally, an estimated 438.9 million people were infected with hookworm in 2010, 819.0 million with *Ascaris lumbricoides,* and 464.6 million with *Trichuris trichiura* (Pullan et al. 2014). These infections are a common public

This article is part of the supplement "Health and social determinants of health in Vietnam: local evidence and international implications".

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Published online: 22 November 2016

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health issue in the most impoverished communities (Karagiannis-Voules et al. 2015). Although the infections decline with the improvement of sanitation (Ziegelbauer et al. 2012), further reductions in children and within their communities are still urgently needed in developing countries (Pullan and Brooker 2012). Indeed, the burden of disease is estimated at 68% of 4.98 million years lived with disability attributable to soil-transmitted helminths (STH), with 67% of such infections occurring in Asia (Pullan et al. 2014).

Helminth infections are acquired from environments contaminated by the worms' infective stages that develop from fertilized eggs. People infected with helminths pass eggs in their feces, which mature in the environment before becoming infective again (Centers for Disease and Prevention 2013). Thus, the storage and handling practices of human excreta are important factors to reduce the risk of infection with helminths (Gulliver et al. 2014). Despite the potential health risks, human excreta are a valuable resource as fertilizer for agricultural production (Jensen et al. 2008, 2010). Excreta contain nutrients such as phosphorus, nitrogen, and potassium, which are essential to plant growth. Hence, application of excreta in agriculture can help communities increase agricultural productivity through the recycling of nutrients while saving on cost for chemical fertilizers, resulting in economic benefits (Jensen et al. 2010).

Reuse of human excreta has long been part of the agricultural tradition in Vietnam. To reduce human health risks associated with excreta use in agriculture, the Vietnamese Ministry of Health has stipulated the time for human excreta storage in latrines to be at least six months before application as fertilizer (Ministry of Health 2005). However, in Central Vietnam, it has been observed that 74% of farmers do not follow the recommended storage time (Jensen et al. 2008). Excreta are often only stored for 3–6 months in vault latrines before reuse. Moreover, kitchen ash and/or lime is added to cover excreta following each latrine use and during the storage period. These practices primarily aim at reducing the moisture content, preventing foul odours and combating flies (Knudsen et al. 2008). However, it is unclear how these practices affect the elimination of viable helminth eggs over time.

The addition of lime to cover excreta in the latrine vaults has been reported to increase pH value, which may inactivate *A. lumbricoides* ova in feces; even in the laboratory with pH >12 in the solution, the efficiency of inactivating *A. lumbricoides* ova was found to be low (Polprasert and Valencia 1981). On the other hand, there has been limited research conducted on the survival of *A. lumbricoides* eggs in fecal material found in latrine vaults. Most studies used *A. suum* eggs, placing them into "tea bags" that were inserted into the vault and monitored for die-off (Jensen et al. 2009; Yang et al. 2002). There is, however, no calibration between the viability of *A. suum* eggs compared to *A. lumbricoides* eggs. As well, eggs in these experimental conditions were not necessarily impacted by the composting process in the same way as the eggs in newly deposited feces. Therefore, it is essential to have a better understanding of how *A. lumbricoides* egg deactivation actually occurs in a real context without too much speculation from experiments, and using the helminth species of interest. The objective of this study was to test the efficiency of excreta storage with additive materials and aeration modalities over time on the die-off of *A. lumbricoides* eggs in an in situ condition. This study will contribute to a more specific understanding on public health implication of helminth intervention in the context of Vietnam and other developing countries.

Methods

Study area

The study site was Hoang Tay commune, Kim Bang district, Ha Nam province. This commune is located at 20°36′ N and 105°54′E in Northern Vietnam. We set up the study on 8th February 2012 in spring season. The population of Hoang Tay commune was estimated at 5735 individuals residing in 1720 households. The main income source was from agricultural production such as rice and other crop cultivation, which frequently used human excreta and manure as fertilizer. Nearly 40% of households had access to hygienic latrines and 50% of households reported using human excreta in agriculture (Pham-Duc et al. 2013). According to a cross-sectional survey in 2008, the prevalence of helminth infections was 61.8%, with *T. trichiura* as the predominant species (60.1%), followed by *A. lumbricoides* (33.5%) and hookworm (2.6%) (Pham Duc 2008).

Experimental design and excreta storage vaults

At the research station in Hoang Tay commune, we constructed 24 identical vaults with a size of 0.4 m (width) × 0.4 m (length) × 0.7 m (height), with bricks and cement for the experiment. Each vault could hold approximately 100 kg of excreta and had a cover made of metal. The constructed vaults were situated in a covered house in a field, and resembled the excreta storage latrines that were commonly used by the local population.

The vaults were randomly assigned into four experimental options: (V1) 100 kg excreta without additive materials; (V2) 97 kg excreta with 3 kg lime; (V3) 90 kg excreta with 5 kg lime and 5 kg rice husk; and (V4) 90 kg excreta with 10 kg lime. In each vault, the mass of excreta

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with or without additive materials was 100 kg. For testing the effect of excreta aeration within the vaults, each option was further assigned to a design with and without air pipes. The air pipe system is illustrated in Fig. 1.

We collected excreta from all households that had single vault or double vault latrines in Hoang Tay commune. The excreta had been stored from three to six months within the vaults, which continuously incorporated fresh excreta as the latrines were used. To make the excreta homogeneous, a mixing process was applied. First, 100 kg of excreta was mixed by machine for 1 min at 36 rpm, which was suitably slow to avoid destroying the structure of excreta. Then, we equally divided excreta into 24 plastic boxes, each with a 240-litre capacity. We repeated the mixing and dividing activity until all collected excreta were allocated to the 24 boxes. Second, lime and rice husks were weighed according to the quantities specified in Table 1, and then added to the excreta in the 24 boxes. Third, the excreta in each plastic box were re-mixed to achieve homogeneity with the lime and rice husks, before moving the content into the experimental vaults.

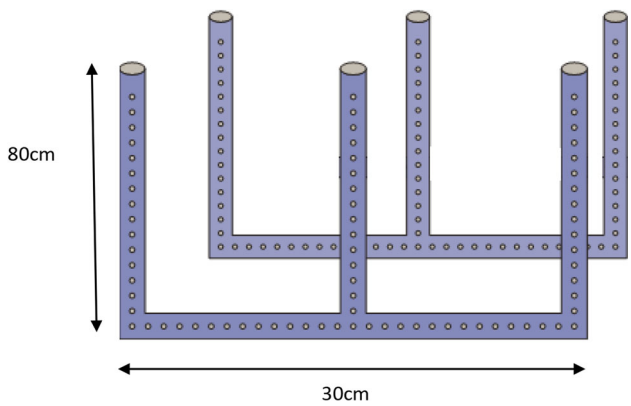

Fig. 1 The aeration system for vaults $V1_{with\ air}$, $V2_{lime3kg\ with\ air}$, $V3_{lime\ riceh\ air}$, and $V4_{lime10kg\ air}$ with three replicates for each option in Vietnam experiment 2012. The tube diameter was 35 mm. The aeration system width was 30 cm and the height was 80 cm. ($V1_{no\ air}$: the vault had 100 kg excreta without air pipe; $V1_{with\ air}$ the vault had 100 kg excreta with air pipe; $V2_{lime3kg}$: the vault had 97 kg excreta and 3 kg lime without air pipe; $V2_{lime3kg\ air}$: the vault had 97 kg excreta and 3 kg lime with air pipe; $V3_{lime\ riceh}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk without air pipe; $V3_{lime\ riceh\ air}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk with air pipe; $V4_{lime10kg}$ had 90 kg excreta and 10 kg lime without air pipe; $V4_{lime10kg\ air}$ had 90 kg excreta and 10 kg lime with air pipe)

Excreta sample collection and experimental data collection

The first samples were collected immediately after excreta and their additives were placed in the experimental vaults. Subsequently, excreta were sampled once every two weeks over the duration of 181 days, amounting to 14 samples per experimental vault, which were then subjected to laboratory analysis for the detection of *A. lumbricoides* eggs. The sampling order 1st, 2nd, 3rd, 4th 5th, 6th, 7th, 8th, 9th, 10th, 11th, 12th, 13th, 14th corresponded to excreta storage days 1, 13, 27, 41, 55, 69, 83, 97, 111, 125, 139, 153, 167 and 181, respectively. Excreta samples were collected by means of sterilized spoons at five different sampling points in the vault at a depth of 15 cm from the surface. The five samples from each vault were pooled and then split into portions: (1) approximately 40 g for *A. lumbricoides* egg analysis; and (2) 200 g for other indexes analysis, reported elsewhere. Samples for analyzing *A. lumbricoides* were temporarily stored in an ice-box and all samples were transported to the laboratory during the same day.

The pH of the vault was recorded directly by pH meter (Hanna-HI99121) during each sampling event. Log Tag Humidity and Temperature Data Loggers were placed inside the vaults and set to automatically record the

Table 1 Experimental options for excreta storage, air pipes, and additive materials in Vietnam experiment, 2012 ($V1_{no\ air}$: the vault had 100 kg excreta without air pipe; $V1_{with\ air}$ the vault had 100 kg excreta with air pipe; $V2_{lime3kg}$: the vault had 97 kg excreta and 3 kg lime without air pipe; $V2_{lime3kg\ air}$: the vault had 97 kg excreta and 3 kg lime with air pipe; $V3_{lime\ riceh}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk without air pipe; $V3_{lime\ riceh\ air}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk with air pipe; $V4_{lime10kg}$ had 90 kg excreta and 10 kg lime without air pipe; $V4_{lime10kg\ air}$ had 90 kg excreta and 10 kg lime with air pipe)

Vault options	Excreta (kg)	Additive material (kg)		Air pipe	Replicates
		Lime	Rice husk (riceh)		
$V1_{no\ air}$ (control)	100	0	0	No	3
$V1_{with\ air}$	100	0	0	Yes	3
$V2_{lime3kg}$	97	3	0	No	3
$V2_{lime3kg\ air}$	97	3	0	Yes	3
$V3_{lime\ riceh}$	90	5	5	No	3
$V3_{lime\ riceh\ air}$	90	5	5	Yes	3
$V4_{lime10kg}$	90	10	0	No	3
$V4_{lime10kg\ air}$	90	10	0	Yes	3

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temperature and humidity every four hours. Another Log Tag was set up to record the ambient temperature inside the house.

Laboratory analyses

Relative humidity (RH) of the excreta was determined by means of the following working steps and by applying formula 'Eq. 1'. One hundred grams of excreta (M1) was placed into an aluminum box with known weight (M2) and dried in an oven at 105 °C until no change in weight was registered. The aluminum box containing excreta was placed into a desiccator for 30 min and then weighed again (M3).

$$\mathrm{RH} = (M1 + M2 - M3) \times 100/M1 \quad (1)$$

M1 is gram of sample, **M2** is gram of aluminum box, **M3** is gram of aluminum box containing sample after 30 min of desiccation.

We used the Romanenko method for analyzing *A. lumbricoides* eggs (Romanenko 1968). The total weight of excreta for each sample varied from 25 to 40 grams and was analyzed using a standardised procedure. Firstly, excreta were placed into five falcon tubes, up to two-thirds capacity, and then NaOH (5%) added until the tubes were nearly full. The mixtures were stirred with a stick for 30 min. Afterwards, the tubes were centrifuged at 1000–1500 rpm for five minutes. The water layer in each tube was eliminated. Next, saturated $NaNO_3$ was added to each tube, stirred for 10 min, and then centrifuged at 1000 rpm for five minutes. Saturated $NaNO_3$ was added until a liquid convex appeared at the top of each tube. Finally, three slides were placed consecutively on each falcon tube for 20 min each. The slides were prepared with one drop of the resultant liquid and one drop of glycerine solution (50%) on top. We used a microscope with lens 40 × to identify the following: viable eggs by normal ova; dead eggs by abnormal ova (i.e., vacuolations inside the ova, congelation and vitreous transformation, irregular over cell sizes, bubbling of the cells, granulations and vacuolations or ova shell-cracked and dilapidated); infertile ova; and fertile unembryonated ova.

Statistical analyses

The percentage of viable *A. lumbricoides* eggs was calculated for each sampling by the number of viable eggs counted in that sample divided by the total number of eggs counted. The percentages of viable eggs for all vault options over the storage period were analyzed in linear regression and compared using the one-way ANOVA test to find the best fit model in repeated measures analysis. R 3.1.0 software was used for the analyses and the statistical significance was assessed using $p \leq 0.05$ (The R project for statistical computing 2016).

Results

Variation of physio-chemical factors: temperature, pH, and humidity

The average temperature in all vaults varied from 20.95 ± 2.01 to 28.44 ± 0.35 °C. The room temperature was lower than in the vaults (approximately 6 °C) during the first week, after setting up the experiment. The average pH value decreased from 10.59 ± 1.85 to 7.85 ± 0.18 (Table 2).

The initial pH values were the highest in vault options $V4_{lime10kg}$ and $V4_{lime10kg\ air}$ (Fig. 2); they decreased from 12.50 ± 0.30 to 7.94 ± 0.18 and from 11.43 ± 0.93 to 7.81 ± 0.12, respectively, over 181 days. The relative humidity (RH) decreased from 56.87 ± 3.41% to 15.28 ± 2.66% over 139 storage days, however, this was not significant.

The pH varied from the first to the ninth sampling (over 111 storage days) in all vault options ($p < 0.001$), except for the vault options $V1_{no\ air}$ and $V1_{with\ air}$ (Fig. 2). However, no significant difference in pH was observed from 111 storage days to 181 storage days. The largest reduction of pH values were in vault options $V4_{lime10kg}$ and $V4_{lime10kg\ air}$ (lime 10%), initially ranging from 12.20–12.80 to 10.38–11.45, which decreased to 7.76–8.11 and 7.71–7.95, respectively.

Die-off rates of *A. lumbricoides* eggs

The average percentage of viable *A. lumbricoides* eggs gradually decreased from 76.72 ± 11.23% on the first day of storage to 8.26 ± 5.20 % on the 181st day of storage (Table 5). This percentage decreased significantly in all vault options ($p < 0.001$) over the full duration of the study. In the control vault (option 11), the average percentage of viable *A. lumbricoides* eggs decreased from 77.27 ± 1.34% to 6.30 ± 6.47% over 181 days. However, there was no difference in reduction of the average percentage of viable *A. lumbricoides* eggs when comparing control vault option $V1_{no\ air}$ with other vault options over 181 days ($p > 0.05$). There were significant reductions in the percentage of viable *A. lumbricoides* eggs over 111 days storage (Table 3). The largest reduction of viable *A. lumbricoides* eggs was observed for the vault options $V4_{lime10kg}$ (mean ± standard deviation reduction: 79 ± 11) in comparison with vault option control $V1_{no\ air}$ (73 ± 4) (coefficient in interaction between sampling occasion and vault is the smallest with $p < 0.001$). The storage days and

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Table 2 Average temperature and pH in all vault options over 181 storage days in Vietnam experiment, 2012

	Storage day						
	1	13	27	41	55	69	83
Average temperature in vaults (°C)	20.95 ± 2.01	19.07 ± 1.03	23.21 ± 1.13	23.20 ± 0.77	23.88 ± 0.60	27.37 ± 0.80	30.58 ± 0.84
Temperature in room (°C)	14.9	18.4	25	23.1	25.1	29.7	30.8
pH	10.59 ± 1.85	9.20 ± 1.63	9.16 ± 1.81	8.86 ± 1.74	8.53 ± 1.29	8.29 ± 0.88	7.92 ± 0.34
Relative humidity (%)	56.87 ± 3.41	NA	52.19 ± 3.07	NA	44.44 ± 3.40	NA	36.19 ± 2.84
	Storage day						
	97	111	125	139	153	167	181
Average temperature in vaults (°C)	30.07 ± 0.58	30.00 ± 0.36	32.03 ± 0.30	31.24 ± 0.39	30.79 ± 0.23	30.52 ± 0.37	28.44 ± 0.35
Temperature in room (°C)	30.5	29.3	33.7	34.1	33.2	27.3	ND[a]
pH	8.03 ± 0.23	7.80 ± 0.24	7.68 ± 0.17	7.68 ± 0.13	7.78 ± 0.17	7.76 ± 0.15	7.85 ± 0.18
Relative humidity (%)	NA	25.15 ± 2.99	NA	15.28 ± 2.66	NA	NA	NA

[a] *NA* not available data; mean ± standard deviation

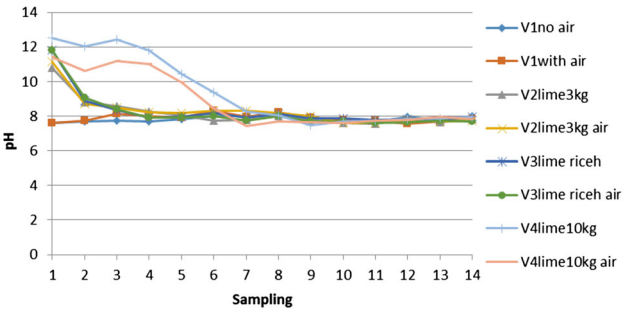

Fig. 2 Reported pH values in vault options following 14 sampling in Vietnam experiment, 2012 ($V1_{no\ air}$: the vault had 100 kg excreta without air pipe; $V1_{with\ air}$ the vault had 100 kg excreta with air pipe; $V2_{lime3kg}$: the vault had 97 kg excreta and 3 kg lime without air pipe; $V2_{lime3kg\ air}$: the vault had 97 kg excreta and 3 kg lime with air pipe; $V3_{lime\ riceh}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk without air pipe; $V3_{lime\ riceh\ air}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk with air pipe; $V4_{lime10kg}$ had 90 kg excreta and 10 kg lime without air pipe; $V4_{lime10kg\ air}$ had 90 kg excreta and 10 kg lime with air pipe)

increased pH values were associated with a significant increase in the percentage of dead *A. lumbricoides* eggs.

Discussion

This study assessed the percentage of viable *A. lumbricoides* eggs in human excreta over 181 storage days with different options of adding locally available materials. The number of eggs in all vault options at 181st storage day was less than 1 egg per gram of total solid excreta, and thus the fecal sludge met the World Health Organization (WHO) safety limit for reuse in agriculture (World Health Organization 2006a). Vault option $V4_{lime10kg\ air}$, with air pipe and 10% lime per total weight, was shown as the best combination of aeration and additive material by consistently meeting the WHO standard by the 111th storage day. This result presents a promising practice for treating excreta by storage and the addition of locally available materials, which reduces treatment time for the safe use of excreta in agriculture.

Parameter influencing *A. lumbricoides* eggs die-off

The percentage of viable *A. lumbricoides* eggs decreased significantly in vault options having a high pH (5 and 10% lime) over 111 storage days (70.08 and 73.66% reduction, respectively). Throughout the 181 storage days, the temperature of the vaults increased from 20.95 to 28.44 °C. During the first 13 storage days, the average temperature in all vaults was higher than the temperature in the room (6 °C), which might be explained by activities of thermobacteria. The values for temperatures within the vault and the room were the same after 13 storage days, and there

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Table 3 Temperature, pH, and effects of vault options and storage time on percentage of live eggs during storage of excreta over 181 storage days and 111 storage days in Vietnam experiment, 2012

	p value over 181 storage days ($n = 336$)			*p* value over 111 storage days ($n = 216$)		
	Percentage of live eggs	pH	Temperature	Percentage of live eggs	pH	Temperature
Vault options	0.058	0.077	<0.001	<0.001	<0.001	<0.001
Sampling (storage time)	<0.001	0.539	<0.001	<0.001	<0.001	<0.001
Vault options × Sampling	0.873	0.120	0.559	<0.001	<0.001	<0.001

Table 4 Average number egg per total solid of excreta in all vault option over 181 storage days among Vietnam experiment, 2012 ($V1_{no\ air}$: the vault had 100 kg excreta without air pipe; $V1_{with\ air}$ the vault had 100 kg excreta with air pipe; $V2_{lime3kg}$: the vault had 97 kg excreta and 3 kg lime without air pipe; $V2_{lime3kg\ air}$: the vault had 97 kg excreta and 3 kg lime with air pipe; $V3_{lime\ riceh}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk without air pipe; $V3_{lime\ riceh\ air}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk with air pipe; $V4_{lime10kg}$ had 90 kg excreta and 10 kg lime without air pipe; $V4_{lime10kg\ air}$ had 90 kg excreta and 10 kg lime with air pipe)

Vault options	Storage day													
	1	13	27	41	55	69	83	97	111	125	139	153	167	181
$V1_{no\ air}$	17 ± 7	22 ± 3	13 ± 2	15 ± 2	10 ± 2	7 ± 3	8 ± 4	3 ± 2	6 ± 2	2 ± 1	2 ± 0	1 ± 1	1 ± 1	0 ± 1
$V1_{with\ air}$	16 ± 11	14 ± 2	13 ± 4	7 ± 2	10 ± 2	8 ± 2	8 ± 5	4 ± 3	3 ± 2	5 ± 1	2 ± 1	1 ± 1	0 ± 1	0 ± 0
$V2_{lime3kg}$	12 ± 4	19 ± 2	13 ± 3	15 ± 5	16 ± 5	71 ± 104	13 ± 2	6 ± 3	4 ± 2	5 ± 4	3 ± 1	2 ± 1	0 ± 1	0 ± 0
$V2_{lime3kg\ air}$	16 ± 7	17 ± 6	15 ± 5	9 ± 6	38 ± 38	11 ± 5	7 ± 4	5 ± 4	2 ± 1	3 ± 2	3 ± 2	1 ± 1	0 ± 1	0 ± 0
$V3_{lime\ riceh}$	13 ± 4	11 ± 5	11 ± 6	11 ± 1	15 ± 3	10 ± 5	5 ± 3	6 ± 6	0 ± 0	4 ± 0	1 ± 0	1 ± 1	2 ± 1	0 ± 1
$V3_{lime\ riceh\ air}$	13 ± 9	10 ± 7	15 ± 4	11 ± 5	9 ± 2	6 ± 3	3 ± 0	4 ± 4	0 ± 0	4 ± 1	3 ± 3	1 ± 1	0 ± 0	0 ± 0
$V4_{lime10kg}$	18 ± 2	15 ± 7	15 ± 2	17 ± 5	6 ± 1	5 ± 0	3 ± 1	3 ± 2	1 ± 2	1 ± 2	1 ± 1	0 ± 0	0 ± 0	0 ± 0
$V4_{lime10kg\ air}$	19 ± 1	15 ± 7	14 ± 5	14 ± 7	12 ± 5	6 ± 1	4 ± 4	3 ± 4	0 ± 0	0 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0

were no significant differences of temperature among all vault options. In this study, the temperatures within the vaults were lower than the temperature range in many composting latrines in other studies (40–65 °C) (Anand and Apul 2014). Hence, the effect of temperature on the die-off of *A. lumbricoides* eggs might be reduced in our study.

The effect of low moisture conditions increased the rate of die-off of *A. lumbricoides* eggs in the current study. Hawksworth et al. (2010) found that at 30 °C and 100% RH, viable *Ascaris* eggs were found after 58 days, and conversely, no viable eggs were found at 0% RH (Hawksworth et al. 2010). In our study, as the RH decreased, the rate of die-off of *Ascaris* eggs increased. However, the RH did not significantly affect the percentage of viable *A. lumbricoides* eggs when vault options were compared because the difference between them was not large (0–3%). The RH of vault options in this study were in the same as those in tropical climates with temperatures ranging from 20 to 30 °C, which yielded survival times of *Ascaris* eggs between 10 and 12 months (Strauss et al. 2003).

Storage time is the main factor explaining the significant decrease of the percentage of viable eggs over 181 storage days. However, Gantzer et al. (2001) found that live nematode eggs can exist after 6 months of storage (Gantzer et al. 2001).

Public health implication of helminth egg die-off from human excreta storage options

After 181 storage days, all vault options were compliant with the maximum number of viable *A. lumbricoides* eggs, as defined by the WHO guideline for safe use of excreta in agriculture (World Health Organization 2006a) (Table 4). Corresponding to the above storage day, the average number of helminth eggs per gram excreta decreased from 15 (range 23–2) to 0 (range 2–0) in 8 vault options (Table 4). The data crossed the horizontal axis at the 1 egg/g point, showing that vault options $V3_{lime\ riceh}$, $V3_{lime\ riceh\ air}$, $V4_{lime10kg}$ and $V4_{lime10kg\ air}$ reached the WHO standard at 111 days of excreta storage. However, vault option

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Table 5 Average number egg per three replicates in all vault option following sampling time among Vietnam experiment, 2012 (sae: survival *Ascaris* egg equal (*Ascaris* survival developed egg) plus (*Ascaris* survival infective egg); dae:dead *Ascaris* egg equal (*Ascaris* dead developed egg) plus (*Ascaris* dead infective egg) ($V1_{no\ air}$: the vault had 100 kg excreta without air pipe; $V1_{with\ air}$ the vault had 100 kg excreta with air pipe; $V2_{lime3kg}$: the vault had 97 kg excreta and 3 kg lime without air pipe; $V2_{lime3kg\ air}$: the vault had 97 kg excreta and 3 kg lime with air pipe; $V3_{lime\ riceh}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk without air pipe; $V3_{lime\ riceh\ air}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk with air pipe; $V4_{lime10kg}$ had 90 kg excreta and 10 kg lime without air pipe; $V4_{lime10kg\ air}$ had 90 kg excreta and 10 kg lime with air pipe)

Vault option	Sampling 1		Sampling 2		Sampling 3		Sampling 4		Sampling 5		Sampling 6		Sampling 7	
Egg status	sae	dae	sae	dae	sae	dae	sae	dae	sae	dae	sae	dae	sae	dae
$V1_{no\ air}$	346	100	564	242	339	247	386	266	246	324	190	363	218	324
$V1_{with\ air}$	259	107	347	162	330	193	176	102	258	249	213	254	200	153
$V2_{lime3kg}$	330	85	492	212	339	231	390	175	419	258	1777	466	346	270
$V2_{lime3kg\ air}$	403	138	442	209	373	247	240	151	951	382	276	305	196	282
$V3_{lime\ riceh}$	301	60	275	124	276	166	293	258	394	216	253	274	140	251
$V3_{lime\ riceh\ air}$	251	93	259	82	393	133	283	162	234	211	161	225	93	188
$V4_{lime10kg}$	394	63	374	174	372	201	427	223	160	274	141	244	95	203
$V4_{lime10kg\ air}$	522	165	375	185	366	168	363	371	304	236	150	198	120	192

Vault option	Sampling 8		Sampling 9		Sampling 10		Sampling 11		Sampling 12		Sampling 13		Sampling 14	
Egg status	sae	dae	sae	dae	sae	dae	sae	dae	sae	dae	sae	dae	sae	dae
$V1_{no\ air}$	80	236	318	314	115	317	88	226	49	185	34	181	43	331
$V1_{with\ air}$	117	254	154	240	219	184	94	223	52	205	29	177	8	121
$V2_{lime3kg}$	156	249	256	626	238	348	157	321	78	197	33	188	18	130
$V2_{lime3kg\ air}$	132	255	138	570	169	338	127	252	48	217	41	241	18	199
$V3_{lime\ riceh}$	146	224	29	284	155	231	49	208	25	101	64	242	31	171
$V3_{lime\ riceh\ air}$	100	194	20	282	138	164	119	176	25	133	25	176	10	96
$V4_{lime10kg}$	75	197	72	327	84	194	49	182	16	112	26	148	7	123
$V4_{lime10kg\ air}$	87	131	15	250	46	214	56	165	33	137	8	119	5	98

$V4_{lime10kg\ air}$ had the best reliability for reducing egg counts below the one egg per gram standard over the shortest time period.

The largest reduction of viable *A. lumbricoides* eggs occurred in vault options $V4_{lime10kg}$ and $V4_{lime10kg\ air}$ (with 10% lime per total weight). However, vault option $V4_{lime10kg\ air}$ reached the WHO standard on the 111th storage day and far exceeded the recommendation of the Ministry of Health of Vietnam to compost excreta for 180 days before using it for agriculture (Ministry of Health 2005).

According to the WHO guidelines for the safe use of excreta and grey water in agriculture, the least time for excreta storage is six months with alkaline treatment (pH > 9), temperature >35 °C and moisture <25% (World Health Organization 2006b). Excreta can exceed pH 9 by the addition of lime or ash (e.g., 200–500 ml, or enough to cover each fresh defecation). In our study, we mixed excreta with lime by the rate 10% lime per total solid. The pH reached approximately 12 after 41 days, which exceeded WHO requirements for pH, and then decreased to 8 on the 83rd day of storage. This helps to explain why the V4 $_{lime10kg\ air}$ vault option created suitable egg reduction after only 4 months of storage.

In Vietnam, Aya Yajima et al. indicated that despite high latrine coverage (98.1%) in their study population, the prevalence of *A. lumbricoides*, *Trichuris trichiura* and hookworm infection was 13.5, 45.2 and 58.1%, respectively (Yajima et al. 2009). According to the report, the use of human excreta as fertilizer was 17.4% in the agricultural population. The farmers added straw to mix with human excreta, and every 4–6 months, removed it to use as fertilizer in agriculture. Therefore, we hypothesize that human excreta stored in this manner still contained viable parasites by the time that it was applied to the field. So, the use of human excreta in this community is an important factor contributing to the high prevalence of infection. In 2004, Jensen et al. conducted a study in Nghe An province showing that when farmers in 24% of the households used excreta for only one crop per year, they were able to compost for periods exceeding 6 months according to the Vietnamese Ministry of Health guidelines (Ministry of Health 2005). However, 93% of the households would conduct between one and three composting periods lasting

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only 3–4 months (Jensen et al. 2008). Most households (80%) composted excreta inside the latrine. Residents of these households added kitchen ash into the vault of latrine after each defecation, and 63% added lime. Of households composting outside the latrine, 99% applied ash, 55% used lime and 6% of the households used green leaves, straw, or other organic materials. They often mixed these materials with the excreta before composting. This manner of composting excreta resembles the method of storing excreta in our study. It means that the application of excreta in the field after 3–4 months storage might be a risk factor associated with helminth infection. A 2014 study showed that 34% of study participants' hands had the presence of helminth eggs, and the concentration ranged from zero to 10 eggs per two hands (Gulliver et al. 2014). Thus, both farmers and their family members can be at risk of helminth infection. In addition, Pham-Duc et al. showed that the use of human excreta for application in the field was associated with an increased risk for helminth infection (OR = 1.5, 95% CI 1.0–2.3) (Pham-Duc et al. 2013). Therefore, treating excreta before it is used as fertilizer in the field could play a role in the control of human helminth infections.

Global public health officials promote quantitative microbial risk assessment for water and sanitation (World Health Organization 2016), including pathogens such as helminths. However, the data related to helminth die-offs in real conditions remains limited. Information describing human contact with and exposure to excreta during agricultural practices [e.g., (Feachem et al. 1983)], is outdated and rare.

Therefore, our study provides new data to the international literature for understanding helminth survival in the local context of Vietnam and offers information for regional and international studies on helminth risk and control. This data is particularly meaningful for countries in South East Asia and South China where the conditions and practices of using excreta in agriculture are still popular.

Our study was set up in conditions that are similar to the local single vault or double vault latrines that are still used in many places in Vietnam, and therefore, provides evidence and implications for when and how a safe reuse of excreta can be achieved. Further assessment on health risk related to these treatment options and handling are undergoing and will provide more evidence for safe waste reuse in Vietnam.

In conclusion, this field study offers evidence on the ideal combination of locally available materials and excreta storage conditions that would yield the microbial safety in excreta. After 111 storage days, a mixture of high pH and the addition of 10% lime already reached the WHO standard for safe reuse of excrete of less than 1 egg/gram total solid material (World Health Organization 2006a). The findings can be useful for studies on risk assessment and epidemiology of helminth infection and control.

Acknowledgements We are grateful for the kind cooperation and participation of the health station staff and community members in Hoang Tay commune. We would like to thank Dr. Do Trung Dung and the staff of the Parasitology and Entomology Department at the National Institute of Malariology for performing the stool examinations for the helminth eggs, as well as Mr. Nguyen Xuan Huan and the staff of the Department of Pedology and Soil Environment at Hanoi National University for performing humidity analysis of excreta samples. Vi Nguyen edited the English of this paper. This work was supported by the Swiss National Science Foundation and the Swiss Agency for Development and Cooperation through the National Centre for Competences in Research (NCCR) North–South program.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethics standards This study was approved by the Ethics Review Board of the Hanoi University of Public Health (020/2012/YTCC-HĐ3).

