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ORIGINAL ARTICLE

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

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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

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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

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.

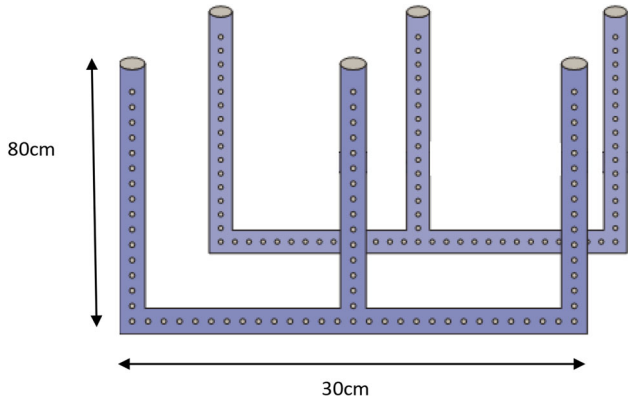

Fig. 1 The aeration system for vaults $V1_{\text{with air}}$, $V2_{\text{lime3kg with air}}$, $V3_{\text{lime riceh air}}$, and $V4_{\text{lime10kg air}}$ with three replicates for each option in Vietnam experiment 2012. The tube diameter was 35 mm. The aeration system width was 30 cm and the height was 80 cm. ($V1_{\text{no air}}$: the vault had 100 kg excreta without air pipe; $V1_{\text{with air}}$ the vault had 100 kg excreta with air pipe; $V2_{\text{lime3kg}}$: the vault had 97 kg excreta and 3 kg lime without air pipe; $V2_{\text{lime3kg air}}$: the vault had 97 kg excreta and 3 kg lime with air pipe; $V3_{\text{lime riceh}}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk without air pipe; $V3_{\text{lime riceh air}}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk with air pipe; $V4_{\text{lime10kg}}$ had 90 kg excreta and 10 kg lime without air pipe; $V4_{\text{lime10kg air}}$ 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 ($V1_{\text{no air}}$: the vault had 100 kg excreta without air pipe; $V1_{\text{with air}}$ the vault had 100 kg excreta with air pipe; $V2_{\text{lime3kg}}$: the vault had 97 kg excreta and 3 kg lime without air pipe; $V2_{\text{lime3kg air}}$: the vault had 97 kg excreta and 3 kg lime with air pipe; $V3_{\text{lime riceh}}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk without air pipe; $V3_{\text{lime riceh air}}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk with air pipe; $V4_{\text{lime10kg}}$ had 90 kg excreta and 10 kg lime without air pipe; $V4_{\text{lime10kg air}}$ 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)		
$V1_{\text{no air}}$ (control)	100	0	0	No	3
$V1_{\text{with air}}$	100	0	0	Yes	3
$V2_{\text{lime3kg}}$	97	3	0	No	3
$V2_{\text{lime3kg air}}$	97	3	0	Yes	3
$V3_{\text{lime riceh}}$	90	5	5	No	3
$V3_{\text{lime riceh air}}$	90	5	5	Yes	3
$V4_{\text{lime10kg}}$	90	10	0	No	3
$V4_{\text{lime10kg air}}$	90	10	0	Yes	3

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

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

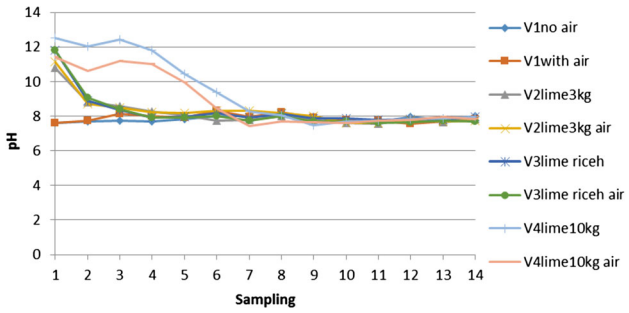

Fig. 2 Reported pH values in vault options following 14 sampling in Vietnam experiment, 2012 ($V1_{\text{no air}}$: the vault had 100 kg excreta without air pipe; $V1_{\text{with air}}$ the vault had 100 kg excreta with air pipe; $V2_{\text{lime3kg}}$: the vault had 97 kg excreta and 3 kg lime without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

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 ($V1_{\text{no air}}$: the vault had 100 kg excreta without air pipe; $V1_{\text{with air}}$ the vault had 100 kg excreta with air pipe; $V2_{\text{lime3kg}}$: the vault had 97 kg excreta and 3 kg lime without air pipe; $V2_{\text{lime3kg air}}$: the vault had 97 kg excreta and 3 kg lime with air pipe; $V3_{\text{lime riceh}}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk without air pipe; $V3_{\text{lime riceh air}}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk with air pipe; $V4_{\text{lime10kg}}$ had 90 kg excreta and 10 kg lime without air pipe; $V4_{\text{lime10kg air}}$ 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
$V1_{\text{no air}}$	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
$V1_{\text{with air}}$	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
$V2_{\text{lime3kg}}$	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
$V2_{\text{lime3kg air}}$	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
$V3_{\text{lime riceh}}$	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
$V3_{\text{lime riceh air}}$	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
$V4_{\text{lime10kg}}$	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
$V4_{\text{lime10kg air}}$	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 $V3_{\text{lime riceh}}$, $V3_{\text{lime riceh air}}$, $V4_{\text{lime10kg}}$ and $V4_{\text{lime10kg air}}$ reached the WHO standard at 111 days of excreta storage. However, vault option

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) ($V1_{\text{no air}}$: the vault had 100 kg excreta without air pipe; $V1_{\text{with air}}$ the vault had 100 kg excreta with air pipe; $V2_{\text{lime3kg}}$: the vault had 97 kg excreta and 3 kg lime without air pipe; $V2_{\text{lime3kg air}}$: the vault had 97 kg excreta and 3 kg lime with air pipe; $V3_{\text{lime riceh}}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk without air pipe; $V3_{\text{lime riceh air}}$ had 90 kg excreta, 5 kg lime and 5 kg rice husk with air pipe; $V4_{\text{lime10kg}}$ had 90 kg excreta and 10 kg lime without air pipe; $V4_{\text{lime10kg air}}$ 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
$V1_{\text{no air}}$	346	100	564	242	339	247	386	266	246	324	190	363	218	324
$V1_{\text{with air}}$	259	107	347	162	330	193	176	102	258	249	213	254	200	153
$V2_{\text{lime3kg}}$	330	85	492	212	339	231	390	175	419	258	1777	466	346	270
$V2_{\text{lime3kg air}}$	403	138	442	209	373	247	240	151	951	382	276	305	196	282
$V3_{\text{lime riceh}}$	301	60	275	124	276	166	293	258	394	216	253	274	140	251
$V3_{\text{lime riceh air}}$	251	93	259	82	393	133	283	162	234	211	161	225	93	188
$V4_{\text{lime10kg}}$	394	63	374	174	372	201	427	223	160	274	141	244	95	203
$V4_{\text{lime10kg air}}$	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
$V1_{\text{no air}}$	80	236	318	314	115	317	88	226	49	185	34	181	43	