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5. ESTIMATION OF INVOLUNTARY EXCRETA INGESTION RATES IN FARMERS DURING AGRICULTURAL PRACTICES IN VIETNAM

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It has been published in the journal Human and Ecological Risk Assessment: An International Journal, 2018: p. 1-11. DOI: 10.1080/10807039.2018.1478274

HUMAN AND ECOLOGICAL RISK ASSESSMENT
https://doi.org/10.1080/10807039.2018.1478274

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Estimation of involuntary excreta ingestion rates in farmers during agricultural practices in Vietnam

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ABSTRACT

Soil-transmitted helminth (STH) infections caused 4.98 million years live with disability globally in 2014, mostly affecting the poor. When farmers handle excreta for reuse in agriculture, involuntary ingestion of excreta particles is an important infection pathway for STH infections. The aim of this study was to quantify farmers' ingestion of human excreta during common agricultural practices in Vietnam. The weight of excreta that remained on farmers' hands, the weight of excreta that would remain on a farmer' mouth after contact with hand, and the frequency of contact between hand to the mouth by observing farmers while they handled excreta were simulated and quantified. Our findings revealed that farmers are on average exposed 5.16 h to excreta handling per year. Based on this average exposure time, it was estimated that farmers ingest 91 mg of excreta per year (95% CI: 73–110 mg). Our study presents for the first time a robust quantitative estimation of excreta ingestion by farmers during excreta handling in agricultural practices. Hence, this paper makes an important contribution to more robust quantitative microbial risk assessment and health impact assessment related to STH infections and diarrhea in Vietnam and other similar settings where excreta is used as fertilizer.

ARTICLE HISTORY
Received 23 March 2018
Revised manuscript
Accepted 15 May 2018

KEYWORDS
human excreta; exposure assessment; occupational health; farmer health; vietnam

Introduction

Human excreta has been used as a source of crop fertilizer for centuries (AgriCultures Network 1994), and currently it is still used by farmers in low- and middle-income countries (LMICs), including Vietnam. Excreta contain key nutrients, such as phosphorus, nitrogen, and potassium that are essential for plant growth. Application of excreta to farm fields increases the agricultural productivity by replacing key nutrients lost during crop harvests while also saving on chemical fertilizers costs, resulting in both economic and environmental

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benefits (Jensen *et al.* 2010). However, this practice poses potential health risks to farmers who handle excreta, as well as to communities and consumers who eat uncooked produce grown with excreta as fertilizers not properly managed (Pham-Duc *et al.* 2013, Fuhrimann *et al.* 2016).

One significant human health risk is soil-transmitted helminth (STH) infections (Lam *et al.* 2015). In 2010, it was estimated globally that 438.9 million people were infected with hookworm, 819 million with *Ascaris lumbricoides*, and 464.6 million with *Trichuris Trichiura* (Pullan *et al.* 2014). Rates of STH infection in Vietnam are considered moderate, with the prevalence estimated at 28.6%, 44.4%, and 23.1% for hookworm, *A. lumbricoides*, and *T. trichiura,* respectively (Van Der Hoek *et al.* 2003). Main risk factors of STH in Vietnam include the use of excreta in vegetable cultivation (Van Der Hoek *et al.* 2003), and living in a household without a latrine (Do *et al.* 2007). While a considerable body of evidence exists on STH infection associated with excreta use, little is known about people exposed to excreta while handling excreta to apply it as crop fertilizer relating to the transmission of helminth infections, in particular *A. lumbricoides*.

Currently, in the peer-reviewed literature no studies can be identified that have attempted to estimate excreta ingestion by farmers during excreta handling in either developed or developing countries. Only two studies investigating the presence of *A. lumbricoides* eggs on farmers' hands are found (Hoa *et al.* 2010, Gulliver *et al.* 2014). In contrast, several studies have sought to estimate soil ingestion in children in the United States (Clausing *et al.* 1987, Davis *et al.* 1990, Davis and Mirick 2006, Xue *et al.* 2010, Wilson *et al.* 2013, Wilson *et al.* 2015, Von Lindern *et al.* 2016, Wilson *et al.* 2016) and one in both adults and children (Davis and Mirick 2006). The data derived from these studies on soil ingestion rates, in combination with data from studies quantifying the number of helminth eggs in soil, was then used in a quantitative microbial risk assessment (QMRA) for the estimation of the associated burden of infection (Schonning *et al.* 2007, Navarro *et al.* 2009, Navarro and Jimenez 2011). Hence, the lack of a robust estimation of excreta ingestion by farmers during excreta handling renders the application of QMRA to estimate the burden of helminth infection in the given context difficult. Therefore, the objective of this paper is to estimate the amount of excreta involuntarily ingested by farmers during excreta handling in Vietnam. The following research questions were pursued: What is the weight of excreta remaining on the hands and the face of farmers during excreta handling? How often do farmers touch their mouth with excreta contaminated hands during transportation, composting, and field application practices? What is the weight of the involuntary ingested excreta?

Methods

Study area

This study took place in Hoang Tay commune, situated in Kim Bang district, Ha Nam Province in northern Vietnam (Figure 1). Approximately 5761 people reside in this area (1762 households) and a considerable part of their source of income is derived from agricultural production such as rice cultivation (Hoang Tay People's Committee 2016). Application of human excreta to fertilize crops was observed in 37% of households in Hoang Tay (Hoang Tay People's Committee 2016). At the time of this study, households mostly relied on very rudimentary forms of on-site sanitation facilities, for instance, 222 septic tanks, 469 pit

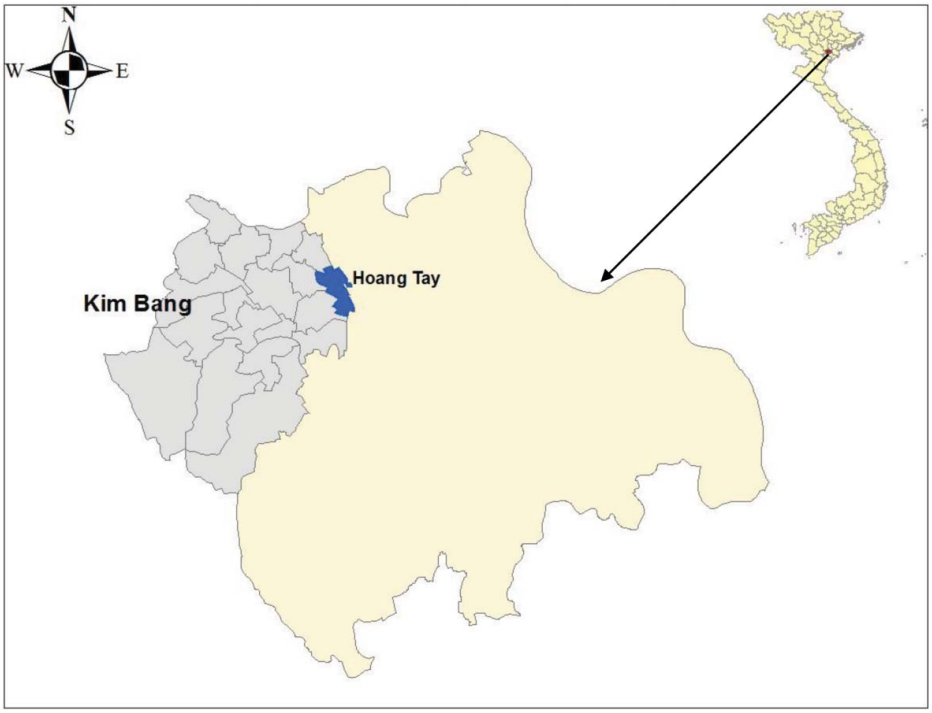

Figure 1. Location of Hoang Tay Commune in Kim Bang district, Hanam province, Vietnam.

latrines, and 250 biogas units. The residents of the study area were found to experience STH infections with hookworms (2%), *A. lumbricoides* (24%), and *T. trichiura* (40%) being the predominant species (Pham-Duc *et al.* 2013).

Laboratory and field sample collection procedure

We estimated involuntary consumption of excreta by farmers in three steps as illustrated in Figure 2. First, we used laboratory simulations and in field to quantify the weight of excreta that remained on a farmer's hands after handling waste. In the laboratory, non-powdered latex gloves were sealed in Ziplock® freezer bags and weighed (TE214S Sartorius scale, ± 100 mg). Three volunteers put the latex gloves on both hands, touched flour (VIMAFLOUR LTD 2017) with equally moisture content of excreta, and then lightly rubbed their hands together to remove large clumps. The gloves were removed, re-sealed in the same Ziplock® bags, and re-weighed to quantify the amount of flour remaining on the gloves. In the field, six farmers and three volunteers were recruited to wear pre-weighed, non-powdered latex gloves while removing excreta from latrines. Following their work, all gloves were placed in unsealed Ziplock® bags, dried for two weeks in the laboratory (average ambient temperature 23°C), and then re-weighed. Second, we estimated the weight of excreta that would remain on a farmer's mouth after contact with a soiled glove. Here, nine volunteers donned pre-weighed, non-powdered gloves, touched wet flour, and then touched their face with one hand. Flour was removed from the face using three pre-weighed plastic adhesive tapes (Ziploc BAG – size 27 × 28 cm) in order to recover all flour. The assumption was that proportion of flour weight remaining on the face after touching once to flour weight remaining

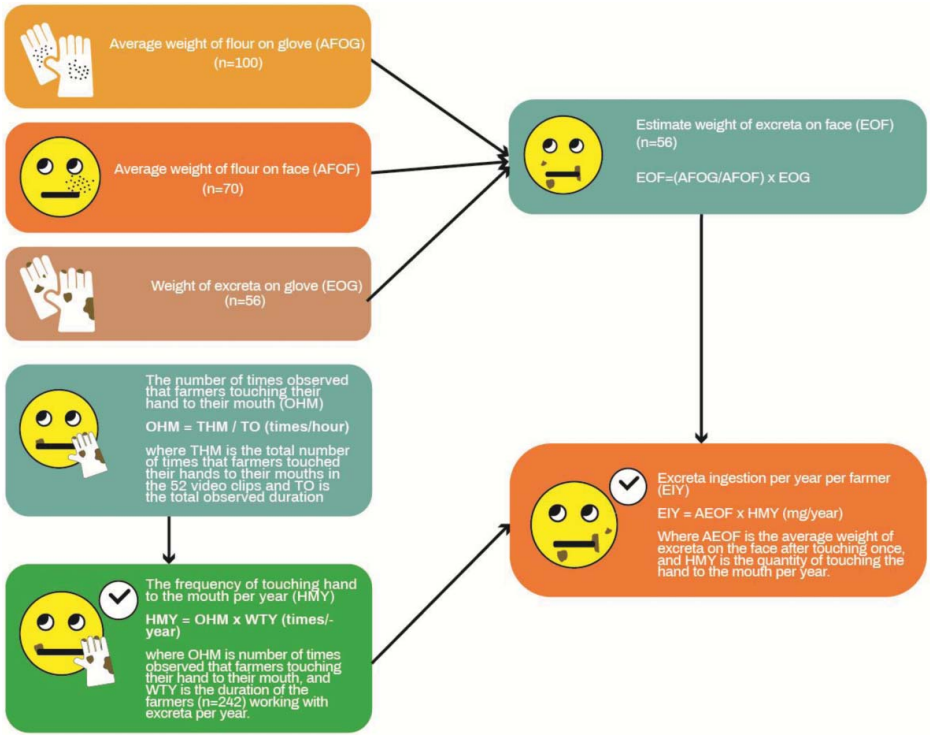

Figure 2. Diagram of estimating involuntary ingestion excreta per year of farmers.

on the gloves equalled the proportion of excreta weight remaining on the face after touching once to the excreta weight remaining on the gloves. The estimated weight of excreta remaining on the face after touching with a soiled glove, was calculated by Eq. (1):

$$\mathrm{EOF} = (\mathrm{AFOF} \,/\, \mathrm{AFOG}) \times \mathrm{EOG}(\mathrm{mg}) \tag{1}$$

where EOF is the weight of excreta on the face after touching once, AFOF is the average weight of flour remaining on the face, AFOG is the average weight of flour remaining on the gloves, and EOG is the weight of excreta remaining on the gloves after touching once.

Third, we quantified the frequency of contact between soiled gloves and mouth by observing farmers while they took excreta from their latrines for composting and field application practices. The farmers were asked to handle excreta as they would normally. Two researchers observed the farmers at work and captured their activities by video recording.

Participant recruitment for video recordings and interviews

In July 2012, 52 farmers (3 men and 49 women) were recruited to participate in the study by local community health workers visiting their households. The health workers introduced the research team and explained that we were investigating how farmers work with excreta. We advised the farmers that video recordings would require participant consent and would only be used for this study and that all data would be kept confidential. Before recording, we documented whether farmers chose to wear personal protective equipment, including

protective clothes, facemasks, gloves, and boots. One researcher worked alongside the farmer, while the other recorded these activities. The researchers reviewed all video clips to quantify behaviors that would increase the risk of STH transmission (e.g. touching mouth, face or furniture with soiled hands; hand-washing with or without soap).

We recorded the number of times observed that farmers touching their hand to their mouth (OHM) by applying formula Eq. (2):

$$\mathrm{OHM} = \mathrm{THM} \,/\, \mathrm{TO}(\mathrm{times} \,/\, \mathrm{hour}) \tag{2}$$

where THM is the total number of times that farmers touched their hands to their mouths in the 52 video clips and TO is the total observed duration.

In addition, we administered a questionnaire to 242 farmers come from households having single and double vault latrines to further examine practices related to excreta use, such as frequency and duration of excreta exposure. The frequency of touching hand to the mouth per year (HMY) was calculated by applying Eq. (3):

$$\mathrm{HMY} = \mathrm{OHM} \times \mathrm{WTY}(\mathrm{times} \,/\, \mathrm{year}) \tag{3}$$

where OHM is number of times observed that farmers touching their hand to their mouth, and WTY is the duration of the farmers working with excreta per year.

Excreta ingestion per year per farmer (EIY) was calculated by applying Eq. (4):

$$\mathrm{EIY} = \mathrm{AEOF} \times \mathrm{HMY}(\mathrm{mg} \,/\, \mathrm{year}) \tag{4}$$

where AEOF is the average weight of excreta on the face after touching once, and HMY is the quantity of touching the hand to the mouth per year.

Statistical analyses

All collected data for the weight of material on gloves and face were put into bootstrap with 1000 times of sampling to identify the 95% confidence interval and the average value. T-test was used to compare average weight of flour and excreta on gloves in different experiments. A p-value of < 0.05 was considered significant. R 3.2.2 software was used for the statistical analyses (© The R Foundation). @risk version 5.0 of Palisade was used to fit the probability density functions (PDF) of different parameters and the best fit PDF were reported.

Results

Weight of excreta that remained on hands and face

The average weight of flour remaining on the pair of gloves after touching the flour in the laboratory simulation was 41 mg (95% CI: 35; 48). The average weight of excreta remaining on the farmer gloves was 302 mg (95% CI: 227; 392). In addition, the average weight of flour touched on the face after one touch was 13 mg (95% CI: 12; 15). The average percentage weight of flour that remained on the face after touching once, per the weight of flour that remained on the gloves was 31.7%. The estimated average weight of excreta on the face after

Table 1. Average weight of flour and excreta remaining on gloves and the face.

Materials	Number of samples	Parameters of probability density functions	Mean (mg) [95%CI][4]	SD	Min	Max	*p* value
Flour on gloves (AFOG)	110	LogLogitic(5.22, 26.17, 2134.40)[1]	41 [35;48]	33	10	230	$p < 0.05$[5]
Excreta on gloves (EOG)	56	LogLogistic(6.64, 1252, 1694.5)[2]	302 [227;392]	341	70	2007	
Flour on face (AFOF)	70	LogLogistic(1.81,9.59, 3292)[3]	13 [12;15]	8	4	61	$p < 0.05$[4]
Estimate excreta on face (EOF)	56		95 [71;127]	108	22	656	$p < 0.05$[4]

[1] [2] [3]LogLogitic means the logistic distribution. Values in parentheses are values of 3 parameters: shift parameter γ, scale parameter β, and shape parameter α.
[4] 95% confidence interval of average weight was calculated on bootstrap sampling 1000 times.
[5]t-test compared average weight of flour and excreta on gloves.

touching once was 95 mg (95% CI: 71; 127). Summary statistics of average weights of flour and excreta remaining on gloves and the face are shown in Table 1.

Frequency of hand to mouth contact and weight of excreta ingested

The total duration of the recorded 52 video clips was 5 h and 20 min. The average age of the farmer was 53 ± 9.8 years (mean ± standard deviation), with an age range of 28–79 years. Most farmers put excreta into a bag for storage until applying it to the field (41/52 farmers), 7 farmers transported excreta immediately to the field for application, and 4 farmers stored excreta in a heap. When farmers (n = 52) worked with excreta, 9 touched their hands to other household items including their hat, face mask, and bicycle. One farmer was observed touching the face of a child, six farmers touched their own heads, and one farmer was seen to wipe their mouth with their arm (Table 2). Most farmers washed their hands (43/52) after handling excreta but only half (25/52) used soap.

The duration farmers worked with excreta ranged from 20 min to 60 h and 50 min per year. The average value was 5 h 10 min (95% CI: 4 h 14 min; 6 h 17 min) as shown in Table 3. The number of touching hand to the mouth incidences per year ranged from 0.062 to 11.394. The weight of excreta ingested per farmer each year ranged from 5 mg to 1082 mg. The

Table 2. Farmers actions when working with excreta.

	Frequency from 52 observed farmers	Percentage
Use of personal protective equipment (PPE)		
Protective clothing	8	15.4
Face mask	22	42.3
Gloves	13	25.0
Boots	1	1.9
All PPE	1	1.9
Touch		
Hand to head	8	11.5
Hand to household items	11	17.3
Arm & hand to mouth	1	1.9
Hand washing		
Soap and water	25	48.1
Water	18	34.6
Wash farm tools	14	26.9

Table 3. Working duration with excreta, frequency of touching hand to mouth, and excreta ingestion per farmer per year (n = 242).

Parameter	Parameters of probability density functions	Mean [95%CI][(3)]	SD	Min	Max
Working duration with excreta (hours/year)	InvGauss(4.99,2.63, RiskShift (0.16))[(1)]	5.16 [4.196;6.242]	8.357	0.333	60.833
Number of times of touching hand to mouth/year (time/year)	InGauss(0.95, 0.49, RiskShift (0.03)[(2)]	0.965 [0.787;1.181]	1.565	0.062	11.394
Excreta ingestion per farmer (mg)		91 [73;110]	148	5	1082

[(1)(2)]InvGauss means the Inverse Gaussian distribution. Values in parentheses are values of 2 parameters: mean μ and shape parameter λ. RiskShift is the shift factor of a distribution.
[(3)] 95% confidence interval of average weight was calculated on bootstrap sampling 1000 times.

average weight of excreta ingestion per year of farmer was found to be 91 mg (95% confidence interval: 73; 110).

Discussion

This study presents for the first time a robust quantitative estimation of involuntary excreta ingestion by farmers during common excreta handling in agricultural practices in Vietnam. Key findings show that the average weight of excreta ingested per year was determined to be 91 mg (95% CI: 73; 110), with a weight range from 5–1082 mg, and a median value of 44 mg. This result is of important contribution to the understanding on human exposure to excreta and to assessment of health risk related to excreta handing.

Based on our review of the current literature, this was found to be the first study investigating excreta ingestion by farmers when excreta are used as fertilizer. We found some studies that estimated soil ingestion for children and adults with values ranging from 1.2 mg/day to 245.5 mg/day (Clausing *et al.* 1987, Davis *et al.* 1990, Davis and Mirick 2006, Wilson *et al.* 2013, Wilson *et al.* 2015). Compared to our result, our estimated excreta ingestion found to be lower if adjusted to a daily value (0.2–0.3 mg/day).

We estimated excreta ingestion only in farmers using excreta in agriculture, but not in farmers who do not directly use excreta but may encounter it during other farming activities and accidently ingest excreta. However, there was an association between the occupational contact with soil and an increase of soil ingestion that was confirmed by Davis and Mirick (2006), so the risk of excreta ingestion may be increased in farmers who use excreta.

In our study, the approach used to estimate excreta ingestion depended on several underlying assumptions. First, it was assumed that when farmers work with excreta, or apply it on the field, they may routinely touch excreta with their bare hands; our results found 75% of farmers did this with only 25% wearing gloves. A second assumption was that these farmers may then touch their mouths with their hands during work; thus, farmers may directly ingest excreta remaining on their hands. During our observations; only one farmer was noted to wipe their mouth using their arm. There was no action of touching hand to the mouth observed, though, wiping the arm across the mouth may occur during farm work. Third, it was assumed that as the time working with excreta increases, the amount of excreta ingestion also increases. Part of the variability observed in our study might be explained by the composition of the study populations. For example, in our study, most surveyed

participants were female (49 women vs. 3 men), therefore the exposure might be different depending on the gender. As the ages of the study participants varied largely from 28 to 79 years, we could expect younger farmers are likely to have less skillful practices than older members, thus considerable differences in actual exposure are anticipated. Finally, the exposure level could also be influenced by various times of measurement with different temperature, and moisture content. In prior studies, Davis and Mirick (2006) estimated the mean soil ingestion rate for adults ranged from 23 to 625 mg/day with working times ranging from 1 to 10 h per day (Davis and Mirick 2006).

By comparison, farmers worked directly with excreta for durations ranging from 20 min to 60 h 50 min per year, with excreta ingestion measured from 5 mg to 1082 mg per farmer each year, indicating ingestion rates are lower when compared to the estimates of Davis and Mirick (2006) (adjusting for working time). This may be explained by that farmers working with excreta are more careful than farmers working with soil because they are aware of the risks related to excreta. Our study also assumed that the proportion of flour remaining on the face to flour remaining on the gloves during the laboratory simulation, equalled the proportion of excreta remaining on the face to excreta remaining on the gloves after hand to the face touches, in the field. Notably, excreta ingestion may have been underestimated because we did not estimate indirect contact from the hand to the mouth. However, the behavioral action of the touching hand to the mouth was observed only once. Similar low frequencies of hand to mouth touching were confirmed in a previous study—twice per day for adults (Michaud *et al.* 1994). Farmers who opt to wear face masks have a physical barrier to excreta ingestion, in our study we observed 42.3% farmers using face masks, effectively minimizing the transmission risk. Based on our video observations fresh excreta was not used directly as fertilizer; rather it was stored and often mixed with additive materials (e.g., lime, ash, rice husk) before being taken out of the latrine. Such treated excreta had little or no smell and may not have been handled as carefully as fresh excreta (Knudsen *et al.* 2008). Our study also showed that adding rice straw and lime helped accelerate the die-off of helminth eggs in human excreta in the same study site (Vu-Van *et al.* 2016). Therefore, the actions of touching the hand to the mouth during the farmers excreta work when they thought the excreta is safe with no smell may be increased. As during normal working, average hand-to-mouth contact frequencies among workers were particularly high 6.3 per hour (Gorman Ng *et al.* 2014).

According to our finding, in comparison involuntary excreta ingestion value was higher than in study of Phuc (2011), 91 mg/year and 60 mg/year (10 mg/event with 6 events/year) respectively (Pham Duc Phuc 2011). In composted excreta, the mean concentration of *Giardia lamblia* was 1.9×10^{-1} (95% CI: 1.2×10^{-2} to 5.1×10^{-1}) and Diarrheagenic *Escherichia coli* was 2.0×10^{-3} (95% CI: 1.1×10^{-4} to 6.1×10^{-3}), Phuc (2011) estimated the annual risks of diarrheal disease by pathogen and exposure per farmer 0.40 and 0.00 respectively for these pathogen while application of excreta in the field (Pham Duc Phuc 2011). Thus, our study confirmed that the assumption of involuntary excreta ingestion of Phuc (2011) and showed there is higher of risks of diarrhoeal disease when the farmer apply excreta in the field.

In conclusion, our paper shows that farmers ingest excreta ranging from 5—1082 mg/farmer/year during agricultural activities where human excreta are routinely used. The data from this study will strengthen future research studying health impacts of excreta handling in agriculture during the reuse processes. At the same time, our findings show that excreta

reuse in agriculture results in a considerable amount of excreta being ingested by farmers. Thus, the promotion of safer excreta handling practices is needed for safeguarding health of farmers in Vietnam.

Acknowledgements

We are grateful for the kind cooperation and participation of the health station staff and community members in Hoang Tay commune. We thank Mr. Nguyen Duy Tien and Mrs Nguyen Bich Thao for supporting the data collection. We thank Mrs Julie Hood (a Canadian VWB/VSF volunteer) and Ms Kylie Cuthbertson (Australian volunteer) for an English language review of this manuscript and Dr Janna Schurer and Ms Lauren MacDonald for providing early comments on the manuscript. This work was supported by the Swiss National Science Foundation and the Swiss Agency for Development and Cooperation through the National Centre for Competences in Research (NCCR) North–South program. HNV was partly funded by the CGIAR research program on Agriculture for Nutrition and Health (A4NH).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Ethics approval

This study was approved by the Ethics Review Board of the Hanoi School of Public Health (020/2012/YTCC-HĐ3) as well as by commune leaders in Hoang Tay.

Funding

This study was conducted within the framework of the Swiss National Centre of Competence in Research (NCCR) North–South: Research Partnerships for Mitigating Syndromes of Global Change. The NCCR North-South is co-funded by the Swiss National Science Foundation (SNSF), the Swiss Agency for Development and Cooperation (SDC), and the participating institutions.

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6. DISEASE BURDEN OF ASCARIS INFECTIONS IN THE CONTEXT OF HUMAN EXCRETA USE IN AGRICULTURE IN VIETNAM

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Abstract

The safe reuse of human excreta in agriculture can conserve nutrient resources, provide environmental benefits and economic savings. However, many developing countries face the challenge of balancing associated health risks with the environmental and economic benefits of this activity. The estimation that 4.98 million years lived with disability are attributable to Soil-Transmitted helminth. However, health risk assessments of excreta management lacks precision and underlining data are often not context specific. Using a quantitative microbial risk assessment (QMRA) methodology we assessed the *Ascaris lumbricoides* annual infection risk and related disease burden in disability-adjusted life years (DALYs) in farmers who handle and use excreta as fertilizer in agriculture in Hoangtay commune, located in the north of Vietnam. In 2012, context specific exposure data were generated both in the field and in the laboratory. 254 farmers were recruited to assess how farmers are exposed to excreta and how many *A. lumbricoides* eggs are on average ingested by farmers handling human excreta. Subsequently,

three scenarios of exposure to different options of excreta management and different storage time durations were considered to characterize the risk. The results show the annual risk of *A. lumbricoides* infection for farmers working with excreta ranged from 0 to 0.1837 depending on the duration of excreta storage. The highest annual disease burden was estimated at 0.000861 DALYs per person per year (pppy) in vault options with 3-5 kg of lime mixed with excreta stored shorter than 3.7 months; the lowest disease burden was estimated at 0.000 DALYs pppy in excreta with 10 kg of lime stored for 3.7 months to 6.0 months and for all vault options if excreta was stored longer 6 months. In conclusion this study could estimate the risk and DALYs due *A. lumbricoides* infection from different types of exposure, and therefore can be used for the management of health risks of farmers and who related to the use of excreta storage in Vietnam.
Keywords: Excreta reuse, helminth, QMRA, agriculture, health risk.

Introduction

Soil-transmitted helminth (STH) infections can be found globally where an estimated 438.9 million people were infected with hookworm, 819.0 million people were infected with *Ascaris lumbricoides* and 464.6 million people were infected with *Trichuris trichiura* in 2010 (Pullan et al. 2014). People infected might have symptoms including abdominal pain and diarrhea, general malaise and weakness, and impaired cognitive and physical development (Centers for Disease Control and Prevention 2013c). Subsequently, 4.98 million years lived with disability (YLDs) are attributable to STH according to the global estimation. Thereof, 65% are caused by hookworm, 22% by *A. lumbricoides* and 13% by *T. trichiura* (Pullan et al. 2014).

STH are transmitted by eggs that are passed through faeces of infected people. These eggs have an incubation period of three weeks to mature in the soil before they become infectious. People that unknowingly ingest infectious eggs become infected with *A. lumbricoides* or *T. trichiura* or some type of hookworm (Centers for Disease Control and Prevention 2013d, a, b). When farmers used excrements in agriculture that had been stored a period in the latrine, which might be accidently ingested an amount of excreta. However, based on current literature, no studies to evaluate the helminth risk to farmers through direct ingestion excreta. There are some studies which applied quantitative microbial risk assessment (QMRA) to analyse the risk of STH infections through the consumption of contaminated vegetable or water with STH eggs (Navarro and Jimenez 2011, Labite et al. 2010, Machdar et al. 2013). A few studies have used accidental soil ingestion to evaluate STH risk by using QMRA (Schonning et al. 2007, Navarro et al. 2009, Navarro and Jimenez 2011, Fuhrimann et al. 2016, Fuhrimann et al. 2017). These estimations mentioned previously might not reflect the risk of using inadequate treatment of excrements by farmers because there is a lack of data on excreta ingestion, especially in cases where protective

clothes are not worn. In addition, using soil ingestion data for excreta ingestion data from literature may result in an under or overestimation of STH infections in farmers. Moreover, all available information on soil ingestions rates were reported from high-income countries such as the USA (Clausing P Fau - Brunekreef et al. 1987, Davis et al. 1990, Davis and Mirick 2006, Wilson et al. 2013). In this context, local ingestion excreta data in developing countries might reflect truly the condition of people with their activities where they operate, which make the health risk assessment is more precise. In response to this, our previous study therefor estimated the average amount of excreta that a farmer accidental ingested during their working time (Tu-Van et al. 2018). In addition, the number of *A. lumbricoides* eggs per stored excreta gram were reported in the experimental excreta, that could be the suitable data for *A. lumbricoides* infection assessment in combination with the involuntary ingestion excreta data (Vu-Van et al. 2016). On the other hand, there is lack of studies that assess helminth risk by using the above data which might help to consider developing the guidelines related to the safe use excreta. Therefore, the present study was designed to assess the *A. lumbricoides* infection risk and estimate the disability adjusted life years (DALYs) due to *A. lumbricoides* infection in farmers, who handle and use excreta as fertilizer in agriculture in Vietnam.

Methods

Study area

The study site was set up at Hoang Tay commune, Kim Bang district, Ha Nam province. This commune is located in Northern Vietnam with a population of about 5,735 individuals residing in 1,720 households (Hoang Tay People's Committee 2012). The main income source was agricultural production such as rice and other crop cultivation, and 50% of households reported using human excreta in agriculture (Pham-Duc et al. 2013). The prevalence of helminth infections was 61.8%, with *T. trichiura* , 33.5% *A. lumbricoides* and 2.6% hookworm (Pham Duc 2008).

Quantitative microbial risk assessment

QMRA is a method for assessing risks from microbial agents. The QMRA methodology includes four steps (Figure 1): (i) hazard identification; (ii) exposure assessment; (iii) dose–response assessment; and (iv) risk characterization (Haas et al. 2014).

1. Hazards identification	***A. lumbricoides***	
2. Exposure assessment	**Source of contamination**	Storage excreta (storage < 3.7 months; 3.7-> 6 months and > 6 months and different adding for storing with the volume of lime: 0 kg/100 kg total solid (V1); 3-5 kg/100kg total solid (V2); and 10kg/100kg total solid (V3)).
	Exposure scenario	 Accidental excreta ingestion along handling excreta in agriculture (with different period of excreta storage and different adding to excreta for storing.
	Exposure group	Community members use excreta in agriculture *n = 2371*
	Exposure events per year	0.062 – 11.394 (times)
	Accidental ingestion of excreta per exposure event	22 – 656 (mg)
3. Dose-response	Probability of infection per farmer per exposure event ⇨ Probability of illness per farmer per exposure event	
4. Risk characterisation	Incidence of helminth infection per year => number of cases ⇨ DALYs per person per year => total DALYs	

Fig. 1. Exposure scenarios considered in the quantitative microbial risk assessment (QMRA) to estimate the burden of *Ascaris lumbricoides* along handling excreta in agriculture in Hoangtay commune.

Hazard identification

A. lumbricoides was selected because it is a roundworm which has the most hardy and resistant ova of all the STH and is extremely common worldwide including Vietnam. In addition, *A. lumbricoides* has reasonable background information about the organism and in theory. One egg might be enough to cause an infection.

Exposure assessment

This QMRA was conducted in the population of Hoang Tay commune. All the farmers in households with single and double vault latrines were surveyed to examine their practices related to excreta use, such as frequency and duration of excreta exposure. This study recruited 254 farmers (79 men and 179 women) who were between 15 and 79 years of age in 2012. This step assessed how farmers are exposed to *A. lumbricoides* and how much *A. lumbricoides* eggs was ingested by exposed cases. Based on our previous study, the estimated average weight of excreta ingestion per one occasion was 95 mg (95% CI**:** 71; 127). The average frequency of involutory ingested excreta was 0.965 times per year, ranging from 0.062 to 11.394 (Figure 1). The number of infectious eggs of *A. lumbricoides* per gram of excreta ranged from 0 to 88 eggs with excreta stored for 3.7 months, and from 0 to 18 eggs with excreta stored for 3.7 - 6 months, and from 0 to 7 eggs with excreta stored for 6 months (Table 6.1).

The data on helminth eggs concentration in excreta was from our previous experiment that was published elsewhere (Vu-Van et al. 2016). In summary, the experiment to examine the die-off of *A. lumbricoides* eggs was conducted in in situ conditions in 24 identical vaults with a size of 0.4 m (width) × 0.4 m (length) × 0.7 m (height). Each vault has the capacity to hold approximately 100 kg of excreta, and in addition to other material as: rice husk, lime, ash. Excreta in all vaults were sampled once every two weeks over the duration of 181 days, amounting to 14 samples per experimental vault, which were then subjected to laboratory analysis for the detection of *A. lumbricoides* eggs. The sampling order 1st, 2nd, 3rd, 4th 5th, 6th, 7th, 8th, 9th, 10th, 11th,12th, 13th, 14th corresponded to excreta storage days 1, 13, 27, 41, 55, 69, 83, 97, 111, 125, 139, 153, 167 and 181, respectively.

We proposed three different scenarios of exposure of farmers to excreta (that was stored in three options) handling as this is likely to happen in the real life of this population:

1. Application of the excreta after storage for 0 months to 3.7 months (excreta were stored from one to 97 days)

2. Application of the excreta after storage for 3.7 months to 6 months (excreta were stored from 97 to 181 days).

3. Application of the excreta after storage above 6 months (excreta were stored longer than 181 days).

Amounts of lime mixed with excreta for storage in above three scenarios were divided into three

options: 0 kg/100 kg total solid (V1); 3-5 kg/100kg total solid (V2); and 10kg/100kg total solid (V3).

Dose–response assessment

The scenario was considered to obtain the dose–response model for *A. lumbricoides*: the accidental ingestion of excreta during the farmers working with excreta in agriculture. For estimation of *Ascaris* risk, the *β*-Poison model was used to fit the data (Navarro et al. 2009) (equation 1)**.**

$\boldsymbol{P(d)}$**=**1-[1+(**d/N**$_{\mathbf{50}}$)**(**$2^{1/\alpha}$-1)]$^{-\alpha}$ (equation 1)

Where *P(d)* is the risk of infection in an individual due to the ingestion of an average number of *A. lumbricoides* egg in a dose, $P_{I(A)}$ is the annual risk of infection in an individual from n exposures per year to *Ascaris* dose; d is the total amount of *A. lumbricoides* eggs accidently ingested, based on *A. lumbricoides* concentration in ingested excreta volume for each exposure scenario; N50 is the median infectious dose. The values of N50 and α were identified to be 35 and 0.104, respectively (Navarro et al. 2009).

$\boldsymbol{P_{I(A)}(d)}$**=**1-[1-$\boldsymbol{P(d)}$]^{n} (equation 2)

Risk characterization

This last step of the QMRA application integrates the information from the three previous steps (hazard identification, dose–response assessment, and exposure assessment) into a single mathematical model to calculate risks as a probability of infection or illness. Annual infection risk is shown in Eq. (2). Annual risk of diarrhea disease is calculated by Eq. (3):

$\boldsymbol{P_{ill}} = \boldsymbol{P_{I(A)}(d)}$ x $\mathbf{P_{ill/inf}}$ (equation 3)

where $P_{ill/inf}$ is the probability of illness given infection. In our study, all *A. lumbricoides* eggs input the QMRA model were live, so we assumed that the $P_{ill/inf}$ = 1.

Estimation of disease burden

The disease burden due to human exposure is expressed in disability adjusted life years (DALYs). This metric combines morbidity (years lived with disability) and premature death (years of life lost) (Murray et al. 2012). For *A. lumbricoides*, DALYs per case of gastrointestinal illness (DALY) are calculated as the sum of the product of the probability of developing disease symptom (i.e. = mild, moderate and heavy symptomatic infection, mild abdominal pelvic

problems, wasting or death) given the illness occurs, relative frequency of the symptom (f), duration of the developed symptom in years (D) and the respective severity factor (S) (Salomon et al. 2012):

DALY = ∑f x D x S (equation 4)

The total disease burden (Total DALYs) per hazard is the product of cases and DALYs per *A. lumbricoides*:

$DALY_s$ = Case x DALY (equation 5)

The total disease burden for all hazards together is

Total $DALY_S$ = ∑Cases x DALY (equation 6)

The disability weight of *A. lumbricoides* was 0.002424 (Barker et al. 2014) . The tolerable *A. lumbricoides* infection risk per person per year (pppy) according the guideline of World Health Organization (WHO) 2006 is given by:

$$\frac{\text{Tolerable DALY loss pppy}}{\text{DALY loss per case of ascariasis}} = \frac{10^{-6}}{2.424 \text{ x } 10^{-3}} = 4.125 \text{ x } 10^{-4}$$

Results

These estimated mean single exposure of infections for farmers involuntarily ingesting excreta stored below 3.7 months were 0.2665, 0.2748 and 0.2560 according to V1 control, V2 lime 3-5kg and V3 lime 10kg respectively (Table 2). With excreta stored from 3.7 months to 6 months the estimated risk of infection for farmers involuntarily ingestion excreta were 0.1544, 0.1330 and 0.0572 according to V1 control, V2 lime 3-5kg and V3 lime 10kg respectively. For excreta stored longer than 6 months the estimated risks of infections for farmers were 0.0316, 0.0316 and 0 according to V1 control, V2 lime 3-5kg and V3 lime 10kg respectively.

Table 6. 1. QMRA model assumptions used to estimate the burden of *Ascaris* infection among farmers along the use of human excreta in agriculture in Vietnam

Description	Unit	Distribution and/or value(s)	Min	Mean[95% CI]	Max	SD	Reference(s)
(Ce) *A.lumbricoides* concentration in excreta							(Vu-Van et al. 2016)
< 3.7 months	Eggs/excreta gram	Negbin(2,0.1379)[(1)]	0	13	88	10	
V1 control	Eggs/excreta gram	Negbin(4,0.2630)	0	11	52	7	
V2 lime 3-5 kg	Eggs/excreta gram	Negbin(2,0.1254)	0	14	87	11	
V3 lime 10 kg	Eggs/excreta gram	Negbin(2,0.1550)	0	11	81	8	
3.7 -> 6 months	Eggs/excreta gram	Negbin(2,0.4989)	0	2	18	2	
V1 control	Eggs/excreta gram	Negbin(5,0.6550)	0	3	15	2	
V2 lime 3-5 kg	Eggs/excreta gram	Negbin(2,0.4701)	0	2	18	2	
V3 lime 10 kg	Eggs/excreta gram	Negbin(1,0.5882)	0	1	10	1	
> 6 months	Eggs/excreta gram	Negbin(1,0.80)	0	0	7	1	
V1 control	Eggs/excreta gram	Negbin(1,0.75)	0	0	6	1	
V2 lime 3-5 kg	Eggs/excreta gram	Negbin(1,0.75)	0	0	7	1	
V3 lime 10 kg	Eggs/excreta gram	0	0	0	0	0	
(V) Volume ingested per exposure event	mg		22	95[71;127]	656	108	(Tu-Van et al. 2018)
(n) Number of exposure per year		InGauss(0.95, 0.49, RiskShift (0.03)))[(2)]	0.062	0.965[0.787;1.181]	11.394	1.565	(Tu-Van et al. 2018)
Dose-response models		Point estimate: $\alpha = 0.0104$; $N_{50} = 35$					(Navarro et al. 2009)
Population at risk exposure scenario	People	Point estimate: 2731					

Disease burden per *A. lumbricoides;* disability-adjusted life years (DALYs) calculated in Table 6.3	DALYs/case	Point estimate: 0.002424	(Barker et al. 2014)

(1) The RiskNegbin distribution is used to generate a nonnegative integer value from the negative binomial distribution. Values in parentheses are values of 2 parameters: independent trials where each trial results in a "success" and probability p.
(2)InvGauss means the Inverse Gaussian distribution. Values in parentheses are values of 2 parameters: mean m and shape parameter λ. RiskShift is the shift factor of a distribution.

Table 6. 2. Single and annual risk of *Ascaris* infection among of farmers exposed with excreta

Option of storage	Min	Mean	Max	SD	5%	95%
Single risk of farmers						
< 3.7 months	0	0.2665	0.4206	0.0700	0.1125	0.3549
V1 control	0	0.2699	0.3883	0.0507	0.1885	0.3349
V2 lime 3-5 kg	0	0.2748	0.4199	0.0689	0.1592	0.3609
V3 lime 10 kg	0	0.2560	0.4156	0.0715	0.1125	0.3457
3.7 -> 6 months	0	0.1245	0.3181	0.0820	0.0000	0.2395
V1 control	0	0.1544	0.3054	0.0708	0.0000	0.2395
V2 lime 3-5 kg	0	0.1330	0.3181	0.0823	0.0000	0.2507
V3 lime 10 kg	0	0.0572	0.2766	0.0720	0.0000	0.1885
> 6 months	0	0.0246	0.2507	0.0503	0.0000	0.1125
V1 control	0	0.0316	0.2395	0.0562	0.0000	0.1592
V2 lime 3-5 kg	0	0.0316	0.2507	0.0562	0.0000	0.1592
V3 lime 10 kg	0	0.0000	0.0000	0.0000	0.0000	0.0000
Annual risk of farmers						
< 3.7 months	0	0.1807	0.9831	0.2350	0.0000	0.6714
V1 control	0	0.1837	0.9863	0.2348	0.0000	0.6649
V2 lime 3-5 kg	0	0.1843	0.9925	0.2384	0.0000	0.6829
V3 lime 10 kg	0	0.1748	0.9960	0.2298	0.0000	0.6501
3.7 -> 6 months	0	0.0907	0.9625	0.1526	0.0000	0.4057
V1 control	0	0.1110	0.9406	0.1661	0.0000	0.4656
V2 lime 3-5 kg	0	0.0959	0.9048	0.1573	0.0000	0.4386
V3 lime 10 kg	0	0.0432	0.8528	0.1051	0.0000	0.2931
> 6 months	0	0.0187	0.7309	0.0691	0.0000	0.1592
V1 control	0	0.0240	0.7682	0.0793	0.0000	0.2123
V2 lime 3-5 kg	0	0.0233	0.7682	0.0746	0.0000	0.2123
V3 lime 10 kg	0	0.0000	0.0000	0.0000	0.0000	0.0000

We estimated the annual risk of *A. lumbricoides* infection for farmers working with excreta based on their risk of involuntary ingestion excreta per year. If excreta were stored shorter than 3.7 months, these mean risks range from 0.1748 to 0.1837 with these storage options.

Excreta stored for 3.7 months to 6 months, the lowest average risk was 0.0432 in vault V3 lime 10kg. Excreta stored longer than 6 months, these mean risks were 0.0233, zero and zero in the vault option V1 control, V2 lime 3-5kg and V3 lime 10kg respectively (Table 6.2). The highest disease burden was estimated at 0.000861 DALYs pppy in vault options V2 lime3-5kg with excreta stored shorter than 3.7 months and the lowest disease burden was estimated at 0 DALYs pppy in vault options V3 lime 10kg with excreta stored for 3.7 months to 6 months and for all vault options if excreta was stored longer 6 months (Table 6.3).

Table 6. 3. QMRA estimates for annual probability of illness, number of cases per year, total DALYs per year, DALY per person per year according to use excreta that had been stored in the vault options

Period storage/ Vault option for excreta storage with Hazard A. lumbricoides	Incidence of Ascaris	Cases	DALYs	DALYs per person per year
<3.7 months	0.29	791	2.31	0.000845
v1	0.28	774	2.26	0.000826
V2lime3-5kg	0.30	807	2.35	0.000861
V3lime 10kg	0.28	755	2.20	0.000807
from 3.7->6 months	0.16	435	1.27	0.000464
v1	0.16	435	1.27	0.000464
V2lime3-5kg	0.16	435	1.27	0.000464
V3lime 10kg	0	0	0	0
> 6months	0	0	0	0
v1	0	0	0	0
V2lime3-5kg	0	0	0	0
V3lime 10kg	0	0	0	0

Discussion

We assess the helminth risk of farmers that handle and work with excreta in agriculture by applying QMRA and used data from our previous study with regard to excreta ingestion data. Our findings suggest that helminth risk to farmers who used excreta stored from 3.7 months to 6 months was lower than those who used excreta stored shorter than 3.7 months (Table 2). Especially in vault options adding 10% percent lime per total solid for excreta storage for 3.7 to 6 months and all vault options of excreta stored longer than 6 months were 0, that meets the tolerable burden disease of DALY pppy is 3.42×10^{-4} according the guideline of WHO (World Health Organization 2006). These results were confirmed by the experimental excreta storage which showed that the vault option, with aeration and 10% lime per total weight and excreta stored longer than 6 months, met the WHO standard for excreta treatment

(<1egg/gram total solid) on the 111th storage day (Vu-Van et al. 2016).

The prevalence of *A. lumbricoides* was 13.5% according the study in Hoa Binh province in 2007 (Yajima et al. 2009). This prevalence rate was in the range of our study with regard to the estimated helminth prevalence even though was no evidence that correlated agricultural use of human faeces with the infection of *A. lumbricoides* and high latrine coverage 98.1% in Hoabinh (Yajima et al. 2009). The prevalence estimates obtained for 29 of the 61 provinces of Vietnam in 2003 showed that 33.9 million people in Vietnam are infected with Ascaris (prevalence 44.4%) (Van Der Hoek et al. 2003), our estimated result in 2012 was lower because there might be the helminth infection decreased in comparison with 2003 and our study did not estimate helminth risk from consumption of contaminated food with *A. lumbricoides* egg which could be studied to further the literature. In Hanam province of Vietnam, the samples of 1,425 individuals from 453 randomly selected households were positive with any helminth (47% of study participants), among them was *A. lumbricoides* was 24% (Pham-Duc et al. 2013). The result of study indicates that people who have close contact with wastewater 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 who did not have contact (Pham-Duc et al. 2013). Our study recruited farmers who worked with excreta, but we did not examine the helminth infection associated with using excreta as fertilizer. Our results were within the same range of Phuc *et al*., study in 2013 which noted that if farmers used excreta stored shorter than 3.7 months, the average order of *Ascaris* single infection risk from high to low as V2 lime 3-5 kg > V1 control > V3 lime 10 kg are 0.2748, 0.2699 and 0.2560 respectively. However, the use of excreta stored for 3.7 months and longer than 6 months led to a significant decrease in the *Ascaris* average single infection risk to be the range from 0 to 0.1544.

The *A. lumbricoides* risk decreases if the farmers used excreta stored longer. The experimental excreta indicated that the average percentage of viable *A. lumbricoides* eggs decreased gradually from 76.72 ± 11.23% (mean ± SD) to 8.26 ± 5.20% in excreta stored from 0 to 181 days that caused by main role of time storage and high pH value (Vu-Van et al. 2016). In addition, in vaults with aeration and 10% lime and all vault options, those met <1egg/gram total solid according WHO standard for excreta treatment on the 111th (3.7 months) and 181st (6 months) storage day. The tolerable helminth risk to farmers that use these excreta in V3lime 10kg and all vault option (stored longer than 6 months) had DALYs pppy of 0. The DALYs pppy for other vault options were higher when the acceptable risk

ranged from 5 x 10^{-4} to 8 x 10^{-4} (Table 3). On the other hand, the average cost-savings were highest for farmers using fresh excreta (847,000 VND), followed by those who stored it for 6 months as recommended by the WHO (312,000 VND) and those who stored or for 3.7 months with lime supplementation 10% (37,000 VND/year) (Tran-Thi et al. 2017). However, the lowest cost-savings was g adding lime to excreta, there is a possibility that farmers could save money from healthcare cost but that was not assessed in the study (Tran-Thi et al. 2017). Our study evaluated *A. lumbricoides* risk of farmers based on direct excreta ingestion and we did not include the probability of using face mask during contact with excreta in model calculating of *A. lumbricoides* risk. In our previous study, 42.3% farmers that were observed, used face mask. The use of face masks may decrease the probability of touching hand to mouth and excreta ingestion volume. However, the face mask that farmers often used were handkerchiefs, so that could limit the effectiveness of protection. Furthermore, our study only considered the farmers exposure to excreta directly from hand to mouth, and not indirect ways of contact. Thus, the helminth risk in our study could be underestimate in comparison with the actual risk to farmers. However, recent study that assessed exposure of farm workers, showed direct exposure to be an important measure for the reliability of risk estimates from QMRA models (Antwi-Agyei et al. 2016).

Hand to mouth touching is an important role in helminth infection of *A. lumbricoides,* in our study the estimate frequencies of hand to mouth touching ranged from 0 to 11 times per year, while for farmer working with excreta ranged from 0.33 to 60.83 hours per year. According to a study in Ghana, the daily working hours ranged from 4 to 13, on average farmers were observed to have 10 hand-to-mouth events per day (Antwi-Agyei et al. 2016). However, the days worked per year ranged from 240 to 360 days with regard to study in Ghana (Antwi-Agyei et al. 2016). The frequency of hand to mouth touching in our study were lower, this may be because of the Hawthorne effect where behaviour might be slightly modified due to observation by a researcher. Our study results documents that in order to minimize the burden of *A. lumbricoides* infection associated with handling and use of human excreta in agriculture, farmers need to treat it at least every 3.7 months with 10% of lime per weight; or at least every 6 months.

Compliance with ethical standards

Conflicts of interest: The authors declare that there is no conflict of interest.

Ethics approval: This study was approved by the Ethics Review Board of the Hanoi School of Public Health (020/2012/YTCC-HĐ3) as well as by commune leaders in Hoang Tay.

Acknowledgements

We are grateful for the kind cooperation and participation of the health station staff and community members in Hoang Tay commune and its. We thank Julie Hood and Elizabeth Lartey Canada VWB/VSF volunteers, for an English language review of this manuscript. This work was supported by the Swiss National Science Foundation and the Swiss Agency for Development and Cooperation through the National Centre for Competences in Research (NCCR) North–South program.

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7. DIARHEA RISKS BY EXPOSURE TO LIVESTOCK WASTE IN VIETNAM USING QUANTITATIVE MICROBIAL RISK ASSESSMENT

This paper has been published in International Journal of Public Health February 2017, Volume 62, Supplement 1, pp 83–91. DOI: 10.1007/s00038-016-0917-6

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Int J Public Health
DOI 10.1007/s00038-016-0917-6

ORIGINAL ARTICLE

Diarrhea risks by exposure to livestock waste in Vietnam using quantitative microbial risk assessment

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Received: 17 May 2016 / Revised: 23 October 2016 / Accepted: 26 October 2016
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Abstract
Objectives The aim of this paper was to assess the diarrhea risks caused by various pathogens among those exposed to biogas wastewater through different activities.
Methods Application of quantitative microbial risk assessment (QMRA) of biogas wastewater was conducted in Hanam Province, Vietnam. A total of 451 representatives from households that use biogas were interviewed about their practices of handling biogas plant and reuse of biogas effluent for irrigation. In addition, 150 samples of biogas wastewater were analyzed for *Escherichia coli, Cryptosporidium parvum,* and *Giardia lamblia.* Risk characterization was calculated using Monte Carlo simulation.
Results The annual diarrhea risk caused by exposure to biogas effluent through irrigation activities ranged from 17.4 to 21.1% (*E. coli* O157:H7), 1.0 to 2.3% (*G. lamblia*), and 0.2 to 0.5% (*C. parvum*), while those caused through unblocking drains connected to biogas effluent tanks were 22.0% (*E. coli*), 0.7% (*G. lamblia*), and 0.5% (*C. parvum*).
Conclusions Further measures are needed to reduce the risk by either improving the microbial quality of biogas effluent or by ensuring the use of personal protective measures when exposed to biogas wastewater.

Keywords Biogas effluent · Diarrhea · QMRA · Wastewater · Exposure · Livestock waste · Vietnam

This article is part of the supplement "Health and social determinants of health in Vietnam: local evidence and international implications".

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Introduction

The global increase in demand for livestock products has led to many concerns about the associated negative impacts of livestock rearing on the environment and on human health. In Vietnam, especially the management of animal waste has become a considerable challenge due to the rapid increase of swine production. A common method to treat animal waste in Vietnam is anaerobic digestion, also called biogas technology. This is a microbiological process whereby organic matter is decomposed in the absence of oxygen. Animal manure as well as human feces can be used as feedstock. The outputs of anaerobic digestion are biogas (a mix of methane and CO_2) and a digestate wastewater, which is the digested slurry exiting the biogas reactor. Biogas is a renewable energy source, and its use also contributes to avoiding burning of fossil or wood, therefore reducing deforestation (Seadi et al. 2008). With these many proven benefits, biogas technology has become widespread throughout Asia.

In recent years, Vietnam has installed about 20,000 biogas reactors annually; reaching more than 100,000 by 2010 (REN21 2011). Biogas plants in Vietnam have often

Published online: 11 November 2016

been installed by farmers individually, and mostly at household scale without much technical support or advice. This frequently leads to biogas plants which are not properly designed, constructed, operated or maintained. This limits the efficiency of microbial removal and thereby affects biogas production. Previous studies on biogas treatment have also shown that the hygienic quality of the biogas effluent does not meet required quality values for discharge into surface water bodies and reuse for irrigation (Huong et al. 2014; Kobayashi et al. 2003; Lohri et al. 2014; Phi et al. 2009). For example biogas digestion only reached 1–2 log reduction for *Escherichia coli*, giving 3.70 ± 0.84 (log10) CFU/mL *E. coli* on average in effluent as compared with raw slurry, whereas the requirement by WHO for wastewater used in agriculture is 10^3–10^5 CFU/100 mL. Nevertheless, a large majority of Vietnam farmers still discharge the biogas effluent into the environment or use it as a valuable source of fertilizer (Huong et al. 2014). Such activities and resulting exposure to pathogens pose potential health risk to humans as diarrhea still remains one of the most important health problems (WHO 2006a). The health risks for communities in Vietnam with biogas plants, when handling or being exposed to biogas wastewater have so far not been assessed.

Ha Nam is a province in the North of Vietnam where there is frequent use of biogas plants with farming households raising pigs. Many of these farmers, most of whom lack understanding around issues of biogas effluent quality, use this effluent for irrigation of vegetables, crops and fruit trees, or then discharge it into drains. This lack of knowledge and awareness are reasons that during these activities of wastewater handling most local people do not pay attention to protective measures and expose themselves to biogas wastewater putting themselves at risk of diseases, especially diarrhea. Poor personal hygiene when in contact with wastewater-based on a theoretical exposure pathway would increase the risk of infection or disease; however, no epidemiological data are available. It is also known that some excreta-related pathogens, such as *E. coli, Cryptosporidium,* and *Giardia* can survive in the environment long enough to pose health risks (WHO 2006a).

The aim of this study was to assess the diarrhea risk of people exposed to biogas wastewater using the quantitative microbial risk assessment (QMRA) method. The results will provide estimations of the health risks associated with exposure to biogas wastewater through different activities. This aims to better understand the current sanitation of biogas plants for further research and interventions; which are geared towards enhancing the environmental and health conditions of communities with biogas plants.

Methods

Study site

The study was carried out in three communities of Ha Nam Province, namely Hoang Tay, Le Ho, and Chuyen Ngoai. Ha Nam is a peri-urban province in Vietnam, situated 60 km South of Hanoi. The three communities comprise a population of 6300 (1700 households), 8800 (2200 households), and 9300 (2300 households), respectively. The economic basis of these three communities relies on agriculture, with equal development of both livestock and crops. The total swine population (not including piglets) in all three communities was around 17,600, and almost all of them are raised at small household scale. The increase of livestock and crop production has unfortunately led to many clearly visible environmental unhygienic conditions with related health issues. These issues have become the concern of local citizens and authorities.

Quantitative microbial risk assessment

QMRA is a method for assessing risks from microbial agents in a framework that defines the statistical probability of an infection from environmental pollution (Haas et al. 2014). QMRA can estimate very low levels of infection or disease risks, and can estimate risks from different exposures pathways and/or from different pathogens that would be difficult to measure using epidemiological studies given the high cost and the large sample sizes needed (WHO 2006a). The QMRA application includes four steps: hazard identification, exposure assessment, dose–response assessment, and risk characterization (Haas et al. 2014). A more updated method of QMRA is presented in WHO (2016), but the essential steps remain similar to Haas et al. (2014).

Hazard identification

Enteric pathogens related to human waste and animal manure cause diarrhea and are transmitted from animal to human waste. It is reported as the second largest contributor to the global burden of diseases, causing an estimated 1.5 million deaths among children under 5 years of age every year (WHO and UN-Water 2010). Our study focused on *E. coli* O157:H7, *G. lamblia,* and *C. parvum*. All three pathogens cause waterborne diseases, especially diarrhea and are very resistant to adverse environmental conditions (Haas et al. 1999). Previous studies carried out in Vietnam showed high load of these pathogens in biogas effluent (Huong et al. 2014; Kobayashi et al. 2003) and wastewater (Phuc 2012) and reported high prevalence of diarrhea in communities (Phuc 2012; Trang et al. 2007).

Exposure assessment

The aim of this step was to determine the intensity and duration of the exposure to biogas wastewater of the populations. In order to achieve this, a survey was conducted in the three above-mentioned communities. From a total of about 1500 households with biogas plants, 451 households were randomly selected. In each of these selected households, a random adult who consented to participate in this research was interviewed about his/her practices of and exposure situation to biogas wastewater. The person was supposed to know best how biogas operates. Therefore, the survey recorded basic characteristics of biogas such as their age, material used (animal manure or with human feces), residency time. The survey results helped quantify the number of people exposed and estimate the frequency of exposure regarding each practice. Among the potential means of exposure to biogas effluent, this study focused on four scenarios as follows: (1) irrigating crops, (2) irrigating fruit trees, (3) irrigating vegetables, and (4) unblocking the open drains connected to effluent tanks. Popular crops and vegetables in the study area included maize, leafy vegetable such as morning glory, coriander, and some herbs that are eaten raw. Answers of each interview may include multiple practices by one individual.

During these scenarios, accidental ingestion of wastewater—by splashing directly into the mouths or indirectly on hands and then to the mouth—was assumed. This assumption was confirmed by observations of most local people using rudimentary tools such as a round scoop with a bucket or better a watering can for irrigation, and a hoe, rake or a wooden stick to unblock the drains without having proper personal protective measures. For this study, we assumed the volume of wastewater of 1 mL that each person involuntarily ingested during one single-exposure while conducting any of these practices (Hoglund et al. 2002; Ottoson and Stenstrom 2003).

Dose–response assessment

The dose–response assessment describes the relationship between the dose (number of pathogens entering the body to cause infection) received and the resulting health effects (diarrhea infection or disease). An exponential and β-Poisson model are the two dose–response models widely used in literature because they fit well to several microorganisms (Haas et al. 2014).

The β-Poisson dose response model (Eq. 1) was applied to estimate the risks of *E. coli* O157:H7:

$$P_{\mathrm{inf}} = 1 - \left[1 + \left(\frac{d}{\mathrm{ID}_{50}}\right)\left(2^{\frac{1}{\alpha}} - 1\right)\right]^{-\alpha}, \quad (1)$$

where P_{inf} is the probability of infection, d is the ingested dose ($d = C \times V$, where C is the concentration of microorganism, and D is the amount of wastewater which a person has involuntarily ingested), ID_{50} = average infecting dose (214.94), and α = parameter of probability function (0.373) (Teunis et al. 2008).

The exponential dose response model (Eq. (2)) was used for *G. lamblia* and *C. parvum*:

$$P_{\mathrm{inf}} = 1 - \exp(-rd), \quad (2)$$

where r = organism specific infectivity (0.0199 for *G. lamblia*, and 0.0042 for *C. parvum*) (Haas and Eisenberg 2001).

Risk characterization

This last step of the QMRA application integrates the information from the three previous steps (hazard identification, exposure assessment, and dose–response assessment) into a single mathematical model to calculate risks as a probability of infection or illness.

Annual infection risk is shown in Eq. (3):

$$P_{\mathrm{inf}(y)} = 1 - (1 - P_{\mathrm{inf}})^n \quad (3)$$

where n is the number of exposures per year.

Annual risk of diarrhea disease is calculated by Eq. (4):

$$P_{\mathrm{ill}} = P_{\mathrm{inf}(y)} \times P_{\mathrm{ill/inf}} \quad (4)$$

where $P_{\mathrm{ill/inf}}$ is probability of illness given infection: $P_{\mathrm{ill/inf}}$ (*E. coli*) = 0.25 (Howard et al. 2006); $P_{\mathrm{ill/inf}}$ (*G. lamblia*) = 0.67 (Rose et al. 1991); $P_{\mathrm{ill/inf}}$ (*C. parvum*) = 0.7 (WHO 2006b).

Sampling strategy

From three communities, 15 households with biogas plants were randomly selected for the study. At each household, two sampling points were identified: the first one at the effluent tank of the biogas plant and the second at the open household drain into which biogas effluent, wastewater and other runoff flow (Fig. 1a). The effluent tank of the biogas plant is a point of exposure as that is where farmers collect effluent for irrigation of fields (see Fig. 1b). The household open drain is also considered a potential point of exposure as this drain often needs to be unblocked by users (see Fig. 1c). Three wastewater samples were collected at each sampling point. Thus, five rounds of sampling gave a total of 150 wastewater samples collected from April to December 2014.

All samples from the biogas effluent tanks were collected at 20 cm depth, and in the center of the tank. Wastewater samples from household drains were collected where the wastewater from the effluent tanks enters the

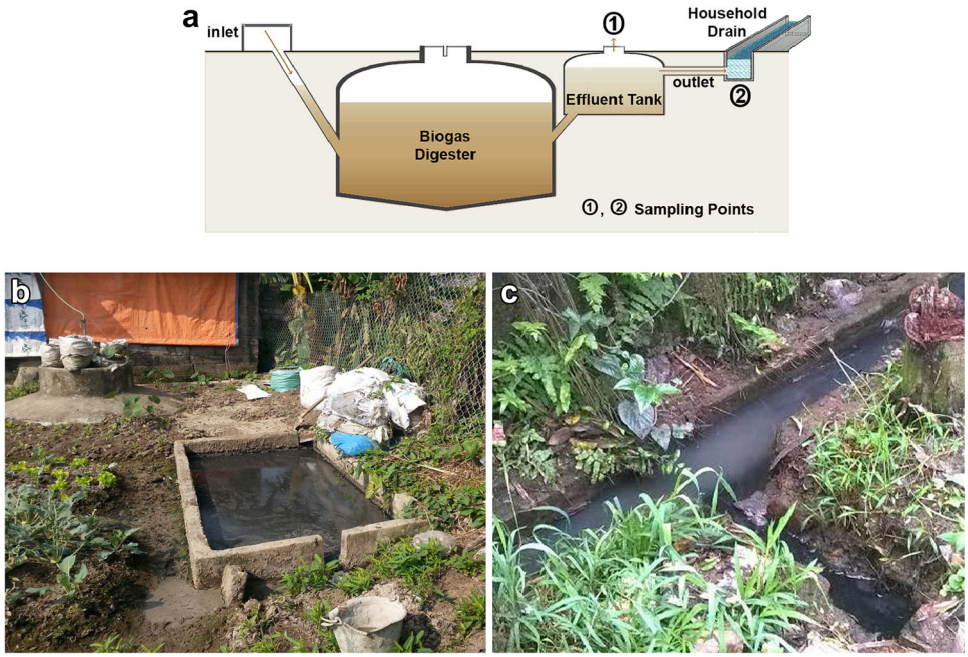

Fig. 1 **a** Scheme of a biogas plant and the two sampling points [*Source* adapted from (Tilley et al. 2014)]. **b** An open effluent tank of biogas plant in Hoang Tay, Hanam Province, Vietnam, 2014 (*Source* Center for Public Health and Ecosystem Research—CENPHER). **c** Open drain receiving biogas effluent in Hoang Tay, Hanam Province, Vietnam, 2014 (*Source* Center for Public Health and Ecosystem Research—CENPHER)

drain. One liter of wastewater was collected for each sample, stored on dry ice (4–8 °C) during transport to the laboratory, and processed within the same day. The samples were analyzed for *E. coli*, *G. lamblia*, and *C. parvum*.

Microbiology analysis

Brilliance *E. coli*/coliform Selective Agar (CM1046, Oxoid) was applied for the detection and enumeration of *E. coli* from wastewater samples. The wastewater samples were diluted 1:10 with 0.1% sterile Peptone Water. The surface of the agar plates was dried before pipetting 0.1 mL of the prepared sample onto each plate and spread over the surface with a sterile spreader. The plates were then incubated for 24 h at 37 °C. The growth of dark purple to indigo blue colonies on agar plates was determined positive for *E. coli.*

Immunofluorescence staining was performed to enumerate *Giardia* spp. and *Cryptosporidium* spp. (Crypto/Giardia CEL; Cellabs Pty Ltd, Australia). Each wastewater sample was vigorously shaken and dispensed into twenty 50-mL sterile test tubes which were centrifuged at 1500*g* for 5 min. The contents of each of the 20 sets of tubes were mixed together after discarding the supernatant, and centrifuged again under the same conditions. A flotation step followed with the remaining sediments in which 10 mL of wastewater volume was mixed with 5 mL of flotation fluid, and centrifuged for 1 min at 100*g*. The remaining flotation fluid in the sample was then removed before concentrating the sample volume to 2 mL. A volume of 200 µl sample was air-dried on Teflon-coated diagnostic slide fixed with acetone and stained with fluorescent monoclonal antibodies to detect *Giardia* spp. and *Cryptosporidium* spp.

In this study, we assumed that 8% of the total *E. coli* population in the wastewater were pathogenic (Haas et al. 1999; Howard et al. 2006). Furthermore, we assumed that all *Giardia* spp. and *Cryptosporidium* spp. found by this testing method were *G. lamblia* and *C. parvum* which are human pathogenic species. This assumption was applied in Mota's study where no information on the strain or the genotype was available (Mota et al. 2009).

Statistical analyses

Data from the 451 household survey were double-entered into Epidata 3.1. The statistical data analysis was done using SPSS 16.0. Non-parametric Mann–Whitney test was applied to compare difference between pathogen concentrations in the effluent tanks and in the drains of the three communities. All the parameters from exposure assessment of the risk model were included as probability density functions (PDF) (Table 1). These PDF were calculated from the original data collected from microbial analyses and from the survey with the best fits. Risks were calculated using estimated PDF randomly sampled by Monte Carlo simulation (10,000 iterations). Monte Carlo analysis gives a full range of possible risks, including average and worst-case events. In the latter, the risks were presented by the mean of 10,000 simulation values of risk. @Risk software (Version 5.5) added on to Microsoft Excel was used to calculate the PDF and to run risk models.

Table 1 Probability density functions of different parameters used in the risk models in Ha Nam Province, Vietnam, 2014

	Parameters of probability density functions[a]		
Sampling points	*E. coli* O157:H7	*G. lamblia*	*C. parvum*
Biogas effluent in effluent tanks	RiskNegBin (1,0.00087069)[b]	NegBin (1,0.050505)	NegBin (1,0.051546)
Drains connected to effluent tanks	NegBin (1,0.0011299)	NegBin (1,0.22388)	NegBin (1,0.078534)
Sampling points	Scenario/exposure activities	Parameters of probability density functions*	
Biogas effluent in effluent tanks	Irrigating crops	NegBin (1,0.20519)	
	Irrigating fruit trees	NegBin (1,0.17590)	
	Irrigating vegetables	NegBin (1,0.093452)	
Drains connected to effluent tanks	Unblocking drains	NegBin (1,0.064715)	

[a] Probability distribution function, fitted with all replicate values of real measurements of *E. coli* O157:H7 (estimated as 8% of *E. coli* analyzed), *G. lamblia* and *C. parvum* as well as the frequency of exposure for pathogens in biogas effluent

[b] Values in parentheses are values of 2 parameters r (>0) and p ($\in$ 0–1) of the negative binominal distribution

Table 2 Mean concentrations of pathogens at two exposure points from 15 households in three communes of Ha Nam Province, Vietnam, 2014

Sampling points	Pathogens	Number of samples	Number of positive samples (%)	Concentrations				
				Unit	Mean	SD	Min	Max
Biogas effluent in effluent tanks	*E. coli*	75	75 (100)	CFU/100 mL	14.7×10^5	34.9×10^5	320	200×10^5
	G. lamblia	75	33 (44.0)	Cysts/100 mL	19	46	0	260
	C. parvum	75	26 (34.7)	Oocysts/100 mL	18	51	0	400
Drains connected to effluent tanks	*E. coli*	75	75 (100)	CFU/100 mL	9.3×10^5	25.7×10^5	10	200×10^5
	G. lamblia	75	18 (24.0)	Cysts/100 mL	4	7	0	30
	C. parvum	75	22 (29.3)	Oocysts/100 mL	12	56	0	480

SD standard deviation

Results

Microbial contamination in biogas effluent tanks and drains

The mean concentration of *E. coli, G. lamblia*, and *C. parvum* from two sampling points are presented in Table 2. All 75 samples from the effluent tanks and 75 samples from drains attached to effluent tanks were positive for *E. coli,* with the mean concentration of 14.7×10^5 CFU/100 mL and 9.3×10^5 CFU/100 mL, respectively. Both sampling points had the same highest concentration of *E. coli* which was 200×10^5/100 mL. *G. lamblia* and *C. parvum* were found to have higher mean concentrations in the effluent tanks (19 cysts/100 mL and 18 oocysts/100 mL) than in the drains connected to these effluent tanks (4 cysts/100 mL and 12 oocysts/100 mL). *G. lamblia* had the highest concentration in the effluent tanks (260 cysts/100 mL), whereas *C. parvum* was highest in drains (480 oocysts/100 mL).

Characteristics and exposure situation of the study population

A total of 451 individuals from 451 households having biogas plants were selected for the exposure survey. 261 (58%) of respondents were females, the mean age of all respondents was 47. The majority of respondents (84%) indicated that their main occupation was working in agriculture. The frequency of exposure, ingestion dose of wastewater, and percentage of the population who participated in each exposure event are shown in Table 3. Using biogas effluent for irrigation was prevalent in the community. These percentages ranged from 24 to 28% depending on each irrigation activity. Similarly, 30% of the people reported participating in unblocking drains. People who were exposed to wastewater in these practices reported performing these activities an average of 24–53 times per year.

Part of the interviewed participants used personal protective measures when handling biogas wastewater. Such personal protective measures would include wearing gloves,

Table 3 Dose assumptions, frequency of exposure and percentage of the exposure population in 3 communes of Ha Nam Province, Northern Vietnam, 2014

Sampling points	Activities	Ingestion dose of wastewater	Average frequency (event/year)	Percentage of population (%)
Biogas effluent in effluent tanks	Irrigating vegetables	1 mL/event[a]	48	26
	Irrigating crops	1 mL/event[a]	32	28
	Irrigating fruit trees	1 mL/event[a]	24	24
Drains connected to effluent tanks	Unblocking drains	1 mL/event[b]	53	30

[a] Microbial risk assessment of source-separated urine used in agriculture (Hoglund et al. 2002; Ottoson and Stenstrom 2003)

[b] Fecal contamination of greywater and associated microbial risks (Hoglund et al. 2002; Ottoson and Stenstrom 2003)

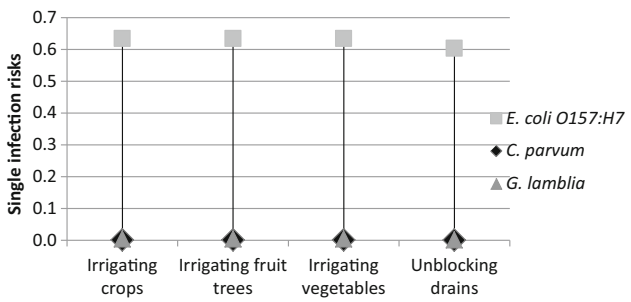

Fig. 2 Single infection risks in the different activities estimated by 10,000-trial Monte Carlo simulations in Vietnam, 2014

wearing face masks, wearing boots and washing hands with soap after work. Amongst all respondents engaging in irrigation activities, 10% indicated that they always use gloves during the practices, 14% always use a face mask, 13% always wear boots, and 25% wash their hands with soap after work. Only 8.9% participants always used all of these four protective measures when using biogas wastewater for irrigation. Similarly, the percentages of people practicing drain-unblocking activities indicating that they always use these protective measures were from 17 to 35%. Around 15% people saying that they always used all of these protective measures when unblocking the drains.

Biogas characteristics and operation

Each household in the survey had one biogas unit. 90.2% of household used pig manure in their biogas, whereas 29.7% used poultry manure. 80.3% of household also discharged human excreta into the biogas due to the proximity of livestock and human areas. The age of biogas was 5.2 ± 3.5 year (0.2–28 years) and the volume of the digester was 11.3 ± 3.1 m^3 (3.0–22.0 m^3). Most of the biogas digesters were built by brick and cement (87%) and plastic (13%).

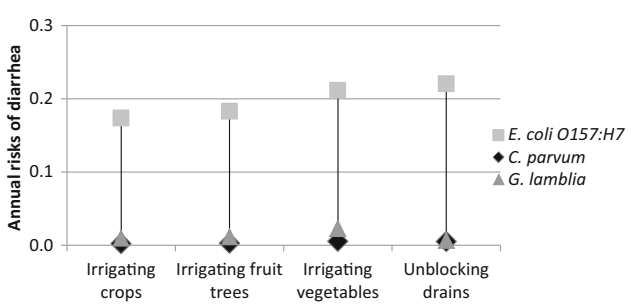

Fig. 3 Annual risks of diarrhea in the different activities estimated by 10,000-trial Monte Carlo simulations in Vietnam, 2014

Risks of infection and diarrhea diseases

Single infection risks of *E. coli* O157:H7, *G. lamblia*, and *C. parvum* in different activities are presented in Fig. 2. The mean single infection risk of these pathogens per individual in all irrigation activities was 0.63, 0.0037, and 0.0008, respectively, and the mean infection risks of these pathogens per individual in unblocking drains was 0.6031, 0.0007, and 0.0005.

With regard to the annual risk of diarrhea, we assume that exposure to biogas wastewater through the selected activities in this study were the only cause of diarrhea. The annual risks of diarrhea caused by the chosen pathogens are presented in Fig. 3. *G. lamblia* caused the highest risk of diarrhea when irrigating vegetables (0.0023) and lowest when unblocking drains (0.007). The annual diarrhea risk of *E. coli* O157:H7 was highest when people were exposed to wastewater through unblocking drains (0.22), and lowest when they were irrigating crops (0.174). Similarly, people who participated in unblocking drains or irrigating vegetables had the highest risk of diarrhea by *C. parvum* (0.005), whereas irrigating crops posed the lowest risk of diarrhea by *C. parvum* (0.002).

Discussion

Results in our study revealed high concentrations of *E. coli*, *G. lamblia,* and *C. parvum* in biogas wastewater. In particular, *E. coli* concentration in the effluent tanks was 14.7–147 times higher than the recommended standard (10^4–10^5 *E. coli*/100 mL) of the WHO guidelines for the safe use of wastewater in agriculture (WHO 2006a). The prevalence of *E. coli* in biogas effluent has also been shown

in previous studies conducted in Vietnam. A study of the hygienic aspects of small-scale biogas plants showed that the mean concentration of *E. coli* in biogas effluent from pig farms was 5000 CFU/mL (Huong et al. 2014), which, however, is almost three times lower than the results from our study. This might be explained by the difference in sampling points in the two studies. Huong et al. (2014) took samples in the second outlet tanks, whereas our study analyzed samples from the first outlet tank (effluent tank) of the biogas plants. Some settling process in the first outlet tank might reduce pathogens concentration in the second outlet tank. Another study in Can Tho Province—in the South of Vietnam—showed a high prevalence and high concentration of *E. coli* in the outflow of biogas plants of pig farms. The concentration of *E. coli* in 4/5 of the samples was greater than 10^5 CFU/mL (Kobayashi et al. 2003). The result is ten times higher than in our studies. However, the Kobayashi's study only collected five samples at each sampling points without repetitions and the study dates back 12 years. During the last 12 years, Vietnamese farmers have improved their knowledge on how to best operate biogas plants which has probably also improved the quality of biogas effluent thanks to several biogas promotions by SNV and the Ministry of Agriculture and Rural Development of Vietnam.

Vögeli and colleagues conducted some case studies to evaluate the quality and the use of biogas effluent in other developing countries, namely India, Tanzania, Nepal and Lesotho (Vögeli et al. 2014). Results showed that these countries present a similar context of applying and using biogas plants. Most of the case study results concluded that quality of the effluent did not meet the requirements for use as fertilizer or discharge into receiving water bodies, although people still use the effluent for irrigation and/or discharge into the surface waters.

Regarding the hygienic conditions of drains, the microbial quality of wastewater in household drains was mentioned in the study of wastewater and excreta use in agriculture in Ha Nam Province during the period of 2009–2010 (Phuc 2012). The wastewater samples were taken not only from households with biogas plants, but also from household drains,. The mean concentration of *E. coli* (78.75×10^6 CFU/100 mL) was much greater than in our study that was 9.3×10^5 CUF/mL. However, the mean concentration of *G. lamblia* (28 cysts/100 mL) and *C. parvum* (28 oocysts/100 mL) in this study was comparable.

Some studies were conducted in other developing countries where local people were exposed to different sources of wastewater to determine the contamination level of the water. For example, wastewater from canals and lagoons in Abidjan, Côte d'Ivoire, were shown to be heavily contaminated with *G. lamblia* and *E. coli* (Yapo et al. 2014), whereas wastewater from canals and sewers at household level in the Klong Luang municipality of Thailand even showed higher contaminations by *G. lamblia* (Ferrer et al. 2012).

Comparing the mean concentration of the three pathogens in the effluent tanks and drains of the three communes, there was no significant difference in *E. coli* and *C. parvum* in these two sampling points ($p > 0.05$; Mann–Whitney test). However, the mean concentration of *G. lamblia* in the effluent tanks was eight times greater than in the drains. This difference is statistically significant ($p < 0.05$; Mann–Whitney test). It might be possible that when biogas effluent flows from the effluent tanks to the drains it is diluted with other wastewater in the drains which reduces the concentration of *G. lamblia*.

The mean infection risk by *G. lamblia* per single exposure in our study was found to be similar to that of the study in the Klong Luang municipality of Thailand which showed that 44 out of 100 people having contact with canal water would have infection by *G. lamblia*, or out 100 times contact with canal water, a person was 44 times at risk of infection by *G. lamblia* (Ferrer et al. 2012). In this study, *Entamoeba histolytica*, another protozoa associated with wastewater, was also shown to have a similar infection risk by a single exposure when having contact with canal water (46%).

Our results were comparable to those obtained from other studies in Vietnam for annual diarrhea risk due to *E. coli* O157:H7. A study that applied the QMRA method to assess diarrhea risks of the exposure activities associated with wastewater and excreta use in agriculture in Ha Nam province of Vietnam showed that the annual diarrhea risks due to *E. coli* O157:H7 was 0.25. This is similar to the overall incidence of diarrheal disease as presented in the same study area of 0.28 per person-year as a result of a cohort study covering over one year of the follow-up period (Phuc 2012). Another cohort study which investigated in the suburb of Hanoi in Vietnam with 18 months follow-up showed similar results in which the incidence rate of diarrheal diseases was 28.1 episodes per 100 person-year counted for the adult population exposed to wastewater in agricultural and aquacultural productions (Trang et al. 2007).

In comparison with our study, the study performed in Thailand by Mamadou and collaborators who assessed the diarrheal risks related to exposure to canal water through irrigation activities, showed comparable results in diarrhea risks caused by *G. lamblia* at 0.67 per person-year (Mamadou et al. 2008). Among the three selected pathogens in this study, the risk of diarrhea due to *E. coli* O157:H7 was also much higher than for *G. lamblia* and *C. parvum.*

The annual diarrhea risks in our study was below the diarrheal disease incidence per person per year (0.4–0.6) among those aged above 5 years in developing countries in 2000, as reported in the WHO guidelines for the safe use of wastewater, excreta and greywater. However, in

comparison with the WHO reference level (10^{-3}) of waterborne disease from drinking water, the annual diarrhea risks in our study were much greater (WHO 2006a).

Our study did not measure the association between the health risks and the use of personal protective measures while participating in activities exposed to wastewater. However, the study on the practices of farmers in some agriculture activities in the Ha Nam province of Vietnam showed that inadequate use of protective measures, and lack of washing hands with soap were associated with increased risks of diarrhea (Phuc 2012). While appropriate treatment methods of biogas effluent are not yet available in Vietnam, improving the use of personal protective measures might be a suitable solution for protecting the health of the exposed population. It was also unfortunate that our study did not measure the temperature of biogas digester to link with pathogen die-off, which needs to be considered in future studies.

In conclusion, *E. coli*, *G. lamblia*, and *C. parvum* remained at high concentrations in the biogas wastewater. The single and annual risks of diarrhea caused by these pathogens in the exposed activities were relatively high. These facts suggest that further actions to improve the biogas effluent quality are required to reduce health risk due to the exposure to biogas wastewater. This study provides evidences to enhance the awareness of people when handling biogas wastewater, and hence promoting practices of using personal protective measures. Results in this study could be taken into consideration to understand the health risks associated with exposure to biogas wastewater through different activities in other areas of Vietnam as well as in other developing countries with a similar context of applying biogas plants.

Acknowledgements The authors thank the numerous individuals, communities, and organizations for providing valuable information and assistance to accomplish this study. Especially, we thank Ms Nguyen Thi Hong for her great contribution to this study. We thank the National Institute of Hygiene and Epidemiology for analyzing the wastewater samples. This study was supported by the Canadian International Development Research Center (IDRC) through the project of Ecohealth Field Building Leadership Initiative (FBLI, Grant No. 106556). TL was supported by an Eawag Partnership Program (EPP). HNV has been supported by the CGIAR Program Agriculture for Nutrition and Health (A4NH) led by IFPRI and by the Chrono-environment laboratory of the Universite de Franche-Comte through a visiting professorship. This study received ethical clearance from the Institutional Review Board of Hanoi School of Public Health No. 051/2004/YTCC-HD3, Code: 014-051/DD-YTCC.

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8. DEVELOPMENT OF NUTRIENT CYCLE THROUGH AGRICULTURAL ACTIVITIES OF A RURAL AREA IN THE NORTH OF VIETNAM

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J Mater Cycles Waste Manag
DOI 10.1007/s10163-016-0557-1

ORIGINAL ARTICLE

Development of nutrient cycle through agricultural activities of a rural area in the North of Vietnam

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Received: 22 September 2015 / Accepted: 24 October 2016
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Abstract Material flow analysis (MFA) has been applied to assess the environmental impact of human activities on nutrient flows at the commune scale. This paper reports the assessment of human excreta and animal manure as a nutrient source for paddy fields and fishponds in Hoang Tay commune, Ha Nam province, Vietnam. The quality of MFA model was confirmed through modified uncertainty analysis, then was used to originally quantify and visualize the interlinks of livestock with the environmental sanitation and agricultural system in terms of nutrients. Currently, half of the pig manure was collected to the biogas, and the remainders were freely discharged to the commune's drainage system (25%) or directly reused in the paddy fields (25%). While wastewater in the drainage system was the biggest source of nitrogen (contributed 46%), paddy field was the biggest source of phosphorous (contributed 55%) discharged to the Nhue River, totaling 57 ± 9 ton N and 29 ± 6 ton P, annually. Consequently, mitigation measures for nutrient resource management were proposed, and reducing half of chemical fertilizers applied and reusing all excreta and manure in the paddy fields were the most effective option.

Keywords Material flow analysis (MFA) · Nutrient recycling · Waste management · Manure · Vietnam

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Introduction

Vietnam is the second largest exporter of rice in Asia. In Vietnam, the area under rice cultivation increased from 4.7 Mha in 1961 to 7.3 Mha in 2007 [9], and the rice yield increased by 146% in this period [24]. Correspondingly, rice production in Vietnam, which was 9.0 million tons in 1961, gradually increased by a factor of four in a period of 46 years (i.e., to 35.6 million tons in 2007). The application of nitrogen fertilizers in Vietnam has increased 7.2% annually since 1985 [9]. Fertilizer consumption has also sharply increased in recent years in Vietnam; it was approximately 8.9 million tons in 2010, almost double of the volume used in 2005 [24]. Paddy fields bring social and economic benefits; however, they have also been recognized as a potential source of environmental pollution due to the overuse of chemical fertilizers. For example, in the Day–Nhue River Basin (DNRB) in the Red River Delta, which is the second biggest 'rice bowl' in Vietnam after the Mekong [17], paddy cultivation constitutes approximately 50% of land use, and the majority of the fertilizer used in the region is applied to paddy fields [3, 4, 15–17]. The Vietnam Ministry of Natural Resources and Environment [18], ADB [1], and Hanh et al. [12] demonstrated that agricultural activities, especially paddy cultivation in DNRB, are the primary sources of nitrogen, which causes environmental contamination. On the other hand, the

Published online: 01 November 2016

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production of livestock in Vietnam has been increasing rapidly, particularly, the pig and poultry populations increased steadily over these two decades [9]. There were 10 million pigs and 50 million chickens in 1980, which increased to 20 million and 130 million, respectively, in 2000. By 2013, these numbers grew to 27 million pigs and 230 million chickens [10]. Subsequently, large amounts of manure are produced. Thus, manure from livestock (pig, poultry) is good nutrients (nitrogen and phosphorous) resource for fertilizing in paddy fields; therefore, chemical fertilizers consumption can be reduced.

Adapted Material Flow Analysis (MFA) is widely known as a useful tool for identifying environmental problems by quantifying mass flows in an environmental sanitation system and for forecasting the impact of possible interventions on the environment [19–22]. The adapted MFA methodology has been successfully applied in Vietnam, in the whole Day–Nhue River Basin [5–7], in Hanoi, an urban area in the DNRB [19, 20], and in Ha Nam, a rural area belongs to DNRB where environmental sanitation and agricultural activities are closely interlinked [8, 23]. Connections of the environmental sanitation and agricultural system in terms of nitrogen and phosphorous were quantified in the case study of Hoang Tay and Nhat Tan communes in Kim Bang district, Ha Nam province, Vietnam [7]. There were significant nutrients flows from pig house (livestock process) to the paddy fields (paddy process).

Recognizing the potential nutrient sources from manure, we originally analyzed the close interlinks of livestock (pig, poultry) with on-site sanitation system and agricultural system (paddy field, fishpond) in a commune located along the bank of the polluted Nhue River, and reported in this paper. We conducted an assessment of human excreta and animal manure management in Hoang Tay commune with three main objectives: (1) to understand and describe the nutrient (nitrogen and phosphorous) fluxes in this commune with regard to considering human excreta and animal manure as center of material flow analysis; (2) to identify potential sources of nutrient loads to the surrounding environments, especially, the Nhue River; and (3) to develop mitigation measures for nutrient loss loads and enhancement of nutrient reuse. The developed MFA model would be widely applied for assessing the potential nutrient sources from human excreta and animal manure for utilizing in paddy fields.

Methodology

Description of the study area

The target site was Hoang Tay commune in Ha Nam province, located about 60 km south of Hanoi, in northern Vietnam (Fig. 1). In the year 2009, the population of this site was 5778, among a total of 1714 households [13]. This low-income commune represents typical land use patterns in northern Vietnam, with residential areas surrounded by aquaculture and paddies. There was four 200 pigs, 38 000 poultries, and 110 cattle in addition to 100 tons of fish in Hoang Tay commune. At the time of this study, households mostly relied on very rudimentary forms of on-site sanitation facilities, for instance, 222 septic tanks, 469 pit latrines, and 250 biogas units. In addition, human and animal feces were often used as fertilizer for paddy fields, and in some cases, were directly dumped into ponds to feed fish. Grey water was released without treatment into drainage networks and then passed into canals or the Nhue River together with the on-site sanitation system effluent.

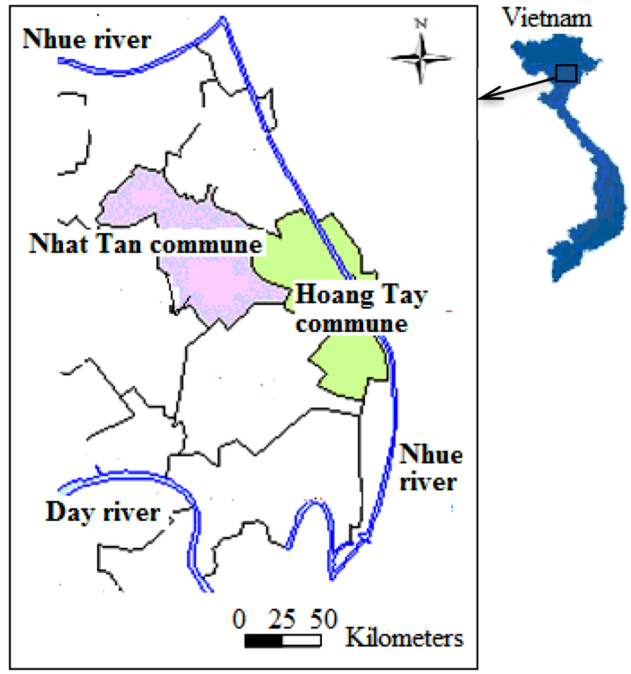

Fig. 1 Study site—Hoang Tay commune in Kim Bang district, Ha Nam province, Vietnam (Vietnam Publishing House of Natural Resources, Environment and Cartography, NARENCA 2008)

Development of the MFA model

The MFA system for Hoang Tay commune was developed on the basis of a previous one developed jointly for Hoang Tay and Nhat Tan communes, which presented in Do-Thu et al. [8]. This study mainly focused on the nutrients (nitrogen and phosphorous) flows of animal manure and human excreta to the environmental sanitation system and agricultural system. The interlinks of livestock with the environmental sanitation system and agricultural system in Hoang Tay commune in terms of nutrients are shown in

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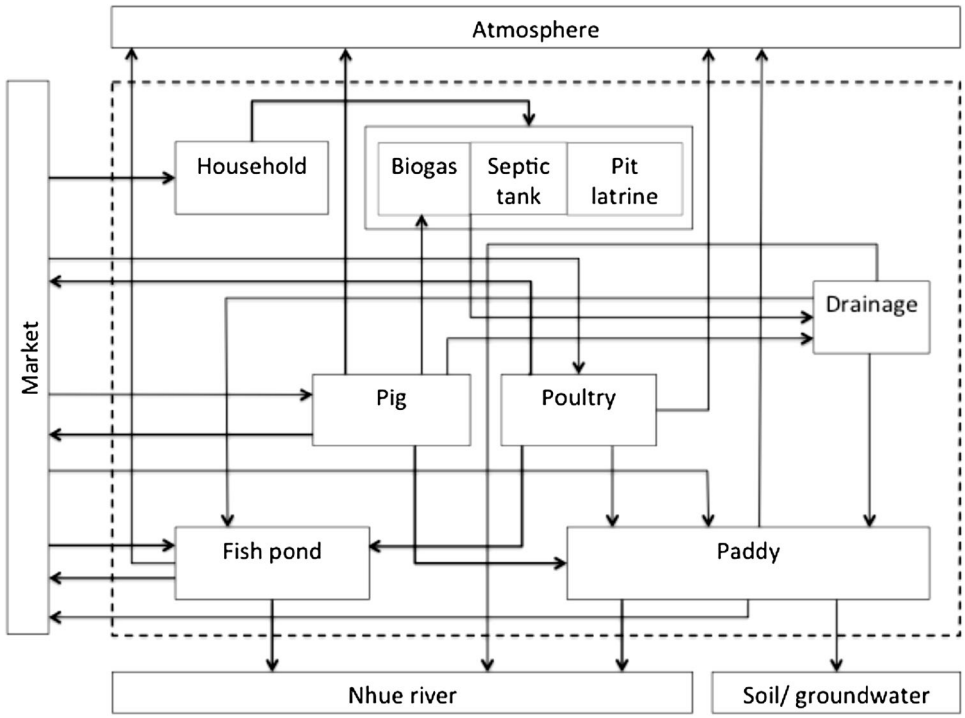

Fig. 2 MFA system showing the nutrient flows among agricultural and environmental sanitation system in Hoang Tay commune, Ha Nam province, Vietnam. *The dotted line* represents the boundaries of the MFA system

Fig. 2. There were nine processes in the MFA system, divided in three main groups: the environmental sanitation system (biogas, septic tank, pit latrine, and drainage), the agricultural system (fishpond and paddy), and livestock (pig and poultry). Market process was not included in the boundary of the MFA system; it was considered as the platform for material exchanges of the processes inside the boundary [19]. The three main environmental processes—atmosphere, the Nhue River, soil/groundwater—were also located outside of the system, as they reflected the destinations for nutrients from the human activities in Hoang Tay commune.

After drafting the model structure, equations were added or updated based on the collected data (called as 'parameter'). The most important data were the statistical data, such as population, area, and number of animals, which were collected from the annual reports of Hoang Tay commune [13]. Other information was in the government reports and documents, such as from the Departments of Natural Resources and Environment [3, 4] or MARD [17], and MONRE [18] and from research institutes and non-government organizations. The secondary data, such as emission rates of nitrogen, got from the results of projects that have been done in the study area or in other areas (Table 1). Two types of equations were used in this model: balance equations and model equations (Table 1). The nitrogen cycle model, proposed in Do and Nishida [7], was applied in the paddy process.

Uncertainty analysis

Modified uncertainty analysis procedure in adapted MFA, which is presented in Do et al. [6], was applied in this case study. The third criterion, with one standard deviation of the budget value as the confidence interval and 68% as the confidence level, could be applied to effectively identify hidden uncertainties in the MFA system; therefore, it was selected for ensuring quality of the current MFA system. Sensitivity analysis for environmental impacts is needed for identifying the influential parameters on outflows to environments, and ultimately, for reducing requirements for data collection [19, 20]. Sensitivity analysis was performed to quantify the effect of a 10% increase in each parameter on the environmental flows, to identify the parameters that had a more significant influence than the others.

Development of scenarios

The validated MFA model was used to develop different scenarios for the target site. Hama et al. [11] and Kim et al. [14] stated that reduction of applied chemical fertilizers and replacing them by another nutrient source would not impact to rice yields. Do and Nishida [7] demonstrated that chemical fertilizers applied in Vietnam could be reduced half possibly [17]. We assumed that the nutrients in manure, excreta, or wastewater in the drainage system could be replaced for half of the currently applied chemical

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Table 1 Parameters and equations for estimating nitrogen flow in pig process

Symbol	Description of data	Data type	Unit	Statistical distribution	Mean		Standard deviation (SD)	
					Value	References	SD/Mean (%)	References
n fat	Number of fattener in the commune	Certain	Head	Normal	3000	[13]	10	Authors' assumption
n sow	Number of sow in the commune	Certain	Head	Normal	1200	[13]	10	Authors' assumption
S paddy	Paddy area in the commune	Certain	ha/year	Normal	590	[13]	10	Authors' assumption
aN man_fat	Daily nitrogen load in fatteners' manure	Certain	gN/head.day	Normal	32	[21, 26–28]	3	[21, 26–28]
aN man_sow	Daily nitrogen load in sows' manure (including piglets)	Certain	gN/head.day	Normal	85	[21, 26–28]	1	[21, 26–28]
aN man_paddy	Nitrogen load in manure supplied for paddy in a crop	Certain	kgN/km^2.crop	Uniform	3000*	[17, 29]	3600**	[17, 29]
a feed_pig	Annual food for pig (both for sow or fattener)	Certain	kg food/head.year	Normal	555	[25]	50	[25]
a feed_piglet	Daily food for piglet	Certain	kg food/head.year	Normal	25	[25]	3	[25]
aN man_fat	Nitrogen load in daily fatteners' manure	Certain	gN/head.day	Normal	35.00	[21, 26–28]	1	[21, 26–28]
aN man_sow	Nitrogen load in daily sows' manure (including piglets)	Certain	gN/head.day	Normal	85.00	[21, 26–28]	1	[21, 26–28]
CN feed_pig	Nitrogen content in commercial food for pig	Certain	gN/kg food	Normal	26	[25]	1	[25]
CN pork	Nitrogen content in pork	Certain	gN/kg meat	Normal	26	[19, 20]	1	[19, 20]
n piglet	Number of piglets per sow annually	Certain	Head/head	Normal	21	[25]	3	[25]
r man_biogas	Ratio of pig manure to biogas system to total pig manure	Uncertain	–	Normal	0.50	Authors' assumption	0.25	Authors' assumption
rN emis_pigman	Ratio of N gas losses to N manure pigs	Uncertain	–	Lognormal	0.2	[25]	0.05	[25]

	Flow description	Flow	Model equation
	Stock change rate of pig process *(Balance equation)*		= IN1 − (OUT1 + OUT2 + OUT3 + OUT4 + OUT5)
Input	Pig feed bought from market	IN1	= [(n fat + n sow) × a feed_pig + n piglet × n sow × a feed_piglet] × CN feed_pig × 10^{-6}
Output	Pig manure put into biogas	OUT1	= (aN man_fat × n fat + aN man_sow × n sow) × (1 − rN emis_pigman) × r man_biogas × 365 × 10^{-6}
	Wastes from pig houses to drainage	OUT2	= (aN man_fat × n fat + aN man_sow × n sow) − OUT1 − OUT3
	Pig manure applied in paddy	OUT3	= aN man_paddy × S paddy × 10^{-3}
	Pig eat sold in market	OUT4	= Y pork × CN pork × 10^{-3}
	Pig manure lost to the atmosphere	OUT5	= (n fat × aN man_fat + n sow × aN man_sow) × rN emis_pigman × 365 × 10^{-6}

* and ** represent minimum and maximum values of the uniform distribution, respectively

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fertilizers in the paddy fields. Therefore, reduction of half of chemical fertilizers applied was added in the following scenario. The first scenario (Scen1) was created a mitigation measure for Hoang Tay commune; all pig manure and human excreta were, respectively, collected in biogas and septic tanks; only wastewater in the drainage system was reused in the paddy fields. That meant nutrient flows from pig process to paddy process or drainage process would be neglected in this scenario. In addition, flows from drainage process to fishpond process or Nhue River process would be ignored, too. Correspondingly, value of parameter r man_biogas in Table 1 would be replaced with 1.00 ± 0.25, represented for 100% of pig manure put into biogas. Second scenario (Scen2) was another solution, and all manure and excreta were reused in paddies, excepting for wastewater. That meant nutrient flows from pig process to biogas process and the ones from drainage process to paddy process were removed. At the same time, the flows from household process to septic tank process would be replaced by flows from household process to paddy process, whereas flows from and to drainage process were kept. Third scenario (Scen3) described the environments when excreta and manure were all reused in the paddies, similar to Scen2; and wastewater was treated in the treatment plant. In this scenario, flows from drainage process to Nhue River process or paddy process would be removed, but adding flows from this process to water treatment plant process, which would be newly added.

Results and discussion

Uncertainty analysis

Plausibility test

The quality of the MFA model was assessed by plausibility test in the uncertainty analysis. The nine processes located inside the MFA boundary passed the plausibility tests

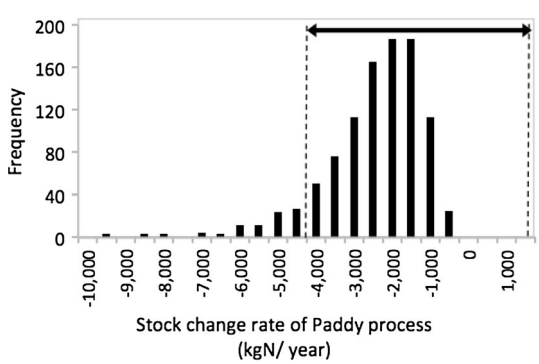

Fig. 3 Plausibility test for nitrogen in the paddy process. *Arrows* represents the plausible range of this process

successfully with the criterion 3: more than 68% of values generated in the range of one standard deviation of the budget value [6]. As can be seen in Fig. 3, 74% of the generated values in Monte Carlo simulation were in the plausible range. Thus, the MFA model developed was of high quality.

Sensitivity analysis

Sensitivity analysis was conducted for nutrient flows from the assessed human activities in Hoang Tay commune to the Nhue River. There were three main sources of nutrients to the Nhue River: wastewater from drainage, overflow water from the fishpond, and overflow water from paddy field. By assessing the variation of total nutrients (N or P) while increasing 10% of each parameter in the MFA model, the most sensitive parameters were identified for total nutrients discharged to Nhue River. Figure 4 revealed that chemical fertilizer (aN fer, aP fer), number of pigs (n pig), number of poultry (n poul), and area of paddy (S paddy) were the most sensitive parameters. The second most sensitive parameters were ratio of households equipped with biogas (r hh_BG), septic tank (r hh_ST), or pit latrines (r hh_PL). In other words, these parameters had a significant impact on the quantifying nutrients flows to the Nhue River.

Nutrient flows in the MFA system

Nutrients flows, connecting livestock to environmental sanitation and agricultural system, were quantified and visualized in Fig. 5.

There were two main sources of on-site sanitation process (biogas, septic tank, and pit latrine): human excreta (13 ± 1 ton N/year, 3 ± 0.1 ton P/year) and pig manure (30 ± 2 ton N/year, 5 ± 0.1 ton P/year). The biggest outflow from this on-site sanitation system was wastewater to drainage (40 ± 3 ton N/year, 8 ± 2 ton P/year). Human excreta was distributed to all three types of on-site sanitation processes, but more than half of this went to pit latrines, because 53% of households were equipped with pit latrines [13]. Therefore, loads of nutrients in pig manure, from pig process to biogas process, were more than double compared with the ones in human excreta, from household process to septic tank and pit latrine process as presented in this figure. However, the wastewater from biogas and septic tanks was not treated completely; it still contained condensed nutrients, which was then released to drainage freely. There was no flow from the on-site sanitation system to the paddy fields in this figure, because the excreta from pit latrines that were reused in paddy fields was very minor compared with other flows (1.2 ± 0.3 ton N/year, 0.3 ± 0.08 ton P/year). Without considering the

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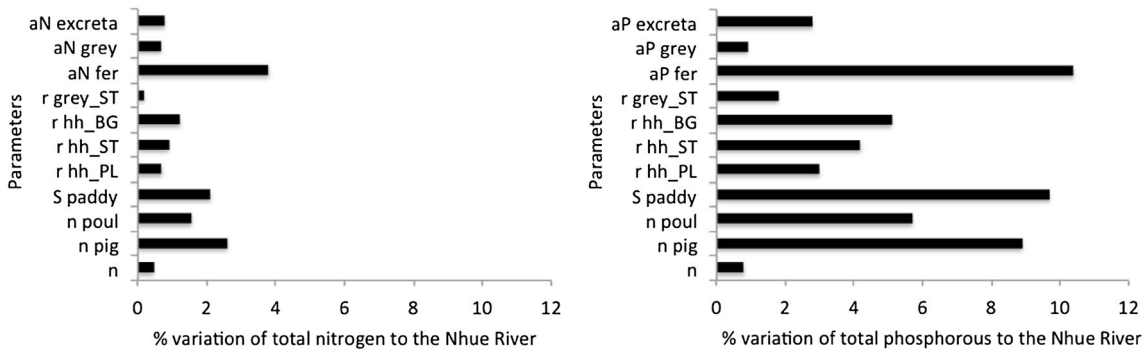

Fig. 4 Sensitivity analysis for nitrogen and phosphorous flows to Nhue River. *Note* aN excreta and aP excreta represent for daily nitrogen (N) and phosphorous (P) load in human excreta; aN *grey* and aP *grey* represent for daily N and P load in grey water; aN fer and aP fer represent for N and P load in chemical fertilizers applied; r *grey*_ST represents for Ratio of *grey* water used in septic tank; r hh_BG, r hh_ST, and r hh_PL represent for ratio of households equipped with biogas latrine, septic tank, and pit latrine to total households; S paddy represents for paddy area; n poul and n pig represent for number of poultry and pig; *n* represents for population in Hoang Tay commune

appropriate methods for storing manure and excreta, the nutrients would not be effectively reused in the paddy fields, but lost through wastewater to the surrounding environments.

As explained above, wastewater from on-site sanitation system was the main source of drainage. The second biggest source of this process was uncollected pig manure (15 ± 2 ton N/year, 5 ± 0.7 ton P/year), which originated from washing pig houses. The uncollected pig manure and wastewater in drainage were then released to paddies (31 ± 5 ton N/year, 7 ± 0.5 ton P/year), fishpond (5 ± 2 ton N/year, 1 ± 0.04 ton P/year), or the Nhue River (26 ± 6 ton N/year, 6 ± 0.5 ton P/year). Thus, just about half of nutrients in pig manure and human excreta were reused in the paddies; the remaining half was discharged into the Nhue River.

The main nutrients input to the pig and poultry processes were from commercial feeds for animals; these were also biggest flows in the whole MFA system. There was 77 ± 11 ton N/year and 22 ± 6 ton P/year for feeding pigs and 30 ± 2 ton N/year and 16 ± 7 ton P/year for feeding poultry. Half of the pig manure was discharged to the biogas, 25% to drainage system, and 25% directly reused in the paddy fields. Unlike pig manure, poultry manure was reused effectively for feeding fish as the main nutrient sources of fishpond (11 ± 3 ton N/year, 5 ± 1 ton P/year). It was also reused in the paddy fields (7 ± 2 ton N/year, 6 ± 3 ton P/year).

Paddy and fishpond were the key processes for manure reuse in the study site. Nutrients contained in chemical fertilizers applied in the paddies were one of the biggest flows in the whole MFA system (74 ± 12 ton N/year, 24 ± 6 ton P/year). Nutrients in the commercial feed for fish were much lower than nutrients from chemical fertilizers applied, but they were also contributed significant input flows (15 ± 5 ton N/year, 2 ± 0.4 ton P/year). As shown in Fig. 5, there were 54 ± 5 ton N/year and 30 ± 4 ton P/year contained in manure and excreta as well as wastewater input to the paddy fields. These contributed 42 and 56% of total N and P input to the paddies fields, respectively. Similarly, poultry manure was the main nutrient source for fish feed (52% N and 63% P). Thus, human excreta and animal manure were the significant sources of nutrients in the agricultural system in Hoang Tay commune.

Many papers on assessing nutrient flows have been published, especially regarding farming system, those have mainly been conducted at global, watershed, and city levels, and a very few have been conducted at the commune level. In addition, those studies have focused mainly on quantifying nutrient discharge from the studied systems and identifying the key sources and paths of pollution while often ignoring the nutrient use efficiency (NUE) in the system. Montangero et al. [19] revealed the assessment of nutrient flows in the urban environmental sanitation system of Hanoi Capital, Vietnam. Do-Thu et al. [8] also applied the MFA for quantifying nutrient flows at Hoang Tay and Nhat Tan commune, Ha Nam province, Vietnam. However, none of them took NUE into account. The MFA model proposed in this research was first assessing NUE in the on-site sanitation and agricultural system at the commune level. Therefore, the estimated NUE was compared with those in case studies with similar agricultural conditions to examine the performance of the proposed MFA model. NUE was estimated from the ratio of nutrient uptaken by rice and total nutrient input, including chemical fertilizers and pig manure. Chen et al. [2] and Wu et al. [30] were also applied similar method for assessing NUE in China. They reported that P load from pig manure contributed

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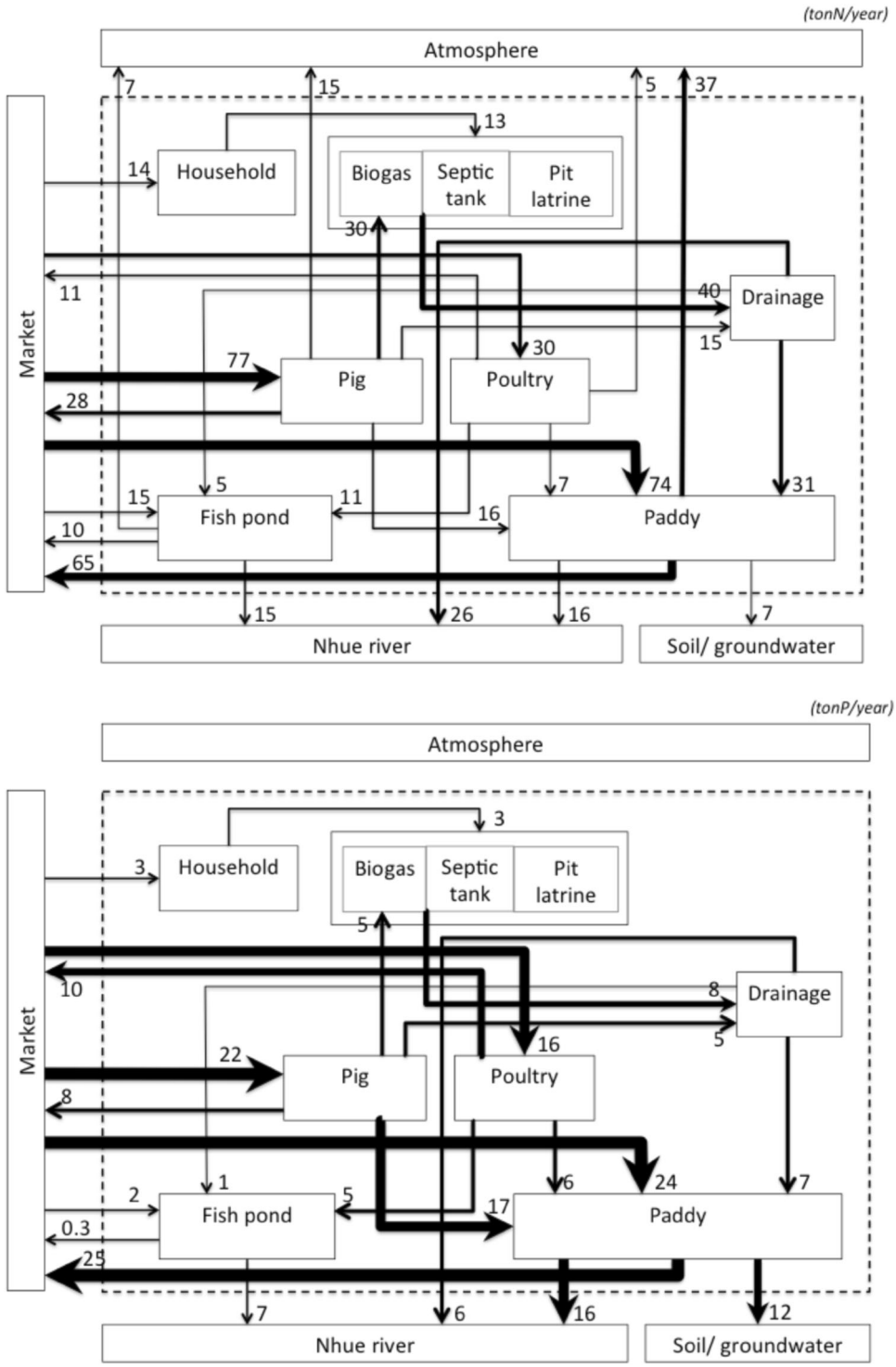

Fig. 5 MFA system showing the values (ton/year) of nitrogen and phosphorous flows, respectively, in the environmental sanitation system and agricultural system in the year 2009 in Hoang Tay commune, Ha Nam province, Vietnam

31.2–42.6% of total P applied in paddy, and NUE value was in the range of 45.7–58.0%. In the case study of Hoang Tay commune, P load from pig manure contributed 41.0% of total P applied in paddy and NUE value was 46.3%. Thus, results from the proposed MFA model were in the range of previous reported results.

Environmental impacts of human activities

In this research, the environmental impacts of agricultural activities were assessed through the interactions of livestock (pig and poultry) with the environmental sanitation system (biogas, septic tank, pit latrine, and drainage) and

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the agricultural system (fishpond, paddy). Figure 5 demonstrated four sources of nitrogen to the atmosphere: pig, poultry, fishpond, and paddy processes. There was 64 ± 12 ton N/year from agricultural and livestock systems in Hoang Tay commune emitted to the air, through manure and chemical fertilizers applied. Among those four processes in the agricultural and livestock system, nitrogen originating from the paddy fields was the largest source (37 ± 8 ton N/year), contributing to 58% of total nitrogen lost to the air. The second largest source was nitrogen in manure from pig process (15 ± 4 ton N/year). Thus, 29 and 19% of nitrogen inputs into paddy and pig processes were lost to the air, respectively. On the other hand, nitrogen lost from poultry process was contributed less to total N lost to the air, but it was 17% of all N inputs into this process.

Fishpond, paddy, and drainage processes were the main sources of nutrients discharged to the Nhue River. There were 57 ± 9 ton N and 29 ± 6 ton P discharged to the river every year. Drainage was the largest source of nitrogen (46%), but paddy fields were the largest sources of phosphorous discharged to the river (55%). In addition, there were 7 ± 0.9 ton N and 12 ± 4 ton P discharging from paddy to soil/groundwater annually.

Development of scenarios

Our results demonstrated that nutrients in the manure and excreta have not yet been effectively reused in the agricultural system, and also have the potential to pollute the surrounding environments. The validated MFA model was used to run three proposed scenarios, and the results presented in Fig. 6 show that the nutrient loads to the surrounding environments were reduced most effectively by applying scenario 3. 55, 70, and 44% of current nitrogen load to the atmosphere, the Nhue River, and soil/groundwater were, respectively, decreased in the scenario 3. In addition, 69 and 52% of the phosphorous load to the Nhue River and soil/groundwater were declined, respectively. Scenario 2 was also a good option for reducing nutrient loads to the environments. The reduction of total nitrogen load to the atmosphere, the Nhue River, and soil/groundwater were 47, 49, and 36%, respectively. In addition, 59 and 44% of total phosphorous load to the Nhue River and soil/groundwater were reduced, respectively. However, collecting all of the pig manure in biogas was not a good solution for our case study of Hoang Tay commune. The reduction in total nutrients loads to the environments was much less in scenario 1. Therefore, reusing excreta and manure in the paddy fields, which was proposed in scenario 2 and 3, would help reduce nutrients losses to the environments, and also reduce half of chemical fertilizers applied as currently.

Conclusions and recommendations

Our study demonstrates that this MFA method is suitable to quantify nutrient flows in an area which is clearly faced with data uncertainty and scarcity. Nutrient flows from livestock to the environmental sanitation and agricultural systems were originally and successfully quantified in this study. In addition, the impact of manure reuse on the surrounding environments was first assessed in a small commune of Vietnam. The MFA results identified the critical control sources of nutrients in Hoang Tay commune, which included the overuse of chemical fertilizers in paddy fields, uncontrolled effluent from the environmental sanitation system, and insufficient management of pig manure. Consequently, options for nutrient resource management were proposed, such as reducing half of chemical fertilizers applied and reusing all excreta and manure in the paddy

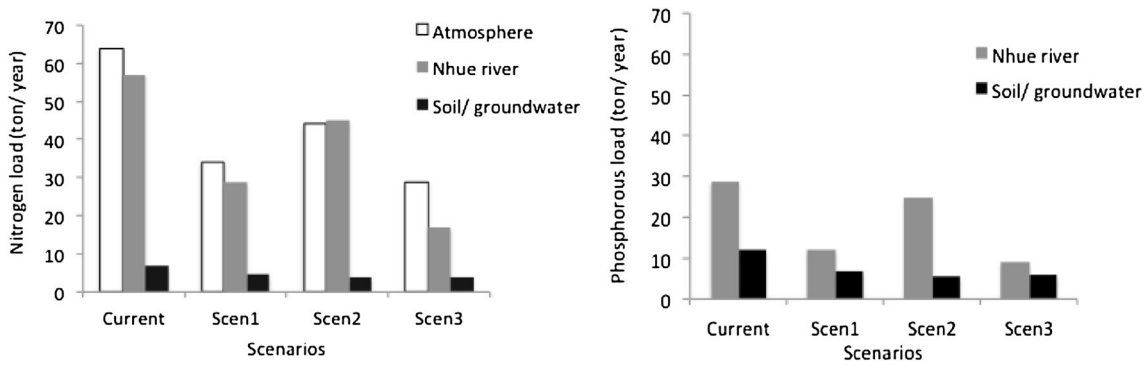

Fig. 6 Nitrogen and phosphorous loads from Hoang Tay commune to the surrounding environments in the year 2009 (Current) and in different scenarios (Scen). *Note* Scen1 represents for all pig manure and human excreta were, respectively, collected in biogas and septic tanks; only wastewater in the drainage system was reused in the paddy fields. Scen2 represents for all manure and excreta that were reused, excepting for wastewater. Scen3 represents for excreta and manure that were all reused in the paddy fields; wastewater was treated in the treatment plant

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fields. However, sustainable sanitation must consider the potential health impacts of applying manure. Therefore, pre-treating manure, through composting for example, 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 mitigation measures on resource consumption and environmental pollution.

Acknowledgements We thank Nguyen Kim Ngan and Nguyen Duy Tien for their technical support in the study and Vi Nguyen for proofreading the manuscript. The Swiss Development Cooperation (SDC) and Swiss National Science Foundation (SNSF) financially supported this research through the PAMS Nr. SEA-3_05 Project within the NCCR North–South program. The field research surveys conducted in this study were funded by the NAFOSTED (Program No. 104.04-2013.37).

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9. TURNING POOP INTO PROFIT: COST-EFFECTIVENESS AND SOIL TRANSMITTED HELMINTH INFECTION RISK ASSOCIATED WITH HUMAN WASTE REUSE IN VIETNAM

This paper has been published in PLOS Neglected Tropical Diseases November 27 2017, https://doi.org/10.1371/journal.pntd.0006088.

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PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Turning poop into profit: Cost-effectiveness and soil transmitted helminth infection risk associated with human excreta reuse in Vietnam

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Abstract

Human excreta is a low cost source of nutrients vital to plant growth, but also a source of pathogens transmissible to people and animals. We investigated the cost-savings and infection risk of soil transmitted helminths (STHs) in four scenarios where farmers used either inorganic fertilizer or fresh/composted human excreta supplemented by inorganic fertilizer to meet the nutrient requirements of rice paddies in the Red River Delta, Vietnam. Our study included two main components: 1) a risk estimate of STH infection for farmers who handle fresh excreta, determined by systematic review and meta-analysis; and 2) a cost estimate of fertilizing rice paddies, determined by nutrient assessment of excreta, a retailer survey of inorganic fertilizer costs, and a literature review to identify region-specific inputs. Our findings suggest that farmers who reuse fresh excreta are 1.24 (95% CI: 1.13–1.37, p-value<0.001) times more likely to be infected with any STH than those who do not handle excreta or who compost appropriately, and that risk varies by STH type (*Ascaris lumbricoides* RR = 1.17, 95% CI = 0.87–1.58, p-value = 0.29; Hookworm RR = 1.02, 95% CI = 0.50–2.06, p-value = 0.96; *Trichuris trichiura* RR = 1.38, 95% CI = 0.79–2.42, p-value = 0.26). Average cost-savings were highest for farmers using fresh excreta (847,000 VND) followed by those who composted for 6 months as recommended by the WHO (312,000 VND) and those who composted for a shorter time (5 months) with lime supplementation (37,000 VND/yr); however, this study did not assess healthcare costs of treating acute or chronic STH infections in the target group. Our study provides evidence that farmers in the Red River Delta are able to use a renewable and locally available resource to their economic advantage, while minimizing the risk of STH infection.

OPEN ACCESS

Citation: Tran-Thi N, Lowe RJ, Schurer JM, Vu-Van T, MacDonald LE, Pham-Duc P (2017) Turning poop into profit: Cost-effectiveness and soil transmitted helminth infection risk associated with human excreta reuse in Vietnam. PLoS Negl Trop Dis 11(11): e0006088. https://doi.org/10.1371/journal.pntd.0006088

Editor: Mark Booth, University of Newcastle, UNITED KINGDOM

Received: March 15, 2017

Accepted: October 31, 2017

Published: November 27, 2017

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Data Availability Statement: All relevant data are within the paper.

Funding: Travel support for RJL, JMS, and LEM was provided by Global Affairs Canada and the Veterinarians Without Borders Volunteer Cooperation Program (https://www.vetswithoutborders.ca/). Stipend support for JMS was provided by the Integrated Training Program in Infectious Diseases, Food Safety and Public Policy (https://www.usask.ca/wcvm/graduate_

programs/ITraP_program/index.php). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Author summary

Each year, hundreds of millions of people worldwide are infected with intestinal worms spread by contaminated soil, also known as soil transmitted helminths (STHs). These worms are most common in tropical climates in areas lacking good hygiene and sanitation, and negatively impact child development, quality of life, and economic wellbeing. Reuse of human excreta for fertilizer is a common practice in many low to middle income countries because farmers require a low cost source of nutrients to grow food crops eaten by people and animals. Excreta can contain microbes, such as STHs, that cause disease in people; however, composting is a known method of killing STHs. Therefore, our goal was to determine if Vietnamese rice farmers involved in this practice are at higher risk of STH infection, and to calculate the amount of money saved by farmers composting for different lengths of time, and supplementing with various commercial fertilizers. We suggest that farmers compost excreta for six months to reduce disease exposure and optimize household savings. Optimizing practices to improve food production and protect farmer health is critical for poverty alleviation in low to middle income countries.

Introduction

Application of human excreta onto rice paddies as fertilizer is a common practice in northern Vietnam, where many farmers use single or double vault latrines, lack access to wastewater infrastructure, and have variable access to commercial inorganic fertilizers [1]. Using organic waste to fertilize fields has clear benefits for crop yield [2]; however, this practice increases certain health risks for farmers and consumers, such as infection by soil transmitted helminths (STHs)[3,4]. The STH group includes *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm spp., which are intestinal parasites that spread between people when sanitation is inadequate or when good hygiene is not practiced [4]. People are infected when they accidentally ingest infective eggs or when their skin contacts infective larvae in contaminated soil. These parasites are particularly prevalent in regions with warm, moist climates, and are included in the category of tropical neglected diseases associated with poverty.

World Health Organization (WHO) guidelines recommend that farmers compost human excreta for six months prior to application in order to inactivate STH eggs and larvae, and thereby reduce spread between people [5]. This practice is not feasible for all Vietnamese farmers, in particular those who harvest multiple crops per year or have single vault latrines that lack a chamber for long-term excreta storage. Current evidence suggests that only one-third of farmers who use human excreta follow the six-month recommendation [6], and that STH infection remains an occupational hazard associated with handling human excreta [3]. It is common practice for household members to add a handful of kitchen ash after using a latrine, as this reduces smell. A recent study characterizing *A*. *lumbricoides* egg die-off during excreta composting suggests that adding lime reliably accelerates egg inactivation so that WHO criteria for safe handling ($<$1 viable egg/g total solids) are met by 153 days [7]. Ascarid eggs can survive longer periods in adverse environmental conditions than other STHs, and for that reason we chose *A*. *lumbricoides* die-off as a proxy for overall STH die-off [8].

Rice farmers in some agricultural regions of Vietnam have shifted their source of fertilizer from human excreta to commercial inorganic products, either wholly or in part. It is unclear whether this trend will become universal as not all farmers are able to afford or access commercial fertilizer, and others consider human excreta a superior source of long-term nutrition for plants and soil [9]. Inorganic fertilizers are primarily imported, and their costs are

influenced by a wide range of factors, including energy prices [10]. Using human waste to fertilize crops is recognized as a way to decrease household expenditures; however, it is unclear how costs and health risks associated with STH infection interact. The goal of this study was to compare the costs and STH risk associated with fertilizing rice paddies in the Red River Delta (RRD).

Methods

Study context

The RRD encompasses eight provinces and two major urban municipalities (Hanoi and Haiphong) in northern Vietnam. The RRD is an agriculturally intense area that produces approximately 15% of the national annual rice output [11]. Throughout the region, farmers use various combinations of human excreta, inorganic fertilizers, and animal manure to replenish soil nutrients and maximize rice yield. To generate cost estimates, we chose four fertilization scenarios: (A) Fresh human excreta ($\leq$ 139 day storage without lime); (B) Composted human excreta (153 day storage with 10% lime as per [7]); (C) Composted human excreta (181 day storage without lime; WHO standard [5]); (D) Inorganic fertilizer. Although three scenarios (A-C) involved human excreta, we assumed that only farmers who handled fresh excreta (A) would experience STH infection risk, as the composting scenarios (B and C) met WHO standards for helminth inactivation. Risk of STH infection for Vietnamese farmers handling fresh excreta was evaluated by systematic review and meta-analysis. Our economic analysis of the four scenarios included the direct costs incurred for composting human excreta (i.e. lime) and supplementing excreta with inorganic fertilizers. Capital costs (i.e. cost to build a double vault latrine) were not included because differences in factors such as materials and design cause costs to vary substantially in the RRD, and would add a high level of uncertainty to our analysis. To estimate the direct costs, we determined nutrient content of organic fertilizer scenarios, conducted a retailer survey of inorganic fertilizers in the study area, and collected economic inputs from published sources specific to the RRD (e.g. household size, excreta production per household, annual harvest frequency, average paddy size).

STH risk estimate

Systematic review. Two systematic review research questions were defined as: (1) "is there risk of infection with any STH type among farmers who work with fresh human excreta to fertilize rice paddies in the RRD, Vietnam"; and (2) "is there risk of infection with STHs, by type of helminth, among farmers who work with fresh human excreta to fertilize rice paddies in the RRD, Vietnam". Two authors (RL and JS) used three search engines (PubMed, Embase, and ProQuest) to search titles/abstracts of English language peer-reviewed publications using the following terms: Vietnam AND ((human excreta) OR (human waste) OR (night soil) OR (fertilizer) OR (manure)). Inclusion criteria for relevance screening were: time period (2006–2016), location (Vietnam), diagnostics (stool sample tested for STH), occupation (farming), exposure (contact with human excreta), and risk assessment reporting (risk estimate and measurement of error). The Vietnamese government, in collaboration with international aid organizations, has conducted targeted STH elimination campaigns in past decades, and we restricted the time period to avoid overestimating risk. One author (NTT) conducted similar searches in two databases managed by the National Agency for Science and Technology Information to identify Vietnamese language publications, projects, theses, and government reports. Authors initially read all titles/abstracts of the search results and then full publications of the subset that appeared to meet the inclusion criteria. As well, citation lists in relevant English and Vietnamese publications were manually searched for additional papers of interest.

Table 1. Quality assessment tool used to evaluate individual publications that met inclusion criteria for reporting STH risk in farmers handling human excreta (modified from [12,13]).

Study Component	Reviewer's Comments	Score
Selection: (≤ 6 stars)		
Representativeness of the sample:		
a) Truly representative of the average '__' in the target population (all subjects or random sampling).**		
b) Somewhat representative of the average '__' in the target population (non-random sampling).*		
c) Selected group of users (e.g. farmers, nurses).		
d) No description of the sampling strategy.		
Sample size:		
a) Justified and satisfactory.*		
b) Not justified.		
Non-respondents:		
a) The response rate is reported and satisfactory.*		
b) The response rate is not reported, or unsatisfactory.		
Assessment of the exposure (risk factor):		
a) Validated measurement tool. **		
b) Non-validated measurement tool, but the tool is available or described.*		
c) No description of the measurement tool.		
Comparability: (≤ 2 stars)		
The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled.		
a) The study justifies inclusion of variables (e.g. stepwise regression), controls for confounders or effect modifiers, and has comparable outcome groups.**		
b) The study satisfied 2 of 3 above listed criteria.*		
c) The study does not provide sufficient detail to assess comparability of outcome groups.		
Outcome: (≤ 5 stars)		
Sample collection:		
a) >1 stool samples collected per respondent within one week period.*		
b) One sample collected per respondent.		
c) No description.		
Diagnostic test:		
a) >1 validated test used or 2+ blinded technicians used the same validated test.**		
b) One validated test used by a trained technician.*		
c) Diagnostic test not described, not validated, or known to misrepresent true infection prevalence.		
Statistical test:		
a) The statistical test used to analyze the data is clearly described and appropriate, and the measurement of association is reported with estimates of error (confidence interval and p-value). **		
b) The statistical test used to analyze the data is clearly described and appropriate, and the measurement of association is reported with a confidence interval or p-value.*		
c) The statistical test is not appropriate, not described, or incomplete.		
Total Score (≤ 13 stars):		

*—indicates 1 point

**—indicates 2 points

https://doi.org/10.1371/journal.pntd.0006088.t001

Duplicate reports (identified by title and primary author) were removed, and all publications that met the inclusion criteria were retained and independently assessed for quality by RL and JMS using a version of the Newcastle-Ottawa scale modified from [12,13] (Table 1). Quality assessment of relevant studies evaluated the materials and methods section of each report for

complete descriptions and appropriate study design, sampling methods, control of confounders, exposure and outcome assessment, inclusion and exclusion criteria, and statistical analysis. Data were extracted on author, language, region, study design, population, exposure, outcome, sample size, measure(s) of association, 95% confidence intervals, p-values, standard errors, confounders adjusted for, and number of: exposed, unexposed, outcome positive and outcome negative, from each publication and recorded in a Microsoft Excel (2013) database.

Meta-analysis. The risk of STH infection associated with handling fresh human excreta was assessed in random-effects meta-analysis using data from relevant studies that met inclusion criteria collected in the systematic review. Unadjusted raw data for disease positive, disease negative, exposed and unexposed were extracted from each manuscript and used to generate pooled relative risks (RRs) for each research question aspoor reporting of necessary sample sizes, p-values, or standard errors precluded inclusion of adjusted risk estimates in the analysis. One study [14] did not report sample size by exposure or outcome, and therefore could not be included in the meta-analysis. Statistical analysis of heterogeneity was assessed by the I^2 statistic, where an I^2 value above 25% denoted significant heterogeneity. Where possible, sensitivity analysis was used to assess the extent to which one or more factors, such as study, might explain observed heterogeneity. All analyses were conducted using STATA 14.

Cost estimates

Nutrient standardization of organic fertilization scenarios. The Vietnamese Rice Knowledge Bank provides recommendations for nitrogen: phosphorus: potassium (NPK) amounts needed to replace soil nutrients per rice harvest [15]. To ensure that the NPK content of each organic fertilizer scenario (A-C) was standardized within the upper and lower bounds of these recommendations, we composted human excreta to monitor changes over the defined composting period. Excreta were stored in concrete vaults meant to simulate double vault latrines, and three samples per vault were analysed every 28 days over a 139 day period. Changes in moisture content (and thereby excreta weight) were also assessed to determine NPK concentrations per kg of excreta. Details of storage conditions, sample collection, and moisture assessment are reported elsewhere [7].

Total nitrogen concentration of composted excreta was assessed by the Kjeldahl method [16]. Total phosphorus content was assessed by mixing 0.5 grams of excreta to 10 mL of 98% sulfuric acid in a dried Kjeldahl flask, and pipetting 1 mL of the mixture into a 50 mL flask where it was combined with 2 mL of 2.5% ammonium molybdate and 1 mL of ascorbic acid. The solution was diluted with 40 mL dH_2O, heated in a sand bath to 300˚C for 10 minutes, cooled, and then measured at 710 nm by ultraviolet–visible spectrometer (Model U-2900, Hitachi High Technologies Corporation, Toyko, Japan). The total potassium content was determined by mixing 0.5 grams of excreta with 10 mL of 98% sulfuric acid in a dried Kjeldahl flask, and measuring the solution by atomic absorption spectrophotometer (Model AA-6800, Shimadzu Corporation, Kyoto, Japan) as per government standard [17].

To generate mean nutrient content for fresh excreta (A), we averaged the NPK and moisture content for excreta sampled over 139 days. For the compost scenarios (B,C), we conducted a simple linear regression to identify significant changes over time in NPK and moisture levels for excreta stored with 10% lime (B), and without lime (C). If no significant changes were evident, we used the last recorded estimates (i.e. 139 days). When NPK and moisture content differed significantly over time, general linear regression was used to predict levels at 153 days (B) and 181 days (C). Using these data, final NPK concentrations were calculated and compared with upper and lower NPK recommendations to identify a suitable application rate for rice paddies, and to inform the cost estimates of scenarios A, B, and C. If excreta did not provide

sufficient N, P, or K, we assumed that farmers would supplement with nutrient specific inorganic fertilizers.

Inorganic fertilizer costs. To estimate the price of recommended NPK fertilizers, we recruited two resident volunteers in each of the eight provinces in RRD. Each volunteer visited at least one retailer in their commune in December 2016 and surveyed the shop owner to determine the price per kilogram for any brand (up to ten) of the recommended fertilizer types. We used the average retail price across all brands and communes to estimate cost-savings.

Cost estimate generation. To quantify the cost-savings available to farmers who substituted human excreta for inorganic fertilizer (A, B, C), we used the nutrient analysis along with estimates of excreta production [18] and household size [19] to determine the amount of organic NPK available to an average RRD household. We then used estimates of annual harvest frequency and average paddy size to calculate the average amount of NPK required per household annually [19]. The amount of organic NPK available was then compared to the average NPK required to determine the weight of human excreta that should be distributed onto rice paddies each year for each fertilization scenario. We assumed that organic NPK levels falling within the upper and lower bounds of recommended inorganic NPK application would lead to optimal rice yield. Finally, we used our estimate of RRD fertilizer prices to calculate the average cost incurred by farmers for each scenario (A-D). Sensitivity analysis was conducted to test upper and lower values for fertilizer prices, rice paddy size and household size, while holding all other values constant.

Results

STH risk estimate

Systematic review. Our search terms identified 513 unique publications for review (113 English, 400 Vietnamese; Fig 1). Of these, four English language publications and zero Vietnamese language publications, met our inclusion criteria. Overall, the quality of reporting was low to moderate, and we noted a high degree of variability in quality scores among selected publications (mean = 8, range = 6–12; Table 2). Two of four papers reported STH risk estimates in risk ratios (RR) and the other two reported odds ratios (OR).

Meta-analysis. Using inverse variance weighting and random effects, our analysis found that farmers who fertilized rice paddies with fresh human excreta were 24% (RR = 1.24, 95% CI: 1.13–1.37, p-value<0.001; Fig 2) more likely to be infected with one or more STHs than farmers who did not handle fresh excreta. However, the infection risks of individual helminths were lower than the risk of any STH, and were not significant. Risk of hookworm infection (RR = 1.02, 95% CI: 0.5–2.06, p-value = 0.96) was lowest compared to other helminth types (*A. lumbricoides* RR = 1.17, 95% CI: 0.87–1.58, p-value = 0.29; *T. trichiura* RR = 1.38, 95% CI: 0.79–2.42, p-value = 0.26; Fig 2). Meta-analyses that examined infection risk with *T. trichiura* or hookworm demonstrated significant heterogeneity (I^2 = 85.9% and I^2 = 87.0%, respectively). Sensitivity analysis for the *T. trichiura* outcome found that removal of Yajima et al., 2009 eliminated the majority of heterogeneity [21]. The *A. lumbricoides* meta-analysis had also low evidence quality due to high heterogeneity among three studies (I^2 = 47.8%); heterogeneity was eliminated by the removal of Yajima et al., 2009 (I^2 = 0%). Sensitivity analysis could not be carried out for hookworm infection risk due to the small number of studies (n = 2) included in the meta-analysis.

Cost estimates

Nutrient standardization of organic fertilization scenarios. The Vietnamese Rice Knowledge Bank recommends spreading inorganic NPK 16-16-8 or 17-12-5 fertilizers at a

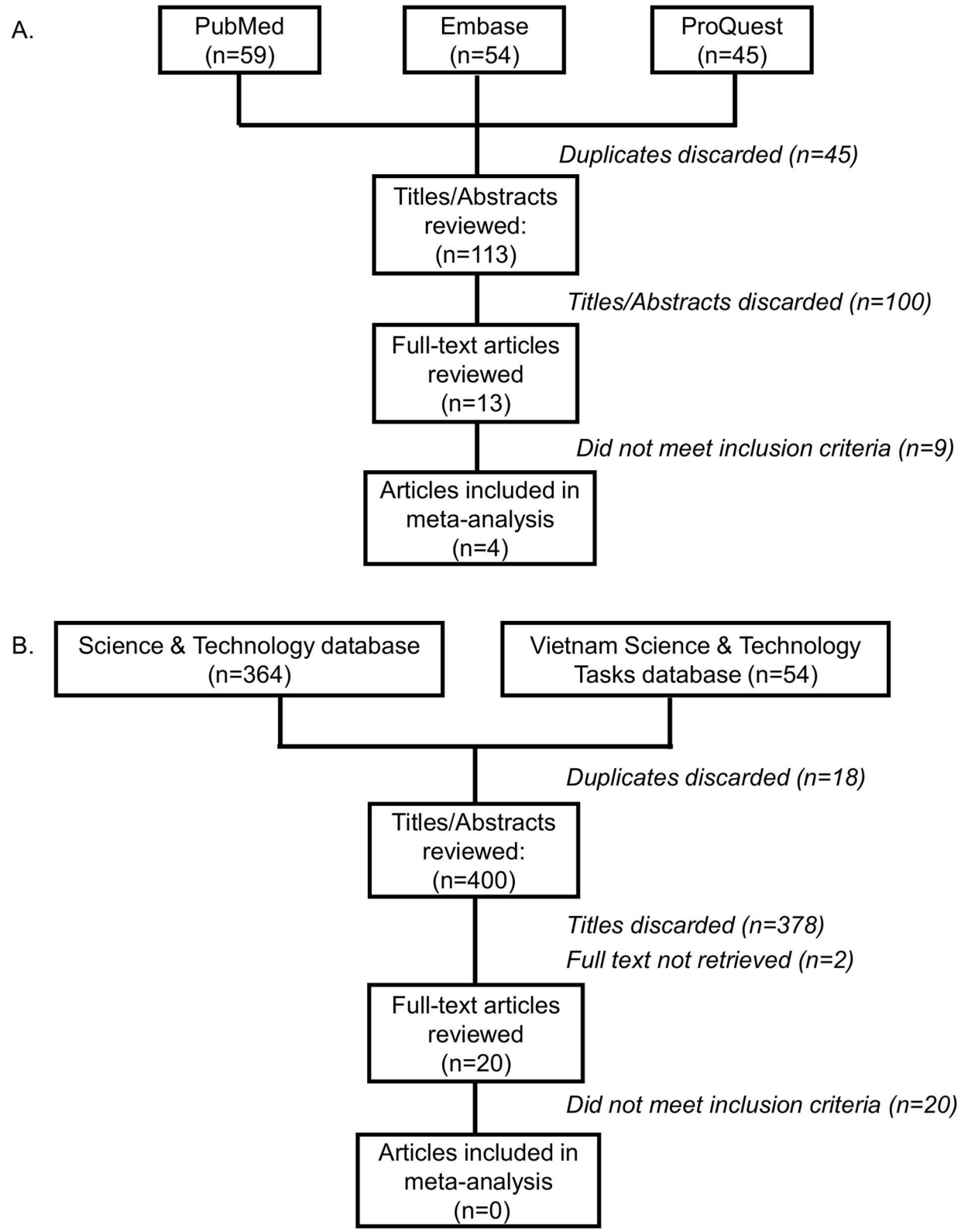

Fig 1. Flow chart of search strategy and study selection for reports of STH infection following use of human excreta in Vietnam (A) English. (B) Vietnamese.

https://doi.org/10.1371/journal.pntd.0006088.g001

Table 2. Characteristics and methodological quality of four English language, cross-sectional studies reporting the association of human excreta use in agriculture on STH infection in Vietnam (2006–2016).

Author	Region	Population Assessed	Exposure (Exposure Assessment)	Outcome (Outcome Assessment)	Sample Size	MOA (95% CI)	p-value	Controlled Confounders	Quality Score
Nguyen, PH et al. 2006 [14]	53 provinces in Vietnam	Non-pregnant women of reproductive age	(1) Using untreated feces for farming (Structured questionnaire)	(a) Hookworm (b) *A. lumbricoides* (c) *T. trichiura* (Kato-Katz technique)	N = 5127 Sample size by exposure and outcome NR.	Multivariate: OR(1,a) NR OR(1,b) = 1.3 (1.0–1.6) OR(1,c) NR	NR	-Models included farming, lack of a closed latrine, zone of residence, untreated feces as fertilizer, helminth coinfection, or women's occupation depending on helminth type model. Household data weighted by zone/commune.	6.5
Trang, DT et al. 2007 [20]	Yen So commune, peri-urban area, Hanoi, Vietnam	Farmers and their families including adults 15–70; children <72 month	(1) Use of fresh human excreta in agriculture (Household interviews)	(a) Any STH (b) Hookworm (c) *A. lumbricoides* (d) *T. trichiura* (Direct smear method)	N = 807 (E, O) +ve: (1,a) n = 77 (1,b) n = 49 (1,c) n = 40 (1,d) n = 22	Univariate RR(1,a) = 1.20 (0.93–1.55) RR(1,b) = 1.46 (1.04–2.05) RR(1,c) = 1.1 (0.76–1.58 RR(1,d) = 1.44 (0.87–2.36) Multivariate: RR(1,a) = 1.19 (0.93–1.53) RR(1,b) = 1.45 (1.03–2.03) RR(1,c) NR RR (1,d) NR	NR	- Multivariable model examined significant relationships of potential covariates including: age, gender, household hygiene practices, waste water practices, socioeconomic status, and animal husbandry for each outcome. However, which covariates were included in the final models was NR.	8
Yajima A et al. 2009 [21]	Tien Xuan commune, Hoa Binh province, Vietnam	Commune residents	(1) Use of human feces in agriculture (Questionnaire)	(a) Hookworm infection (b) *A. lumbricoides* (c) *T. trichiura* (Kato-Katz technique)	N = 101 (E, O) +ve: (1,a) n = 13 (1,b) n = 1 (1,c) n = 10	Univariate only: RR(1,a) = 0.7 (0.46–1.06) RR(1,b) = 0.21 (0.03–1.54) RR(1,c) = 0.74 (0.43–1.27)	NR	-n/a	6
Phuc PD et al. 2013 [22]	Nhat Tan and Hoang Tay communes, Hanam province, Vietnam	Commune residents >12 months of age among both individuals with primary occupation of agriculture work and individuals whose primary occupation was non-agriculture work	(1) Use of human excreta for application in field (Questionnaire)	(a) Helminth spp. (formalin-ether concentration technique) (b) *A. lumbricoides* (c) *T. trichiura* (Kato-Katz technique)	N = 1425 (E, O) +ve: (1,a) n = 381 (1,b) n = 197 (1,c) n = 330	Univariate: OR(1,a) = 1.5 (1.2–2.0) OR(1,b) = 1.4 (1.1–2.0) OR(1,c) = 1.5 (1.2–2.0) Multivariate: OR(1,a) = 1.3 (0.9–2.0) OR(1,b) = 1.3 (0.8–2.0) OR(1,c) = 1.5 (1.0–2.3)	(1,a) = 0.18 (1,b) = 0.33 (1,c) = 0.04	-Adjusted for age, gender, season	12

NR = not reported; MOA = Measure of Association

E,0 +ve = Sample size of individuals positive for both exposure (E) and outcome (O). Numbers in parentheses correspond to the combination of exposure and outcome assessed.

n/a = not applicable

OR: Odds Ratio

RR: Risk Ratio

MV: Multivariate

Quality score derived from mean score of 2 reviewers using quality assessment tool (Table 1) designed for cross-sectional studies. Maximum score of 13 points.

https://doi.org/10.1371/journal.pntd.0006088.t002

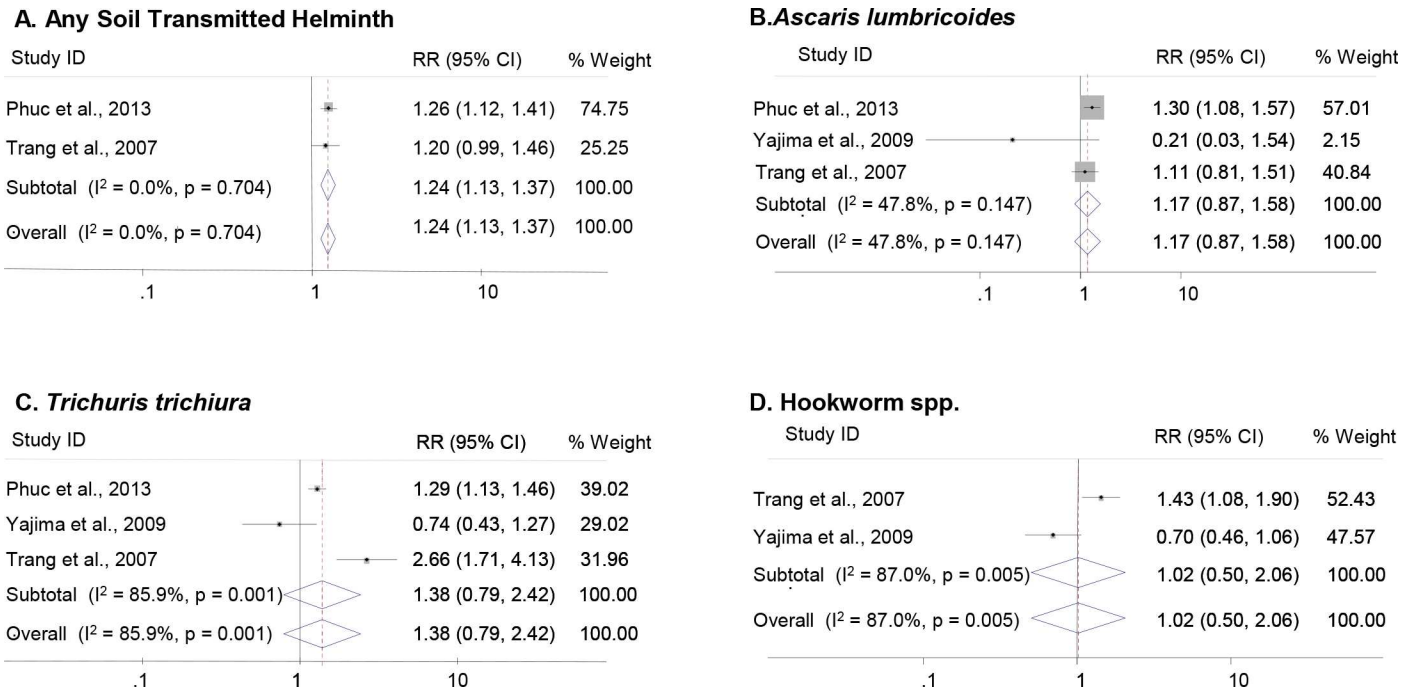

Fig 2. Meta-analysis forest plot describing individual and pooled RR estimates of STH infection in farmers who handle fresh human excreta in Vietnam. [A] Any Soil Transmitted Helminth [B] *Ascaris lumbricoides* [C] *Trichuris trichiura* [D] Hookworm spp. Note: Weights are from random effects analysis.

https://doi.org/10.1371/journal.pntd.0006088.g002

rate of 415–550 kg/ha for each rice harvest in the RRD [15]. These percentages are substantially higher than all NPK levels observed in organic human excreta, regardless of whether it was fresh or composted (Tables 3 and 4). On average, fresh excreta contained higher nitrogen, phosphorus, potassium, and moisture levels (1.66%, 3.23%, 2.44%, and 42.2%, respectively) than composted excreta. For excreta stored without lime (A,C), NPK levels and moisture content all decreased significantly over time (p-value<0.01 each). When excreta was composted with 10% lime (B), only nitrogen and moisture changed significantly over time (p-value <0.001 each); however, this storage method resulted in lower NPK than excreta composted without lime (C).

We compared the amount of organic NPK available per household to the annual RRD soil requirements and found excreta to be a good source of phosphorus and potassium but not nitrogen. We determined that farmers should apply fresh excreta (A) at a rate of 3459 kg/ha to meet but not exceed phosphorus and potassium requirements. At this rate, 425 kg of fresh excreta would be used to fertilize an average sized rice paddy, which is 65% of the total excreta produced by an average RRD family per year. Only 43% of the minimum nitrogen requirement would be met, indicating that farmers would need to supplement a nitrogen-based fertilizer (urea or diammonium phosphate (DAP)) for optimal rice production. Nutrient and moisture loss during composting meant that average RRD households did not produce sufficient excreta to meet soil requirements after 153 days or 181 days of storage. Excreta composted with 10% lime (B) or without lime (C) could be combined with inorganic NPK fertilizer at 6:1 or 5:1 ratios (organic: inorganic), respectively, in order to meet all nutrient requirements.

Inorganic fertilizer costs. Our retailer survey collected cost data for the two recommended NPK fertilizers (16-16-8 and 17-12-5) and two nitrogen-based fertilizers (urea and

Table 3. Summary of data input sources used to assess cost and STH risk associated with four rice fertilization methods in RRD, Vietnam.

Input	Estimate	Source(s)
STH risk for farmers who handle fresh human excreta, RR (95% CI)	1.24 (95% CI: 1.13–1.37)	Systematic review and meta-analysis
NPK[1] fertilizer recommendation for rice paddies in RRD[2]	16-16-8 or 17-12-5	[15]
Recommended NPK application rate, kg/ha per rice harvest	415–550	[15]
Recommended compost period to inactivate STH[3] ova	153 days (with ash and 10% lime)	[7]
	181 days (with ash)	[5]
Total nitrogen in human excreta, % (A, B, C)[4]	1.66, 1.29, 1.32	Nutrient analysis
Total phosphorus in human excreta, % (A, B, C)	3.23, 1.67, 2.92	Nutrient analysis
Total potassium in human excreta, % (A, B, C)	2.44, 1.82, 2.23	Nutrient analysis
Moisture content in composted human excreta, % (A, B, C)	42.16, 9.16, 6.83	Nutrient analysis
Moisture content in newly evacuated excreta, %	93	[18]
Average excreta production, kg/person/day	0.998	[18]
Average number household members in RRD, n ± sd	Agricultural: 3.66 ± 1.38	[19]
	Not agricultural: 4.38 ± 1.19	
Average rice paddy size in RRD, ha ± sd	0.12 ± 0.096	[19]
Number of rice harvests per year	2	[19]
Retail fertilizer price in RRD, Average (min-max) '000'VND/kg	16-16-8: 9.2 (4–15)	Retailer survey
	17-12-5: 7.96 (3.5–12)	
	DAP: 7.7 (3–15)	
	Urea: 7.8 (4.5–12)	
Estimated retail price of lime, '000'VND/kg	2	Expert opinion
Average lime weight (10% of excreta), kg/year	133.33	Nutrient analysis

[1] Nitrogen-phosphorus-potassium

[2] Red River Delta

[3] Soil transmitted helminth

[4] A = Fresh human excreta (≤ 139 day storage without lime); B = Composted human excreta (153 day storage with 10% lime; C = Composted human excreta (181 day storage without lime)

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DAP) from 35 fertilizer retailers across eight provinces in the RRD. All retailers agreed to participate in the survey, although not all could provide prices for each fertilizer type. We obtained cost estimates for 41 NPK 16-16-8 products from 27 retailers, 25 NPK 17-12-5 products from 17 retailers, 74 urea products from 34 retailers, and 38 DAP products from 24 retailers. Among all products and brands, fertilizer prices ranged from 3,000 to 15,000 VND per kilogram (Table 3). When ranked by average price, NPK 16-16-8 was most expensive (9,000 VND/kg), followed by NPK 17-12-5, urea, and DAP (all approximately 8,000 VND/kg).

Cost estimate generation. Rice paddies of average size in the RRD require 118.4 kg per year of inorganic NPK 16-16-8 or 17-12-5 fertilizer. Under scenario D, farmers applying one of the recommended NPK fertilizers in this amount incur an average annual cost of 1,004,000 VND (range: 416,000–1,783,000 VND; Fig 3). Farmers using fresh excreta would need to purchase a nitrogen-based fertilizer (urea or DAP), which would cost an average of 157,000 VND/year if applied up to the minimum recommended level. This implies an annual cost savings of 847,000 VND for scenario A. Under scenario B, farmers incurred costs for lime (average: 267,000 VND/year) and NPK fertilizer (average: 701,000 VND/year), representing a household saving of 37,000 VND annually. Farmers operating under scenario C incurred costs for NPK fertilizer only (average: 693,000 VND/year), and could save on average 312,000 VND annually. According to the sensitivity analysis, costs varied according to rice paddy size, household size,

Table 4. Observed nutrient and moisture content of human excreta composted by two methods in a simulated double vault latrine system over 139 days with linear regression to predict values at 153 and 181 days.

	Sampling day						Average (≤139 days)	Predicted	P-value
	1	27	55	83	111	139			
Ash								(181 days)	
Total Nitrogen (%)	1.937	1.770	1.647	1.587	1.527	1.497	1.661[1]	1.317[2]	0.000
Total Phosphorus (%)	3.537	3.314	3.174	3.152	3.124	3.105	3.234[1]	2.918[2]	0.000
Total Potassium (%)	2.704	2.427	2.412	2.388	2.370	2.360	2.443[1]	2.228[2]	0.002
Moisture (%)	61.39	56.47	48.31	39.63	28.60	18.54	42.155[1]	6.830[2]	0.000
Ash + 10% Lime								(153 days)	
Total Nitrogen (%)	1.820	1.643	1.550	1.477	1.413	1.377	1.547	1.290[3]	0.000
Total Phosphorus (%)	1.838	1.696	1.628	1.614	1.608	1.604	1.665[3]	N/A	0.565
Total Potassium (%)	2.054	1.865	1.849	1.835	1.822	1.816	1.874[3]	N/A	0.477
Moisture (%)	53.40	48.81	40.38	32.83	21.43	11.96	34.802	9.163[3]	0.000

[1]Model inputs for Scenario A (≤ 139 day storage without lime)

[2]Model inputs for Scenario C (181 day storage without lime)

[3]Model inputs for Scenario B (153 day storage with 10% lime)

https://doi.org/10.1371/journal.pntd.0006088.t004

and fertilizer costs; however, unless NPK fertilizer becomes significantly cheaper than the cost of lime, the fertilizer scenarios maintain their ranking with regard to cost savings.

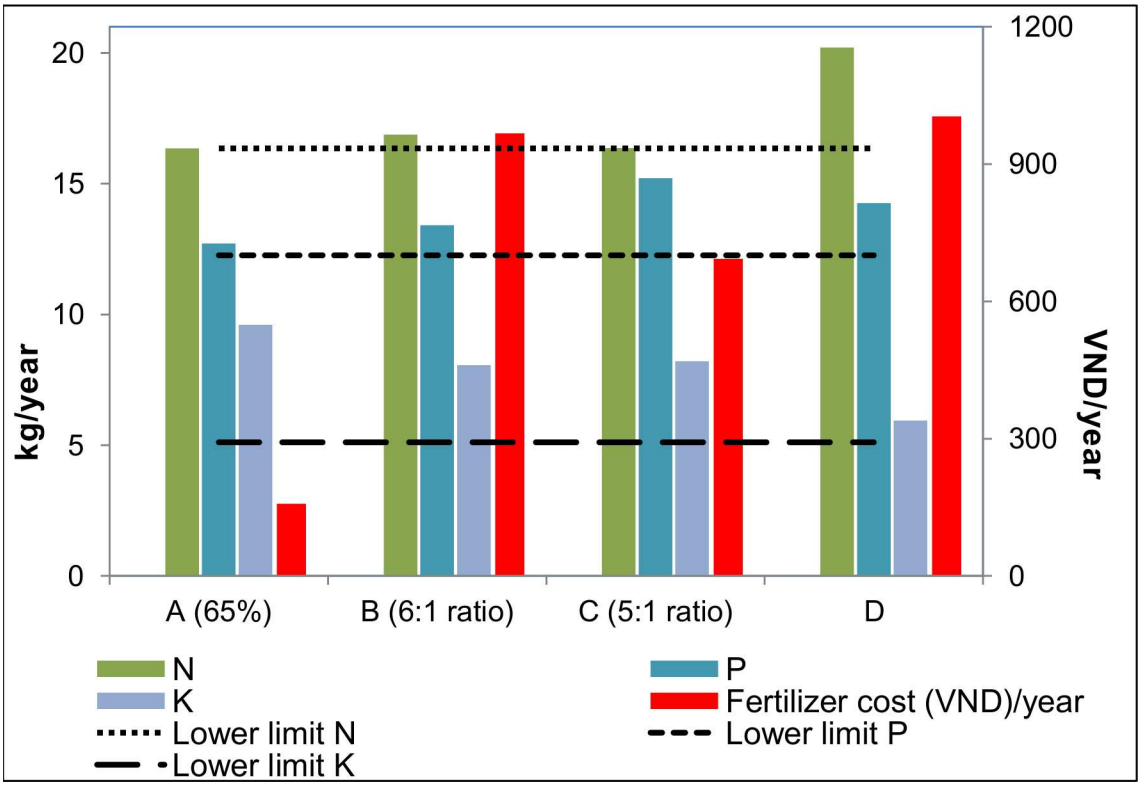

Fig 3. Nutrient content and annual cost of four fertilization scenarios utilizing human excreta and/or commercial fertilizer in Vietnam. Nitrogen (N), phosphorus (P), potassium (K). Scenarios: A -Fresh human excreta (≤ 139 day storage) supplemented with nitrogen; B—Composted human excreta (153 day storage with 10% lime) supplemented with NPK; (C) Composted human excreta (181 day storage) supplemented with NPK; (D) Inorganic NPK fertilizer.

https://doi.org/10.1371/journal.pntd.0006088.g003

Discussion

This study adds to current knowledge about the opportunities and risks associated with reusing human excreta to fertilize rice plants in one region of Vietnam. Our finding that handling fresh excreta increases the risk of STH infection in farmers (RR = 1.24, 95% CI: 1.13–1.37) emphasizes the importance of adequately treating excreta to inactivate STH life stages. Furthermore, the risk is not limited to farmers as fresh excreta reuse facilitates STH spread to other commune residents, and ultimately to consumers, through food, water, and environmental transmission routes. This practice is one factor contributing to the high prevalence of *A. lumbricoides* (44.4%; N = 34 million), *T. trichiura* (23.1%; N = 17.6 million) and hookworm (28.6%; N = 21.8 million) infections in Vietnam [23]. Our study did not assess healthcare costs associated with STH prevention, treatment, or chronic disability. Individuals with low intensity infections are often asymptomatic; however, those with high intensity infections can experience a variety of acute or chronic conditions (e.g. diarrhea, abdominal discomfort, anemia and rectal prolapse) that reduce quality of life and may require costly medical interventions to treat [4]. Long-term sequelae of chronic infections, such as impaired cognitive development and growth faltering, can negatively impact lifelong earnings and contribute to the cycle of poverty in low resource communities. Although our study showed that farmers using fresh excreta benefitted from the largest cost-savings in fertilizer expenditure, the direct and indirect societal costs incurred due to prolonged STH infection would likely outweigh these savings.

Despite laws that prohibit use of human excreta for agriculture in Vietnam, this practice remains common among certain farming groups [24]. Human excreta is perceived as more valuable than animal manure due to differences in dietary protein content, and it is believed to improve soil structure more sustainably than inorganic fertilizers [9]. Although many farmers compost excreta, WHO recommendations for hygienic composting are not commonly followed, as farmers harvest multiple crops per year and are unwilling or unable to store excreta for six months prior to use [5,6]. Some misperceptions about the reasons for safe composting might influence farmer willingness to use fresh excreta. For example, focus group participants in the RRD emphasized ease of application and benefits to soil structure, rather than the benefits of composting to protect human health [1]. Our alternative to the WHO standard, composting for 153 days with 10% lime to accelerate STH inactivation, was not a reasonable alternative for farmers prioritizing cost savings. However, for farmers less concerned about cost savings, the 10% lime compost strategy could be further accelerated to 111 days by adding aeration to latrines, which would allow excreta to be safely handled at more frequent intervals [7].

Human excreta use in crop agriculture was previously estimated to represent 83 million USD in fertilizer import savings to the Vietnamese economy [6], which is one-fifth of the 2014 net expenditure on inorganic fertilizer importation (384 million USD) [25]. Our cost analysis indicated that farmers could save 37,000–847,000 VND/yr (1.48–37.28 USD, $2017) [26] by using human excreta. While these savings might appear low, they represent 1–22% of a farmer's average annual income in the RRD [27]. Furthermore, the savings could represent a higher percent of annual income in regions that are less fertile, where rice yields are lower, or in remote locations where transportation challenges result in higher commercial fertilizer costs. Another report, suggesting that household excreta traded on the domestic market could contribute up to 15% of household income for those in the lowest income quintile, is in line with our analysis [6]. Therefore, it is unlikely that low-income rural farmers would be willing to universally replace organic fertilizers of human origin with inorganic commercial fertilizers. This was previously demonstrated by farmers who were given non-composting latrines and who ultimately broke the seals open to access the excreta [24].

Our nutrient analysis of human excreta originating from the RRD and composted over time demonstrated excreta to be an adequate organic source of phosphorus and potassium, but not nitrogen, for plant growth. Therefore, all of the scenarios using human excreta (A-C) required additional inorganic fertilizer in order to meet the recommendations for optimizing rice yield. It is not clear how far outside Vietnam these results should be extrapolated as differences in dietary intake directly influence NPK excretion, and soil supplementation requirements vary regionally. Furthermore, our analysis was based on total excreta collected in a double vault latrine, rather than waste separated into liquid and solid components, as occurs in some other regions that use excreta. However, beyond the immediate economic and agricultural gains to reusing excreta, there are global benefits to nutrient recycling. It is estimated that the demand of phosphate rock will outweigh supply by the mid-21st century, which has important consequences for food security as phosphorus is essential for plant growth [28]. As access to a hygienic toilet (flush, pour flush, sulabh or double vault latrine) is still regionally variable in Vietnam (61.6–96.7% of homes containing a latrine), an opportunity currently exists to optimize nutrient recovery infrastructure in homes requiring sanitation upgrades [27].

Our systematic review and meta-analysis found a statistically significant higher risk of infection with any STH among Vietnamese farmers who use human excreta, and highlighted the limited volume of evidence to describe this association. Only four studies met our inclusion criteria, despite searching academic and grey-literature sources in Vietnamese and English. Of these, only three were included in meta-analysis due to poor reporting quality. Out of a possible score of 13, two studies achieved a quality score of 50% or lower. Our findings showed that studies often did not report descriptions of appropriate sample size determinations, confounders controlled for, sample size for positive exposure and/or outcomes, as well as risk estimates or associated p-values. However, aside from one study, all studies were from the RRD study area, included participants of similar age and gender, and estimated exposure and outcomes using similar methods. Each study had slightly different definitions for agricultural use of human excreta, and therefore our meta-analysis included both individuals whose primary occupation was rice farming, but also those who worked with human excreta in other ways aside from direct field application. Inclusion of Yajima et al., 2009 in the meta-analyses of both *T*. *trichiura* and *A*. *lumbricoides* produced significant heterogeneity in the estimates of infection risk. This study had a small sample size and very low prevalence of STHs (i.e. one case of *A*. *lumbricoides* detected), leading to low risk ratios and wide confidence intervals. The study did not provide information on approaches used to measure or control for potential confounders, further adding to the difficulty in interpretation of protective properties of human excreta use in STH. Pooled results by STH type revealed the lowest risk for hookworm infection, and significant variation in studies combined in meta-analysis for this outcome. It was not possible to examine potential factors contributing to heterogeneity in hookworm risk estimates due inclusion of only two studies in meta-analysis. Therefore, in order to better understand factors influencing infection and to substantiate the limited body of evidence on STH risk in the RRD of Vietnam, additional research employing high methodological rigour is warranted.

Although our study attempts to represent the typical situation in the RRD, much of our data comes from Ha Nam province exclusively which may differ in relevant ways from other RRD provinces. The estimate of risk was limited by the number of estimates reported in the literature and we assumed that STH risk was equal in scenarios B-C. Human excreta was collected from various households and mixed before analysis. Thus, results may not accurately reflect the nutrient and moisture content of unmixed excreta if collected and analysed from time of defecation. Costs related to the removal of excess human excreta, latrine construction and maintenance, and personal safety equipment were not explored.

Conclusions

Our study confirmed that human excreta is a significant and sustainable source of nutrients needed for crop fertilization. Its use as agricultural fertilizer, a common practice in Vietnam, offers direct benefits to rice farmers. Human health, agricultural productivity, household earnings are optimized when farmers follow WHO standards for excreta use and government standards for crop fertilization; however current policies prohibit excreta use altogether and therefore may need to be revisited. Furthermore, our results suggest that farmers and the Vietnamese economy would benefit by forward thinking public health messaging promoting STH prevention, such as safe excreta handling strategies, personal protective equipment (e.g. gloves and boots) and regular anthelmintic prophylaxis, rather than an outright ban on excreta use. This study highlights agricultural policies needing further attention, and demonstrates the value of promoting research that provides innovative solutions for safely and economically extracting nutrients from human excreta.

Acknowledgments

We extend our thanks to Mr. Nguyen Xuan Huan and the staff of the Department of Pedology and Soil Environment at Hanoi National University for determining the moisture content of the excreta samples. We are also very grateful to Ms. Le Thuy An and Ms. Nguyen Thi Hien for their diligent assistance in identifying Vietnamese fertilizer companies.

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10. DISCUSSION

The use of animal manure and human excreta has a benefit for agricultural production. However, there are environmental and health risks associated with these practices. First, we experimented on storing excreta and assessed the health, economic and environmental impacts associated with the use of excreta for target intervention to ease these negative effects (Chapter 4, 6 & 9). Second, we applied QMRA methodology to quantify the infection risks of helminth and diarrhea related to pathogens in livestock waste and excreta use in agriculture (Chapter 5, 6 & 7). Third, we applied a MFA approach to establishing interconnections between the environmental sanitation and agricultural systems and quantifying the discharge of nutrients N and P to the environment. In the Chapter 10, the main findings of our investigations are summarized and discussed, and we will draw links between methods used in this study to propose future integrative experimental studies, microbial risk assessment and material flow analysis.

10.1 Ascaris lumbricoides egg die-off: Efficiency of treatment for safe use of human excreta in agriculture

In Chapter 4, we set up 24 vaults in an experimental house for storage and study the die-off of *A. lumbricoides* eggs and whether it is affected by storage time, additive materials, temperature and aeration. The nutrient indexes of the samples were also evaluated. In our experiment, the number of eggs in all vault options at 181st storage day was less than 1 egg per gram of total solid excreta, and thus the fecal sludge met the World Health Organization (WHO) safety limit for reuse in agriculture (World Health Organization 2006a). As reported, the percentage of viable *A. lumbricoides* eggs decreased significantly in vault options which has a high pH (5 and 10% lime) over 111 storage days (70.08 and 73.66% reduction, respectively). Throughout the 181 storage days, the temperature of the vaults increased from 20.95 to 28.44 °C. During the first 13 storage days, the average temperature in all vaults was higher than the room temperature (6 °C), which might be explained by activities of thermo-bacteria. The values for temperatures within the vault and the room were the same after 13 storage days, and there were no significant differences in temperature among all vault options. In this study, the temperatures within the vaults were lower than the temperature range in many composting latrines in other studies (40–65 °C) (Anand and Apul 2014). Hence, the effect of temperature on the die-off of *A. lumbricoides* eggs might be reduced in our study.

The effect of low moisture conditions increased the rate of die-off of *A. lumbricoides*

eggs in the current study. Hawksworth et al. (2010) found that at 30 °C and 100% relative humidity (RH), viable *Ascaris* eggs were found after 58 days, and conversely, no viable eggs were found at 0% RH (Hawksworth et al. 2010). In our study, as the RH decreased, the rate of die-off of Ascaris eggs increased. However, the RH did not significantly affect the percentage of viable *A. lumbricoides* eggs when vault options were compared because the difference between them was not large (0–3%). The RH of vault options in this study were in the same range as those in tropical climates with temperatures ranging from 20 to 30 ° C, which yielded survival times of *Ascaris* eggs between 10 and 12 months (Strauss et al. 2003).

Storage time was the main factor explaining the significant decrease of the percentage of viable eggs over 181 storage days. However, Gantzer et al. (2001) found that live nematode eggs can exist after 6 months of storage (Gantzer et al. 2001).

There were 8 vault options including 4 with air pipe and 4 without air pipe designated. Each vault options and air pipe had three replicates. The data showing the vault options $V3_{lime\ riceh}$, $V3_{lime\ riceh\ air}$, $V4_{lime10kg}$ and $V4_{lime10kg\ air}$ reached the WHO standard at 111 days of excreta storage. However, vault option $V4_{lime10kg\ air}$ had the best reliability for reducing egg counts below the one egg per gram standard over the shortest time period. The largest reduction of viable *A. lumbricoides* eggs occurred in vault options $V4_{lime10kg}$ and $V4_{lime10kg\ air}$ (with 10% lime per total weight). However, vault option $V4_{lime10kg\ air}$ reached the WHO standard on the 111th storage day and far exceeded the recommendation of the Ministry of Health in Vietnam to compost excreta for 180 days before using it for agriculture (Ministry of Health 2005a).

According to the WHO guidelines for the safe use of excreta and grey water in agriculture, the least time for excreta storage is six months with alkaline treatment (pH>9), temperature >35 °C and moisture <25% (World Health Organization 2006a). Excreta can exceed pH 9 by the addition of lime or ash (e.g., 200–500 ml, or enough to cover each fresh defecation). In our study, we mixed excreta with lime by the rate 10% lime per total solid. The pH reached approximately 12 after 41 days, which exceeded WHO requirements for pH, and then decreased to 8 on the 83rd day of storage. This helps to explain why the $V4_{lime10kg\ air}$ vault option created a suitable egg reduction after only 4 months of storage. Therefore, our study provides evidence on the helminth survival, the ideal combination of locally available materials and excreta storage conditions that would yield the microbial safety in excreta.

10.2 Health risks associated with the use of human excreta and animal manure in agriculture

Reuse and application of human excreta onto rice paddies as fertilizer is a common

practice in northern Vietnam, where many farmers use single or double vault latrines, lack access to wastewater infrastructure, and have variable access to commercial inorganic fertilizers (Phuc et al. 2006). Using organic waste to fertilize fields has clear benefits for crop yield (Takashi Asano 1998); however, this practice increases certain health risks for farmers and consumers, such as infection by soil transmitted helminths (*Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm spp.,) (Centers for Disease Control and Prevention 2013a, Pham-Duc et al. 2013) or some excreta related pathogen, such as *E. coli*, *Cryptosporidium* and *Giardia* (World Health Organization 2006). They are all intestinal parasites that spread between people when sanitation is inadequate or when good hygiene is not practiced (Centers for Disease Control and Prevention 2013) and can survive in the environment long enough to pose health risks (World Health Organization 2006a). People can be infected when they accidentally ingest infectious eggs/(oocysts) bacteria or viruses during common excreta handling, eat contaminated food due to the use of human excreta and animal manure in agriculture or when their skin comes into contact with infectious larvae in contaminated soil. These parasites are particularly prevalent in regions with warm, moist climates, and are included in the category of tropical neglected diseases.

The risk of transmission of helminth infection with excreta during agricultural practices is well known and has been described in many parts of the world. The practice is frequent in Asia in general and rural Vietnam in particular. The risk of transmission and route of intestinal parasitic infection in high risk areas are not well known, particularly, the accidental ingestion of infectious eggs during common excreta handling. The result from the quantitative estimation of involuntary excreta ingestion rates in farmers during agricultural practices indicated that the average weight of excreta ingested per year was 91 mg (95% CI: 73; 110) in Chapter 5. This result was lower than finding of 1.2mg/day – 245.5mg/day in several studies that estimated soil ingestion for children and adults (Clausing P Fau - Brunekreef, Brunekreef B Fau - van Wijnen, and van Wijnen 1987, Davis et al. 1990, Davis and Mirick 2006, Wilson et al. 2013, Wilson et al. 2015) but higher than the estimates for a study conducted by Phuc (2011) at 60mg/day (10mg/event with 6 event/year). It is noted that our 5.16 hours exposure to excreta handling per year is much lower than another study done by Davis and Mirck, 2006; where they found soil ingestion rate for adults in the range of 23-625 mg/day with working time between 1-10 hours/day. Thus, the amount of excreta ingestion varied widely in different study areas and may depend on the period of contact, frequent contacts with irrigation practices might increase the risk of helminth infection. This

is confirmed by a study in Hanoi which indicated that people who frequently had contact with irrigation water throughout the year rather than seasonally had a higher risk of infection with *T. trichura* (Trang et al. 2007). Furthermore, we observed the frequency of hand to the mouth touching from 0-11 times per year in our study which is also mentioned in another study in Ghana (Antwi-Agyei et al. 2016). The practice will play an important role in the helminth infection as it may decrease the accidental ingestion volume of excreta due to its low frequency.

An important result of our study was that adequate treatment of human excreta before use as fertilizer (storage of longer than 3.2 months with the addition of 10% lime per total solid) could decrease the disease burden to 0 (Chapter 6). This finding indicated that safe composting of human excreta should be intensively promoted in agricultural settings with recommendation of no shorter than 3 months of composting. Our result is comparable with study done by Jensen et al. 2009, where they indicated an approximate compost duration of 3-4 months under the condition of high pH and low moisture could provide a safe compost product for application in the field (Jensen et al. 2009a).

Among exposures, we identified the exposure to livestock waste (Chapter 7) as important risk factors for diarrheal episodes. Our study demonstrated that the annual diarrhea risk caused by exposure to biogas effluent through irrigation activities ranged from 17.4 to 21.2% (*E. coli 157:H7*), 1.0 to 2.3% (*G. lamblia*) and 0.2 to 0.5% (*C. parvum*). The annual diarrhea risks in our study was below the diarrheal diseases incidence per person per year (0.4-0.6) among those aged above 5 years in developing countries in 2000 (WHO guidelines for the safe use of wastewater, excreta and grey water). However, in comparison with the WHO reference level (10^{-3}) of waterborne disease from drinking water, the annual diarrhea risks in our study were much greater (WHO, 2006). In fact, the quality of biogas effluent can explain the observed differences from country to country. Indeed, several studies in India, Tanzania, Nepal and Lesotho showed that the quality of the effluent did not meet the requirement for use as fertilizer or discharge into receiving water bodies, however people still used the effluent for irrigation and/or discharge into the surface waters (Vögeli et al. 2014).

10.3 Economic benefit of human excreta

Reuse of human excreta for fertilizer is a common practice in many low to middle income countries because farmers require a low-cost source of nutrients to grow food crops eaten by people and animals. We investigated the cost-savings where farmers used either inorganic fertilizer or fresh/composted human excreta supplemented by inorganic fertilizer to

meet the nutrient requirements of rice paddies in the Red River Delta, Vietnam (Chapter 9).

In fact, the use of human excreta for agriculture remains common among certain farming groups (Jensen et al. 2005b). Human excreta is perceived as more valuable than animal manure due to differences in dietary protein content, and it is believed to improve soil structure more sustainably than inorganic fertilizers (Jensen et al. 2008). Although many farmers compost excreta, WHO recommendations for hygienic composting are not commonly followed, as farmers harvest multiple crops per year and are unwilling or unable to store excreta for six months prior to use (World Health Organization 2006a, Jensen et al. 2008). Some misperceptions about the reasons for safe composting might influence farmer willingness to use fresh excreta. For example, focus group participants in the Red River Delta emphasized ease of application and benefits to soil structure, rather than the benefits of composting to protect human health (Phuc et al. 2006). Our alternative to the WHO standard, composting for 153 days with 10% lime to accelerate soil-transmitted helminth inactivation, was not a reasonable alternative for farmers prioritizing cost savings. However, for farmers less concerned about cost savings, the 10% lime compost strategy could be further accelerated to 111 days by adding aeration to latrines, which would allow excreta to be safely handled at more frequent intervals (Vu-Van et al. 2016).

Human excreta use in crop agriculture was previously estimated to represent 83 million USD in fertilizer import savings to the Vietnamese economy (Jensen, Phuc, and West 2010), which is one-fifth of the 2014 net expenditure on inorganic fertilizer importation (384 million USD) (FPT Securities 2007). Our cost analysis indicated that farmers could save 37,000–847,000 VND/yr (1.48–37.28 USD, $2017) (Vietcombank 2016) by using human excreta. While these savings might appear low, they represent 1–22% of a farmer's average annual income in the Red River Delta (General Statistics Office of Viet Nam 2016). Furthermore, the savings could represent a higher percent of annual income in regions that are less fertile, where rice yields are lower, or in remote locations where transportation challenges result in higher commercial fertilizer costs. Another report, suggesting that household excreta traded on the domestic market could contribute up to 15% of household income for those in the lowest income quintile, is in line with our analysis (Jensen, Phuc, and West 2010). Therefore, it is unlikely that low-income rural farmers would be willing to universally replace organic fertilizers of human origin with inorganic commercial fertilizers. This was previously demonstrated by farmers who were given non-composting latrines and who ultimately broke the seals open to access the excreta (Jensen et al. 2005a).

The nutrient analysis of human excreta originating from the Red River Delta and compost over time demonstrated excreta to be an adequate organic source of phosphorus and potassium, but not nitrogen, for plant growth. Therefore, using human excreta required additional inorganic fertilizer in order to meet the recommendations for optimizing rice yield. It is not clear how far outside Vietnam these results should be extrapolated as differences in dietary intake directly influence NPK excretion, and soil supplementation requirements vary regionally. Furthermore, our analysis was based on total excreta collected in a double vault latrine, rather than waste separated into liquid and solid components, as occurs in some other regions that use excreta. However, beyond the immediate economic and agricultural gains to reusing excreta, there are global benefits to nutrient recycling. It is estimated that the demand of phosphate rock will outweigh supply by the mid-21st century, which has important consequences for food security as phosphorus is essential for plant growth (Cordell, Drangert, and White 2009). As access to a hygienic toilet (flush, pour flush, sulabh or double vault latrine) is still regionally variable in Vietnam (61.6–96.7% of homes containing a latrine), an opportunity currently exists to optimize nutrient recovery infrastructure in homes requiring sanitation upgrades (General Statistics Office of Viet Nam 2016)

Our study confirmed that human excreta is a significant and sustainable source of nutrients needed for crop fertilization. Its use as agricultural fertilizer, a common practice in Vietnam, offers direct benefits to rice farmers. Human health, agricultural productivity, household earnings are optimized when farmers follow WHO standards for excreta use and government standards for crop fertilization. The study highlights agricultural policies needing further attention and demonstrates the value of promoting research that provides innovative solutions for safely and economically extracting nutrients from human excreta.

10.4 Environmental impact of (human excreta and animal manure use in agriculture

In the Chapter 8, an adapted Material Flow Analysis (MFA) has been applied to assess the environmental impact of human activities on nutrient flows at the commune scale. The quality of the MFA system showing the values (ton/year) of nitrogen, phosphorus flows, respectively, in the environmental sanitation system and agricultural system in Hoang Tay commune, Ha Nam province, Vietnam. There were nine processes in the MFA system, divided in three main groups: the environmental sanitation system (biogas, septic tank, pit latrine, and drainage), the agricultural system (fishpond and paddy), and livestock (pig and poultry). The main source of nutrient N and P inputs to the system are chemical fertilizer and commercial feed for animals. In addition, the major nutrient N and P source affecting surface

water (Nhue river) and the agricultural system (fishpond and paddy) in Hoang Tay originates from human excreta (through the discharge of wastewater of on-site sanitation) and animal manure (through pig and poultry processes).

The environmental impacts of agricultural activities were assessed through the interactions of livestock (pig and poultry) with the environmental sanitation system (biogas, septic tank, pit latrine, and drainage) and the agricultural system (fishpond, paddy). There were four sources of nitrogen to the atmosphere: pig, poultry, fishpond, and paddy processes. There was 64 ± 12 ton N/year from agricultural and livestock systems in Hoang Tay commune emitted to the air, through manure and chemical fertilizers applied. Among those four processes in the agricultural and livestock system, nitrogen originating from the paddy fields was the largest source (37 ± 8 ton N/year), contributing to 58% of total nitrogen lost to the air. The second largest source was nitrogen in manure from pig process (15 ± 4 ton N/year). Thus, 29 and 19% of nitrogen inputs into paddy and pig processes were lost to the air, respectively. On the other hand, nitrogen lost from poultry production contributed less to total N lost to the air, but it was 17% of all N inputs into this process.

Fishpond, paddy, and drainage processes were the main sources of nutrients discharged to the Nhue River. There were 57±9 ton N and 29±6 ton P discharged to the river every year. Drainage was the largest source of nitrogen (46%), but paddy fields were the largest sources of phosphorous discharged to the river (55%). In addition, there were 7 ± 0.9 ton N and 12 ± 4 ton P discharged from paddy to soil/groundwater annually.

Our results demonstrated that nutrients in the manure and excreta have not yet been effectively reused in the agricultural system, and also have the potential to pollute the surrounding environments. The MFA results identified the critical control sources of nutrients in Hoang Tay commune, which included the overuse of chemical fertilizers in paddy fields, uncontrolled effluent from the environmental sanitation system, and insufficient management of pig manure. Consequently, options for nutrient resource management were proposed, such as reducing half of chemical fertilizers applied and reusing all excreta and manure in the paddy fields. However, sustainable sanitation must consider the potential health impacts of applying manure. Therefore, pretreating manure, through composting for example, 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 mitigation measures on resource consumption and environmental pollution.

10.5 Strengths and limitations of the studies

10.5.1. Strengths

The main strength of this thesis relies on an integrated approach to understanding the complex issue of excreta in Vietnam from multiple perspectives such as biological, health, environmental and economic. This work was built up from the conceptual framework developed by our group on an integrated assessment of environmental sanitation and health (Nguyen-Viet et al. 2009) which was tested and validated in different contexts.

First, the PhD work could provide new understanding on how helminth eggs die-off varied in different options of excreta storages that are both currently applied by local farmers or proposed by the research team in under *in situ* conditions. This experiment was very laborious and required extensive follow-ups both in setting up and analyses of data to generate locally new information on helminth behaviour in Southeast Asia (Vu-Van et al. 2016). Much of the information was dated ranging from 1970s and 1980s in Africa and Latin America in (Richard et al. 1983). The data in Vietnam can help modify the current regulations on excreta management that suggest 6 months of composting whereas our data suggest 115 days with the proposed option to meet the standard of MOH at 1 helminth egg/ gram of excreta (Vu-Van et al. 2016).

Second, our study generated empirical data on human exposure to excreta when handling excreta for reuse in agriculture. The innovation of the study was the combination of field observation coupled with experiment in the laboratory and modelling to quantitatively estimate the voluntary excreta ingestion by farmers. This is particularly novel in Vietnam, and complements similar data produced in the USA, although the studies from the USA may not be relevant to developing countries in risk assessment.

Finally, the environmental and economic study of excreta management and reuse could provide insight on the environmental impact of excreta management and financial saving if excreta is used according to scenarios of treatment from our studies. From a methods point of view, this analysis was very daring in the context of environmental sanitation and health research in developing countries.

10.5.2. Limitations

Our studies have several limitations. First, our studies could not conclusively conclude on the balance of minimizing the health impact versus the economic benefit of excreta management. Indeed, there is an economic benefit of excreta reuse as compared to chemical fertilizers but our cost savings analysis did not include the health savings through the prevention of human

diseases. This will offer new direction for future work in the area of cost of illnesses related to sanitation. Such a study was successfully applied to estimate healthcare costs caused by food borne diseases/ illness (Hoang et al. 2015).

In the study of human exposure to excreta, we estimated excreta ingestion only in farmers using excreta in agriculture, but not in farmers who do not directly use excreta but may encounter it during other farming activities and accidently ingest excreta. The approach we used to estimate excreta ingestion depends on several underlying assumptions. Firstly, it assumed that when farmers work with excreta, or applied it on the field, they may routinely touch excreta with their bare hands; our results found 75% of farmers did this with only 25% wearing gloves. A second assumption was that these farmers may then touch their mouths during work; thus, farmers may directly ingest excreta remaining on their hands. Thirdly, it assumed that as the time working with excreta increases, the amount of excreta ingestion also increases. Part of the variability observed in our study might be explained by the composition of the study populations.

11. CONCLUSIONS AND PERSPECTIVES

11.1. Conclusion

This thesis work presented the estimation of involuntary excreta ingestion rates in farmers during agricultural practices in Vietnam in Chapter 5. The results showed that farmers involuntarily ingest an annual average weight of 91 mg. In Chapter 4 the retention time of *A. lumbricoides* eggs were indicated. Egg die-off occurred after more than 3 months of storage with added 10% lime per total weight, or after at least 6 months storage days without any added matter. These die-off times already reached the WHO standard for safe reuse of excreta, less than 1 egg/gram total solid material. We applied QMRA to estimate the burden of *A. lumbricoides* infection associated with the handling and use of human excreta in agriculture. The burden was 0 if farmers treated excreta that had been stored more than 3 months with 10% of lime per total weight or if farmers simply waited for 6 months, with no added lime. In Chapter 7, the risk of diarrhea through exposure to livestock waste, or reuse of biogas effluent for irrigation was examined. The findings showed that the single and annual risks of diarrhea caused by *E. coli, G. lamblia* and *C. parvum* in the aforementioned activities were relatively high.

Furthermore, this thesis also identified the critical control sources of nutrients in the study site, where chemical fertilizers were overused in paddy fields, effluent from the environmental sanitation system was uncontrolled, and management of pig manure was insufficient. Human excreta was distributed to all three types of on-site sanitation processing (biogas, septic tank, and pit latrine) but more than half of it went to pit latrines. The results proposed to reduce half of chemical fertilizers used, and to reuse all excreta and manure in the paddy fields. Finally, our study confirmed that human excreta is a significant and sustainable source of nutrients needed for crop fertilization. Our studies show that in order to optimize the human health, economic and nutrient benefits, farmers should follow WHO guidelines for excreta use in agriculture. Vietnamese Government policies on the use of excreta are out of date and need to be reviewed.

11.2. Perspectives and research needs

11.2.1. Adapting human excreta treatment process

Our results indicated that human excreta and animal manure are commonly composted and used in agriculture. Meanwhile, a large amount of animal manure is either deposited into a biogas digester, with the sludge product to be used on paddy fields or is released into fish ponds or public rivers for fish farming. Besides the undeniable benefits of

reusing human and animal waste by both aerobic and anaerobic digestions, (e.g., soil improvement with high nutrients, reduce agricultural input from chemical fertilizers, being a source of energy, avoid burning fossil fuel or wood and reduce deforestation, cost saving etc.), it is worth mentioning important environmental health concerns related to waste reuse, such as emissions to air (e.g. methane, ammonia and nitrous oxide) and water pollution through the mechanism of leaching. Disease risk to the general public is also a matter of concern. Hence, health economic analysis and evaluation are important tools in helping policy makers understand the balance between acceptable health risks and nutrient and economic benefits. It is also necessary to develop guidelines on the treatment of human excreta and animal manure before they are used in agriculture.

11.2.2. Process for estimation of excreta ingestion during excreta handling in agricultural practices

To our best knowledge, this is the first study being conducted to estimate the excreta unintentionally ingested during the handling of excreta in agriculture practices. However, it is necessary to conduct future research with other objects, time of exposure (frequencies and seasons) and different exposure places to verify and generate results for different context. In addition, the results of quantitative microbial risk assessments usually over or under estimate the actual risks. Thus, the results obtained in this study from the exposure assessment will ensure the results of QMRA will be close to those that would be obtained from epidemiological studies.

11.2.3. Applying One Health/Ecohealth approach to optimize the benefits of human excreta

The study shows that minimizing health risks and maximizing nutrient and economic benefits of human excreta should be based on inter-sectoral collaboration and transdisciplinary research. In the case of optimization of human excreta use in agriculture, a single sector will not have the specific expertise and skills required to implement effective and sustainable programs. Therefore, the emphasis should be on creating an interdisciplinary research team that would support and accommodate the different health, economic, nutrient, societal and environmental priorities.

Agricultural intensification can bring economic benefits; however, the practice may have harmful consequences for human health and the environment. In order to develop sustainable agriculture for the long term, all three dimensions (the environment, human health, and economic benefit/production levels) need to be addressed in a balanced and

integrated way, with due regard given to meeting both present as well as future needs. The three dimensions should thus be seen as mutually enforcing, interdependent entities of sustainability. This is an approach to secure the necessary resources for safeguarding global food production, biodiversity reserves, recreation needs, water quality, well developed rural areas and wildlife areas. It can also be an effective means of poverty reduction as well as a way of mitigating climate change. The approach is also about ethical treatment and animal welfare, as well as ensuring the quality of food and feed. In other words, to be effective, similar studies in the future should apply transdisciplinary approach and represent a new holistic outlook on the ecosystem health and the development of sustainable agriculture.

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ANNEXES

ANNEX 1. Questionnaire, Informed consent and Pictures

Informed consent for the study of recording video clips regard to handling excreta in agriculture.

PHIẾU CAM KẾT CỦA HỘ GIA ĐÌNH THAM GIA NGHIÊN CỨU

Được sự cho phép của Bộ Y tế, sự chấp thuận của Sở Y tế tỉnh Hà Nam. Trường Đại học Y tế Công Cộng phối hợp với Trung tâm Y tế huyện Kim Bảng, Ủy ban nhân dân, Trạm Y tế xã Hoàng Tây tiến hành triển khai thực hiện đề án **"Xác định nguy cơ sức khỏe liên quan đến quá trình sử dụng phân người trong sản xuất nông nghiệp tại xã Hoàng Tây".**

Sự tham gia của gia đình anh/chị vào đề án nghiên cứu sẽ góp phần quan trọng vào việc phòng chống dịch bệnh, nâng cao sức khỏe người dân, góp phần vào việc phát triển kinh tế hộ gia đình.

Chúng tôi, cán bộ nhóm nghiên cứu phối hợp chặt chẽ với Trung tâm Y tế huyện Kim Bảng, Ủy ban nhân dân, Trạm Y tế xã Hoàng Tây và các hộ gia đình được chọn tiến hành quan sát và lưu lại hình ảnh quá trình sử dụng phân người trong sản xuất nông nghiệp để phục vụ công tác nghiên cứu.

Đề nghị hộ gia đình giữ lại phân trong hố xí từ ngày 01/7/2012 đến ngày 15/8/2012 và tham gia một trong các hoạt động sau: lấy phân từ nhà vệ sinh (hố xí) ra ngoài, ủ phân, trộn phân với chất độn, vận chuyển phân ra ngoài đồng ruộng, bón phân ngoài đồng ruộng.

Kính mong nhận được sự quan tâm hợp tác của hộ gia đình anh/chị để đề án đạt kết quả cao.

Xin chân thành cảm ơn!

Kim Bảng, ngày tháng 07 năm 2012

XÁC NHẬN	**XÁC NHẬN**	**HỘ GIA ĐÌNH**
ỦY BAN NHÂN DÂN	**TRẠM Y TẾ XÃ**	

Questionnaire for recording 52 video clips of farmers handling excreta in agriculture.

Phụ lục 1: Câu hỏi phỏng vấn

1. PV Họ tên chủ hộ

   1.1. Chồng:……..Tuổi……..

   1.2. Vợ:……Tuổi……..

2. PV Địa chỉ: Xóm:…….Xã……

3. PV Điện thoại:…….....

4. PV Số người thường xuyên sử dụng nhà vệ sinh:

5. PV Loại nhà vệ sinh

   5.1. Một ngăn: 1. Có tách nước tiểu 2. Không tách nước tiểu

   5.2. Hai ngăn 1. Có tách nước tiểu 2. Không tách nước tiểu

6. PV Sau mỗi lần đi vệ sinh, anh/chị có thêm chất độn gì?.....Nếu có thì mỗi một lần khoảng bao nhiêu (ước tính theo thể tích của bát)……

7. PV Số lần lấy phân ra khỏi nhà vệ sinh:……/năm

8. PV Thời gian mỗi lần lấy phân:……

9. PV Thời điểm lấy phân gần nhất:…….....tháng……..tuần…...ngày.

10. PV Bao nhiêu người tham gia lấy phân?

11. PV Ai là người tham gia lấy phân1. Nam 2 Nữ

12. PV Phân được ủ tại đâu……

13. PV Khi ủ có trộn thêm chất độn nào khác không?_____

14. PV Thời gian ủ phân……

15. PV Bao nhiêu người tham gia ủ phân?.....

16. PV Ai là người tham gia ủ phân 1. Nam 2. Nữ

17. PV Phân được bón cho cây trồng gì:……

18. PV Vận chuyển phân ra đồng bằng phương tiện gì…….

19. PV Khi vận chuyển thì phân đã được ủ bao lâu?.....

20. PV Bao nhiêu người tham gia vận chuyển?.....

21. PV Ai là người tham gia vận chuyển? 1. Nam 2. Nữ

22. PV Mỗi lần vận chuyển phân hết bao nhiêu lâu?

23. PV Khi bón phân cho đồng ruộng/cây trồng thì phân đã được ủ bao nhiêu lâu?.....

24. PV Thời gian một lần bón phân là bao nhiêu lâu?…….....

25. PV Bao nhiêu người tham gia bón phân?.....

26. PV Ai là người tham gia bón phân? 1. Nam 2. Nữ

Phụ lục 2: Phiếu quan sát người dân lấy phân từ nhà vệ sinh

1. LP Số người tham gia:……. (Nếu có 2 người trở lên thì các thông tin quan sát tiếp theo cần phải quan sát và ghi lại cụ thể cho từng người một).

2. LP Ai là người tham gia? 1. Nam 2. Nữ

3. LP Bảo hộ lao động sử dụng

3.1. Loại quần áo bảo hộ lao động:……..

3.2. Sử dụng ủng:

3.2.1. Có: Loại ủng:……..

3.2.2. Không

3.3. Sử dụng găng tay:

3.3.1. Có: Loại găng tay:……...

3.3.2. Không

3.4. Sử dụng khẩu trang :

3.4.1. Có: Loại khẩu trang :……..

3.4.2. Không

3.5. Sử dụng kính:

3.5.1. Có: Loại kính :…….

3.5.2. Không

3.6. Sử dụng mũ:

3.6.1. Có: Loại mũ :……

3.6.2. Không

4. LP Dụng cụ lao động sử dụng lấy phân

4.1. Cuốc:

4.2. Xẻng:

4.3. Cào:

4.4. Dụng cụ khác:

5. LP Đồ vật chứa phân:

5.1. Bao tải:

5.2. Thúng:

5.3. Đồ vật khác:

6. LP Làm việc liên quan tới phân người:

6.1. Thời gian từ:……..h……..đến.....h.....

6.2. Số lần quyệt mồ hôi bằng cẳng tay:……..

6.3. Số lần chạm vào mặt bằng bàn tay:.....

7. LP Quan sát đặc điểm của phân:

7.1. Có chất độn:	1. Tro	2. Tro	3. Trấu	4. Khác:
7.2. Độ ẩm:		1. Khô	2. Ướt	3. Ẩm
7.3. Có mùi hôi không:		1. Có	2. Không	
7.4. Có ruồi bay xung quanh không?		1. Có	2. Không	
7.5. Có gia súc, gia cầm ở gần đó không?		1. Có	2. Không	

8. LP Hoạt động sau lấy phân

8.1. Thay quần áo	1. Có	2. Không
8.2. Rửa tay xà phòng	1. Có	2. Không

Phụ lục 3: Phiếu quan sát người dân ủ phân

1. UP Số người tham gia:……. (Nếu có 2 người trở lên thì các thông tin quan sát tiếp theo cần phải quan sát và ghi lại cụ thể cho từng người một).

2. UP Ai là người tham gia? 1. Nam 2. Nữ

3. UP Bảo hộ lao động sử dụng

3.1. Loại quần áo bảo hộ lao động:……..

3.2. Sử dụng ủng:

3.2.1. Có: Loại ủng:……..

3.2.2. Không

3.3. Sử dụng găng tay:

3.3.1. Có: Loại găng tay:……...

3.3.2. Không

3.4. Sử dụng khẩu trang :

3.4.1. Có: Loại khẩu trang :……..

3.4.2. Không

3.5. Sử dụng kính:

3.5.1. Có: Loại kính :…….

3.5.2. Không

3.6. Sử dụng mũ:

3.6.1. Có: Loại mũ :……

3.6.2. Không

4. UP Đào hố ủ: 1. Có 2. Không

5. UP Chất liệu hố ủ:

5.1. Bằng đất

5.2. Bằng xi măng

5.3. Khác:

6. UP Dụng cụ lao động sử dụng để ủ phân:

6.1. Cuốc:

6.2. Xẻng:

6.3. Cào:

6.4. Dụng cụ khác:

7. UP Đồ vật chứa phân:

7.1. Bao tải:

7.2. Thúng:

7.3. Đồ vật khác:

8. UP Thời gian ủ phân từ:……..h……..đến.....h.....

9. UP Quan sát đặc điểm của phân:

9.1. Độ ẩm: 1. Khô 2. Ướt 3. Ẩm

9.2. Có mùi hôi không: 1. Có 2. Không

9.3. Có ruồi bay xung quanh không? 1. Có 2. Không

9.4. Có gia súc, gia cầm ở gần đó không? 1. Có 2. Không

10. UP Chạm tay lên mặt:

10.1. Số lần quyệt mồ hôi bằng cẳng tay:……..

10.2. Số lần chạm vào mặt bằng bàn tay:.....

11. UP Hoạt động sau ủ phân

11.1. Thay quần áo 1. Có 2. Không

11.2. Rửa tay xà phòng 1. Có 2. Không

Phụ lục 4: Phiếu quan sát người dân vận chuyển phân ra đồng

1. VC Số người tham gia:……. (Nếu có 2 người trở lên thì các thông tin quan sát tiếp theo cần phải quan sát và ghi lại cụ thể cho từng người một).

2. VC Ai là người tham gia? 1. Nam 2. Nữ

3. VC Bảo hộ lao động sử dụng:

3.1. Loại quần áo bảo hộ lao động:……..

3.2. Sử dụng ủng:

3.2.1. Có: Loại ủng:……..

3.2.2. Không

3.3. Sử dụng găng tay:

3.3.1. Có: Loại găng tay:……...

3.3.2. Không

3.4. Sử dụng khẩu trang :

3.4.1. Có: Loại khẩu trang :……..

3.4.2. Không

3.5. Sử dụng kính:

3.5.1. Có: Loại kính :…….

3.5.2. Không

3.6. Sử dụng mũ:

3.6.1. Có: Loại mũ :……

3.6.2. Không

4. VC Đồ vật chứa phân:

4.1. Bao tải:

4.2. Thúng:

4.3. Đồ vật khác:

5. VC Cách vận chuyển phân:

5.1. Quang gánh

5.2. Xe cải tiến

5.3. Xe máy

6. VC Thời gian vận chuyển phân:

6.1. Thời gian từ:……..h……..đến.....h.....

6.2. Số lần quyệt mồ hôi bằng cẳng tay:……..

6.3. Số lần chạm vào mặt bằng bàn tay:.....

7. VC Quan sát đặc điểm của phân:

7.1. Có chất độn:	1. Tro	2. Tro	3. Trấu	4. Khác:
7.2. Độ ẩm:		1. Khô	2. Ướt	3. Ẩm
7.3. Có mùi hôi không:		1. Có	2. Không	
7.4. Có ruồi bay xung quanh không?		1. Có	2. Không	

8. VC Hoạt động sau vận chuyển phân:

8.1. Thay quần áo	1. Có	2. Không
8.2. Rửa tay xà phòng	1. Có	2. Không

Phụ lục 5: Phiếu quan sát người dân bón phân

1. BP Số người tham gia:……. (Nếu có 2 người trở lên thì các thông tin quan sát tiếp theo cần phải quan sát và ghi lại cụ thể cho từng người một).

2. BP Ai là người tham gia? 1. Nam 2. Nữ

3. BP Bảo hộ lao động sử dụng

3.1. Loại quần áo bảo hộ lao động:……..

3.2. Sử dụng ủng:

3.2.1. Có: Loại ủng:……..

3.2.2. Không

3.3. Sử dụng găng tay:

3.3.1. Có: Loại găng tay:……...

3.3.2. Không

3.4. Sử dụng khẩu trang :

3.4.1. Có: Loại khẩu trang :……..

3.4.2. Không

3.5. Sử dụng kính:

3.5.1. Có: Loại kính :…….

3.5.2. Không

3.6. Sử dụng mũ:

3.6.1. Có: Loại mũ :……

3.6.2. Không

4. BP Dụng cụ lao động sử dụng bón phân

4.1. Cuốc:

4.2. Xẻng:

4.3. Cào:

4.4. Dụng cụ khác:

5. BP Đồ vật chứa phân:

5.1. Bao tải:

5.2. Thúng:

5.3. Đồ vật khác:

6. BP Thời gian bón phân:

6.1. Thời gian từ:……..h……..đến.....h.....

6.2. Số lần quyệt mồ hôi bằng cẳng tay:……..

6.3. Số lần chạm vào mặt bằng bàn tay:.....

7. BP Quan sát đặc điểm của phân:

7.1. Có chất độn:	1. Tro	2. Tro	3. Trấu	4. Khác:
7.2. Độ ẩm:		1. Khô	2. Ướt	3. Ẩm
7.3. Có mùi hôi không:		1. Có	2. Không	
7.4. Có ruồi bay xung quanh không?		1. Có	2. Không	
7.5. Có gia súc, gia cầm ở gần đó không?		1. Có	2. Không	

8. BP Hoạt động sau bón phân

8.1. Thay quần áo	1. Có	2. Không

8.2. Rửa tay xà phòng 1. Có 2. Không

Informed consent for the exposure study regard to handling human excreta and manure in agriculture

GIẤY ĐỒNG Ý THAM GIA NGHIÊN CỨU
CHƯƠNG TRÌNH CÔNG CỤ ĐÁNH GIÁ NGUY CƠ SỨC KHỎE MÔI TRƯỜNG TỔNG HỢP TRONG QUẢN LÝ CHẤT THẢI TỪ CON NGƯỜI VÀ ĐỘNG VẬT TẠI VIỆT NAM

Xin hãy đọc kỹ các thông tin trong Giấy đồng ý này trước khi anh/chị đồng ý tham gia nghiên cứu này.

Mục tiêu của nghiên cứu

Đây là một cuộc điều tra do Hội Y tế công cộng Việt Nam phối hợp với Trạm Y tế xã Hoàng Tây tổ chức. Điều tra này nhằm thu thập những thông tin có liên quan đến thực hành của người dân xã Hoàng Tây, tỉnh Hà Nam trong việc xử lý và tái sử dụng nguồn phân động vật trong chăn nuôi (phân lợn, gà, vịt, ngan, ngỗng v.v…) và phân người của những hộ gia đình. Những thông tin thu thập được sẽ giúp Hội YTCC Việt Nam đánh giá thực trạng quản lý và sử dụng phân động vật và phân người tại xã cũng như những nguy cơ ảnh hưởng tới sức khỏe người dân từ nguồn chất thải này.Những thông tin có được sẽ chỉ được sử dụng cho mục đích nghiên cứu.

Thời gian tham gia

Tổng thời gian của cuộc phỏng vấn kéo dài khoảng 30 phút.

Tự nguyện tham gia vào nghiên cứu

Sự tham gia vào nghiên cứu này là hoàn toàn tự nguyện. Anh/chị hoàn toàn có thể từ chối tham gia phỏng vấn mà không gặp phải trở ngại và không có bất cứ ảnh hưởng nào đến bản thân anh/chị. Anh/chị thông báo với người phỏng vấn rằng anh/chị không muốn tham gia nữa và không cần nêu lý do.

Địa chỉ liên hệ

Nếu anh/chị có câu hỏi gì về nghiên cứu này xin liên hệ với: Bác sĩ Lê Văn Tuấn – Trạm trưởng Trạm y tế xã Hoàng Tây - Điện thoại :0986609902. Hội Y tế Công cộng Việt Nam – 138 Giảng Võ – Ba Đình – Hà Nội - Điện thoại:0437368065

Sự chấp thuận tham gia nghiên cứu: Tôi đã được giải thích một cách đầy đủ và rõ ràng về mục đích của nghiên cứu và tôi đồng ý tham gia vào nghiên cứu này. Tôi nhận thức được rằng tôi có thể từ chối tham gia mà không có bất kỳ ảnh hưởng gì với bản thân.

Chữ ký người tham gia nghiên cứu Ngày

Chữ ký điều tra viên Ngày

ID: ___ ___ ___ ___ ___ ___

BỘ CÂU HỎI PHỎNG VẤN CÁ NHÂN

(Người phỏng vấn sẽ hỏi chủ hộ gia đình hoặc người lớn từ 15 – 65 tuổi, người mà biết rõ các thông tin liên quan đến các câu hỏi trong bộ câu hỏi này)

Điều tra viên giới thiệu thông tin về bản thân (tên họ, nơi đang công tác)

Giới thiệu về nghiên cứu và bộ câu hỏi:

> Đây là một cuộc điều tra do Hội Y tế công cộng Việt Nam phối hợp với Trạm Y tế xã Hoàng Tây tổ chức. Điều tra này nhằm thu thập những thông tin có liên quan đến thực hành của người dân xã Hoàng Tây, tỉnh Hà Nam trong việc xử lý và tái sử dụng nguồn phân động vật trong chăn nuôi (phân lợn, gà, vịt, ngan, ngỗng v.v…) và phân người của những hộ gia đình. Những thông tin thu thập được sẽ giúp Hội YTCC Việt Nam đánh giá thực trạng quản lý và sử dụng phân động vật và phân người tại xã cũng như những nguy cơ ảnh hưởng tới sức khỏe người dân từ nguồn chất thải này. Những thông tin có được sẽ chỉ được sử dụng cho mục đích nghiên cứu. Việc phỏng vấn sẽ được tiến hành trong khoảng thời gian từ 20 – 30 phút.
>
> Rất mong được sự hợp tác của Anh/chị.

hóa bằng số.

Chú ý:

1. Tiêu chuẩn lựa chọn đối tượng:

Chọn hộ gia đình: theo danh sách hộ gia đình

Chọn đối tượng điều tra: Người thường thực hiện việc xử lý phân động vật trong quá trình chăn nuôi hoặc phân người từ các hố xí 1 hoặc 2 ngăn 16 đến 60 tuổi, có khả năng trả lời các câu hỏi của điều tra viên.

2. Thay thế đối tượng: Trong trường hợp không gặp, không có nhà, không đồng ý trả lời hoặc không có khả năng trả lời: chọn đối tượng nhà liền kề bên tay phái của hộ gia đình đó)

Ngày phỏng vấn: ___ ___/___ ___/ *2012 (ngày/tháng/năm)*

Họ tên người phỏng vấn: _____

Mã số hộ gia đình _____ (Dựa trên số thư tự ghi trong danh sách hộ gia đình do nghiên cứu đã chọn ra)

Tỉnh: Hà Nam Huyện: Kim Bảng Xã: Hoàng Tây Thôn (xóm): _____

Sự tham gia phỏng vấn: ☐ có ☐ không

Nếu không, lý do tại sao?

☐ Vắng nhà (sau 3 lần đến thăm)

☐ Lý do khác (ghi rõ)

_____

PHẦN 1. THÔNG TIN CƠ BẢN

Q.101. Họ tên người trả lời phỏng vấn: _____

Tuổi (năm dương lịch) _____ Giới: ____ (1.Nam, 2.Nữ)

Q.102. Quan hệ với chủ hộ _____ (1.Chủ hộ, 2.Vợ(chồng), 3.Bố(mẹ), 4.Ông(bà), 5.Con)

Q.103. Nghề nghiệp: (1.Làm nông nghiệp, 2.Buôn bán, 3. Nghề thủ công, 4.Công nhân, 5.Khác:.....)

Q.104. Trình độ học vấn: 1. Cấp I 2. Cấp II 3. Cấp III 4. Cấp III trở lên 5. Khác:.....

Q.105. Tổng số người trong hộ gia đình *bao gồm những người sống cùng trong nhà và ăn chung trong vòng 3 tháng qua*.....người

Q.107. Sống ở xã Hoàng Tây bao nhiêu năm rồi?.....năm

Q.108. Gia đình ta có những loại tài sản sau đây không và số lượng từng tài sản? (Câu hỏi nhiều lựa chọn, ĐTV đọc các tình huống trả lời và ghi số lượng bên cạnh)

Tài sản		**Số lượng**		**Tài sản**	**Số lượng**
1	Ô tô		13		
2	Xe máy		14		
3	Ti vi		15	Gà	
4	Đầu đĩa (VCD, DVD)		16	Vịt	
5	Đài		17	Ngan	
6	Tủ lạnh		18	Ngỗng	
7	Điều hòa nhiệt độ		19	Máy phục vụ sx nông nghiệp	
8	Có Radio, cassette		20	Sập gụ/sofa	
9	Máy giặt		21	Điện thoại cố định	
10	Lợn nái		22	Điện thoại di động	
11	Lợn thịt		23	Máy tính	
12	Trâu, bò		25	Khác:…….	

Q109. Trung bình một tháng gia đình anh/chị thu nhập được bao nhiêu (quy ra tiền, bao gồm cả các sản phẩm tự làm ra) ?***(Ước tính)***……. đồng/ tháng

Q 110. Nhà ở CHÍNH của gia đình Bác/Anh/Chị là loại nhà nào ? (Chỉ chọn một lựa chọn)

1. Nhà tranh tường đất
2. Nhà ngói cấp 4
3. Nhà trần 1 tầng
4. Nhà trần gác (nhà trần hơn 1 tầng)
5. Nhà gỗ tạm
6. Nhà gỗ kiên cố
7. Khác (ghi rõ)……

Q 111. Gia đình ta sử dụng những nguồn nước CHÍNH nào cho ăn uống (không kể nước dùng cho tắm giặt, sinh hoạt khác)? (Chỉ chọn một lựa chọn)

1. Nước mưa
2. Nước giếng khơi
3. Nước giếng khoan
4. Nước hồ, ao
5. Nước sông, suối
6. Nước máy
7. Khác(ghi rõ)……..

Q 112. Gia đình Bác/Anh/Chị dùng loại bếp nào để nấu là chính (Chỉ chọn một lựa chọn)?

1. Bếp lò
2. Bếp củi/rơm
3. Bếp dầu
4. Bếp điện
5. Bếp ga
6. Khác(ghi rõ)……

Q 113. Gia đình anh/chị có sử dụng bể Biogas không? 1. Có (chuyển Q 115) 2. Không (Chuyển Q 117)

Q 114. Thể tích bể Biogas của gia đình là:.....m3

Q 115. Bao lâu gia đình anh/chị hút bể Biogas một lần: .....tháng

Q 116. Mỗi lần hút bỏ thì khối lượng bùn thu được là bao nhiêu:.....m3

Q 117. Gia đình anh/chị tham gia các hoạt động nào sau đây

1. Trồng lúa (diện tích_____)
2. Trồng hoa màu
3. Nuôi cá (diện tích_____)
4. Nuôi gia súc (lợn, trâu, bò...)
5. Nuôi gia cầm (gà, vịt, ngan, ngỗng

PHẦN 2. QUẢN LÝ PHÂN TỪ NGƯỜI TẠI CÁC HỘ GIA ĐÌNH

Q201. Hố xí của gia đình anh/chị là loại nào?

1. Một ngăn, hố xí khô/tạm 2. Hai ngăn 3. Khác:.....

Q 202. Anh/chị có ủ phân không 1.Có 2. Không

Q203. Anh/chị có ủ ở trong hố xí không? 1.Có 2. Không (chuyển Q205)

Q 204. Thời gian ủ trong hố xí là bao lâu? .....tháng

Q 205. Anh /chị có ủ ở ngoài hố xí? 1.Có 2. Không (chuyển phần III)

Q 206. Anh/chị ủ ở khu vực nào phía ngoài?

1. Gần chuồng nuôi 2. Sau vườn 3.Hầm Biogas 4.Khác:.....

Q 207. Thời gian ủ ở bên ngoài là bao lâu? .....tháng

Q 208. Anh/chị sử dụng chất độn nào trong quá trình ủ?

1. Vôi bột 2.Tro 3. Trấu 4.Mùn cưa 5.Không sử dụng 6.Khác:.....

Q. 209. Anh/chị ủ phân để làm gì?

1. *Bón ruộng vụ mùa*
2. *Bón ruộng vụ chiêm(sau tết)*
3. *Bón cho rau*
4. *Cho cá ăn*
5. *Khác:.....*

PHẦN III. QUẢN LÝ PHÂN TỪ ĐỘNG VẬT TẠI CÁC HỘ GIA ĐÌNH

Q 301. Anh/chị có sử dụng phân chuồng để bón không? 1. Có 2. Không

Q 302. Anh/chị có ủ phân không 1.Có 2. Không

Q 303. Anh/chị có ủ ở trong khu vực chuồng không? 1.Có 2. Không (chuyển Q305)

Q 304. Thời gian ủ trong chuồng là bao lâu? .....tháng

Q 305. Anh /chị có ủ ở ngoài chuồng không? 1.Có 2. Không (chuyển phần IV)

Q 306. Anh/chị ủ ở khu vực nào phía ngoài?

1. Gần chuồng nuôi 2. Sau vườn 3.Hầm Biogas 4.Khác:.....

Q 307. Thời gian ủ ở bên ngoài là bao lâu? .....tháng

Q 308. Anh/chị sử dụng chất độn nào trong quá trình ủ?

2. Vôi bột 2.Tro 3. Trấu 4.Mùn cưa 5.Không sử dụng 6.Khác:.....

Q. 309. Anh/chị ủ phân để làm gì?

1.Bón ruộng vụ mùa
2.Bón ruộng vụ chiêm(sau tết)
3.Bón cho rau
4.Cho cá ăn
5.Khác:.....

Q. 310. Anh/chị thấy việc sử dụng phân ủ để bón cho cây trồng sẽ giúp gia đình tiết kiệm được bao nhiêu tiền một tháng so với việc không sử dụng? .....nghìn đồng/tháng

PHẦN IV . ĐÁNH GIÁ PHƠI NHIỄM **Họ tên người tham gia hoạt động: _____ID: ___ ___ ___ ___ ___ __**

Năm sinh _____ Giới: (1.Nam, 2.Nữ) **Quan hệ với chủ hộ** _____ (1.Chủ hộ, 2.Vợ(chồng), 3.Bố(mẹ), 4.Ông(bà), 5.Con)

Sử dụng phân bắc bón cho lúa

C1	**Anh chị có sử dụng phân bắc bón cho lúa không?**	Có	**1**	***C2: Nếu có,* Anh/chị đã làm những công việc cụ thể nào sau đây:**	(KN=không nhớ thì khoanh tròn vào số 99)		(KN= không nhớ thì khoanh tròn vào số 99)	(Điều tra viên cùng người trả lời ước lượng thời gian)	(KN= không nhớ thì khoanh tròn vào số 99)
		Không	**2**	**Chuyển→ C3**					
C2.1	Lấy phân từ nhà tiêu ra ngoài	Có	1	Thời gian lưu trữ phân trong nhà tiêu	……tháng	Tổng số lần lấy trong năm?	……lần	Mỗi lần trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C2.2	Thực hiện công việc ủ phân	Có	1	Thời gian lưu trữ phân trước khi ủ	……tháng	Tổng số lần ủ trong năm?	……lần	Mỗi lần ủ trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C2.3	Vận chuyển phân từ nhà ra ruộng	Có	1	Thời gian ủ phân trước khi vận chuyển	……tháng	Tổng số đợt vận chuyển trong năm?	……đợt	Mỗi đợt trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C2.4	Trộn phân và các chất khác để bón	Có	1	Thời gian ủ phân trước khi trộn	……tháng	Tổng số đợt trộn trong năm?	……đợt	Mỗi đợt trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C2.5	Bón phân trên ruộng	Có	1	Thời gian ủ phân trước khi bón	……tháng	Tổng số đợt bón phân trong năm?	…đợt	Mỗi đợt trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99

Sử dụng phân bắc bón cho cây trồng

C3	**Anh chị có sử dụng phân bắc bón cho cây trồng không?**	Có	**1**	***C4: Nếu có,* Anh/chị đã làm những công việc cụ thể nào sau đây:**	(KN= không nhớ thì khoanh tròn vào số 99)		(KN= không nhớ thì khoanh tròn vào số 99)	(Điều tra viên cùng người trả lời ước lượng thời gian)	(KN= không nhớ thì khoanh tròn vào số 99)
		Không	**2**	**Chuyển→ C5**					
C4.1	Lấy phân từ nhà tiêu ra ngoài	Có	1	Thời gian lưu trữ phân trong nhà tiêu	……tháng	Tổng số lần lấy trong năm?	……lần	Mỗi lần trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C4.2	Thực hiện công việc ủ phân	Có	1	Thời gian lưu trữ phân trước khi ủ	……tháng	Tổng số lần ủ trong năm?	……lần	Mỗi lần ủ trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C4.3	Vận chuyển phân từ nhà ra ruộng	Có	1	Thời gian ủ phân trước khi vận chuyển	……tháng	Tổng số đợt vận chuyển trong năm?	……đợt	Mỗi đợt trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C4.4	Trộn phân và các chất khác để bón	Có	1	Thời gian ủ phân trước khi trộn	……tháng	Tổng số đợt trộn trong năm?	……đợt	Mỗi đợt trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C4.5	Bón phân cho cây trồng	Có	1	Thời gian ủ phân trước khi bón	……tháng	Tổng số đợt bón phân trong năm?	…đợt	Mỗi đợt trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99

Sử dụng phân bắc nuôi cá

C5	**Anh chị có sử dụng phân bắc nuôi cá không?**	Có	**1**	***C6: Nếu có,* Anh/chị đã làm những công việc cụ thể nào sau đây:**	(KN= không nhớ thì khoanh tròn vào số 99)		(KN= không nhớ thì khoanh tròn vào số 99)	(Điều tra viên cùng người trả lời ước lượng thời gian)	(KN= không nhớ thì khoanh tròn vào số 99)
		Không	**2**	**Chuyển→ C7**					
C6.1	Lấy phân từ nhà tiêu ra ngoài	Có	1	Thời gian lưu trữ phân trong nhà tiêu	……tháng	Tổng số lần lấy trong năm?	……lần	Mỗi lần trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C6.2	Vận chuyển phân ra ao cá	Có	1			Tổng số đợt vận chuyển trong năm?	……đợt	Mỗi đợt trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2				KN: 99		KN: 99
C6.3	Cho cá ăn	Có	1			Tổng số đợt cho cá ăn trong năm?	…đợt	Mỗi đợt trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2				KN: 99		KN: 99

Sử dụng phân chuồng bón cho lúa

C7	**Anh/chị có sử dụng phân chuồng bón cho lúa không?**	**Có**	**1**	***C8: Nếu có,* Anh/chị đã làm những công việc cụ thể nào sau đây:**	(KN= không nhớ thì khoanh tròn vào số 99)		(KN= không nhớ thì khoanh tròn vào số 99)	(Điều tra viên cùng người trả lời ước lượng thời gian)	(KN= không nhớ thì khoanh tròn vào số 99)
		Không	**2**	**Chuyển→ C9**					
C8.1	Lấy phân ra từ chuồng gia súc, gia cầm	Có	1	Thời gian lưu trữ phân trong chuồng	……tháng	Tổng số lần lấy trong năm?	……lần	Mỗi lần trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C8.2	Thực hiện công việc ủ phân	Có	1	Thời gian lưu trữ phân trước khi ủ	……tháng	Tổng số lần ủ trong năm?	……lần	Mỗi lần ủ trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C8.3	Vận chuyển phân từ nhà ra ruộng	Có	1	Thời gian ủ phân trước khi vận chuyển	……tháng	Tổng số đợt vận chuyển trong năm?	……đợt	Mỗi đợt trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C8.4	Trộn phân và các chất khác để bón	Có	1	Thời gian ủ phân trước khi trộn	……tháng	Tổng số đợt trộn trong năm?	……đợt	Mỗi đợt trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C8.5	Bón phân trên ruộng	Có	1	Thời gian ủ phân trước khi bón	……tháng	Tổng số đợt bón phân trong năm?	…đợt	Mỗi đợt trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99

Sử dụng phân chuồng bón cho cây trồng

C9	**Anh/chị có sử dụng phân chuồng bón cho cây trồng không?**	**Có**	**1**	***C10: Nếu có,* Anh/chị đã làm những công việc cụ thể nào sau đây:**	(KN= không nhớ thì khoanh tròn vào số 99)		(KN= không nhớ thì khoanh tròn vào số 99)	(Điều tra viên cùng người trả lời ước lượng thời gian)	(KN= không nhớ thì khoanh tròn vào số 99)
		Không	**2**	**Chuyển→ C11**					
C10.1	Lấy phân ra từ chuồng gia súc, gia cầm	Có	1	Thời gian lưu trữ phân trong chuồng	……tháng	Tổng số lần lấy trong năm?	……lần	Mỗi lần trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C10.2	Thực hiện công việc ủ phân	Có	1	Thời gian lưu trữ phân trước khi ủ	……tháng	Tổng số lần ủ trong năm?	……lần	Mỗi lần ủ trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C10.3	Vận chuyển phân từ nhà ra ruộng	Có	1	Thời gian ủ phân trước khi vận chuyển	……tháng	Tổng số đợt vận chuyển trong năm?	……đợt	Mỗi đợt trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C10.4	Trộn phân và các chất khác để bón	Có	1	Thời gian ủ phân trước khi trộn	……tháng	Tổng số đợt trộn trong năm?	……đợt	Mỗi đợt trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C10.5	Bón phân cho cây trồng	Có	1	Thời gian ủ phân trước khi bón	……tháng	Tổng số đợt bón phân trong năm?	…đợt	Mỗi đợt trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99

Sử dụng phân chuồng cho cá ăn

C11	**Anh/chị có sử dụng phân chuồng nuôi cá không?**	**Có**	**1**	***C12: Nếu có,* Anh/chị đã làm những công việc cụ thể nào sau đây:**	(KN= không nhớ thì khoanh tròn vào số 99)		(KN= không nhớ thì khoanh tròn vào số 99)	(Điều tra viên cùng người trả lời ước lượng thời gian)	(KN= không nhớ thì khoanh tròn vào số 99)
		Không	**2**	**<u>Kết thúc phỏng vấn</u>**					
C12.1	Lấy phân ra từ chuồng gia súc, gia cầm	Có	1	Thời gian lưu trữ phân ở chuồng	……tháng	Tổng số lần lấy trong năm?	……lần	Mỗi lần trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2		KN: 99		KN: 99		KN: 99
C12.2	Vận chuyển phân ra ao cá	Có	1			Tổng số đợt vận chuyển trong năm?	……đợt	Mỗi đợt trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2				KN: 99		KN: 99
C12.3	Cho cá ăn	Có	1			Tổng số đợt cho cá ăn trong năm?	…đợt	Mỗi đợt trung bình hết bao nhiêu thời gian?	…..giờ …..phút
		Không	2				KN: 99		KN: 99

Pictures of setting up the experimental excreta storage

The inside of experimental house

The vault with air pipe for storing excreta

The vault of experimental excreta storage with the metal cover

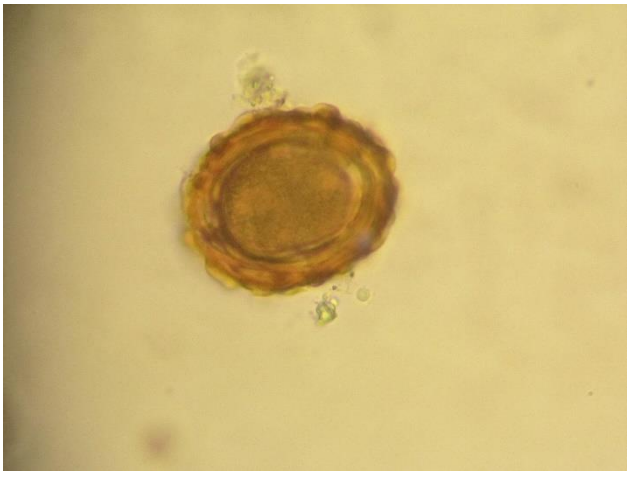
Mixing excreta and adding material before adding to the vault of experiment storage

Pictures of *Ascaris lumbricoides* eggs and analysing the status of them in the laboratory

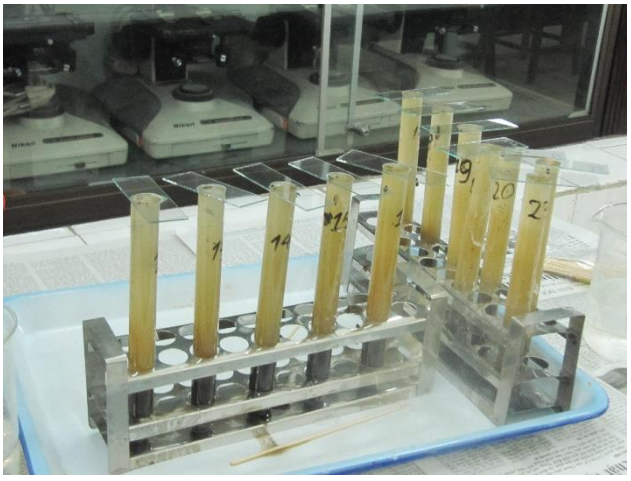
***A. lumbricoides* egg in developed stage**

Dead *A. lumbricoides* egg

Live *A. lumbricoides* egg



Dead *A. lumbricoides* egg



Floatation method for getting *Ascaris lumbricoides* eggs for analysing

Floatation method for getting *Ascaris lumbricoides* eggs for analysing (Romanenko method)

Pictures of exposure excreta study

Scaling the paire of gloves in exposure excreta study.

Taking off excreta from vault of latrine after storage.

Taking off excreta from vault of latrine after storage for composting in heap.

Application excreta as fertilizer on a field.

ANNEX 2. Curriculum vitae

PROFILE	
	Tu Vu Van, MD, MPH Born October 17, 1974; Married since 2001; Address in Vietnam: No.314/22, Dong Tien, Hoa Binh City, Hoa Binh Province Mobile: +(84) 982 326 558 Email: vuvantu@gmail.com; vantu.vu@unibas.ch; vantu.vu@swisstph.ch
EDUCATION	
1991-1997 2007-2009 2010-2017	Medical Doctor**,** Thai Nguyen Medical University, Vietnam Master of Public Health, Hanoi School of Public Health, Vietnam PhD student, University of Basel, Switzerland. Field works in Vietnam
PRESENT APPONTMENTS	
2010-2017	Work at Hoa Binh Provincial General Hospital PhD student belong the NCCR North-South project on "Productive Sanitation" Course taken: Epidemiology concept, Epidemiology method, Exposure assessment, Human and Animal Infection, Quality course, Quality research, R, Statistic.
CONFERENCES & WORKSHOPS ATTENDED	
	Workshop: World Toilet Day commemoration and introducing research results in the field of sanitation and health in Vietnam, Hanoi, Vietnam, 11/2011. Workshop: Getting papers published in peer-reviewed journals, Vietnam National University Ho Chi Minh City Vietnam, 8/2011. Training course: Using R in sciencetific research, Vietnam National University Ho Chi Minh City Vietnam, 12/2010. Training course**:** Environmental health risk assessment of microbiological hazards in water and foods in Vietnam**,** Hanoi School of Public Health, 8/2010. Training workshop**:** "Environmental behaviour and Human health risk assessment associated with the use of agricultural pesticides in Vietnam"**,** Hanoi School of Public Health, 7/2010.

LANGUAGES	
	Vietnamese – mother tongue English - good
COMPUTER SKILLS	
	Microsoft Office (Word, Excel, PowerPoint, Access), Epi-Info, SPSS, R, Internet.
PUBLICATION ARTICLES PUBLISHED IN ISI PEER-REFERRED JOURNALS	
	Vu-Van, T., P. Pham-Duc, M. S. Winkler, C. Zurbrügg, J. Zinsstag, H. Le Thi Thanh, T. H. Bich and H. Nguyen-Viet (2017). "Ascaris lumbricoides egg die-off in an experimental excreta storage system and public health implication in Vietnam." International Journal of Public Health **62**(1): 103-111. Le-Thi, T., P. Pham-Duc, C. Zurbrügg, T. Luu-Quoc, H. Nguyen-Mai, **T. Vu-Van** and H. Nguyen-Viet (2017). "Diarrhea risks by exposure to livestock waste in Vietnam using quantitative microbial risk assessment." International Journal of Public Health **62**(1): 83-91. Nga, D. T., T. T. Thao, **V. Van Tu**, P. D. Phuc and H. Nguyen-Viet (2016). "Development of nutrient cycle through agricultural activities of a rural area in the North of Vietnam." Journal of Material Cycles and Waste Management: 1-9. Tran-Thi, N., R. J. Lowe, J. M. Schurer, **T. Vu-Van**, L. E. MacDonald and P. Pham-Duc (2017). "Turning poop into profit: Cost-effectiveness and soil transmitted helminth infection risk associated with human excreta reuse in Vietnam." PLOS Neglected Tropical Diseases 11(11): e0006088. **Van Vu, T.**, P. D. Pham, M. S. Winkler, C. Zurbrügg, J. Zinsstag, B. H. Tran and H. Nguyen-Viet (2018). "Estimation of involuntary excreta ingestion rates in farmers during agricultural practices in Vietnam." Human and Ecological Risk Assessment: An International Journal:1-11.

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	Ngan, N. K., Bich, N. N., Pham-Duc, P., **Vu-van, Tu**., Schelling, E., & Nguyen-Viet, H. (2013). Towards an integrated management of human and animal waste in Viet Nam: Regulation and health and environmental impacts. *Vietnam Journal of Public Health 28*, 4 - 12. **Vu-Van, Tu**., Huong, L. T. T., Pham-Duc, P., Thao, N. B., & Nguyen-Viet, H. (2011). Managing, using human excreta and community health in Vietnam. *Vietnam Journal of Public Health, 22*, 4 - 13. **Vu-van, Tu.**, Huong, N., Pham-Duc, P., Nguyen-Viet, H., & Zurbrugg C. (2011). Developing a questionnaire to measure awareness and behaviours of people in relation to wastewater use in agriculture at Hoang Tay commune and Nhat Tan commune. *Vietnam Journal of Public Health, 22*, 14 - 20.
BOOK CHAPTERS	
	Hung Nguyen-Viet, Phuc Pham-Duc, Vi Nguyen, Marcel Tanner, Peter Odermatt, **Tu Vu-Van**, Minh V. Hoang, Christian Zurbrüg, Esther Schelling, Jakob Zinsstag. 2015. A One Health perspective for integrated human and animal sanitation and nutrient recycling. In J. Zinsstag, E. Schelling, D. Waltner-Toews, M. Whittaker & M. Tanner (Eds.), *One Health: The Theory and Practice of Integrated Health Approaches*. CAB International, Wallingford, UK. 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.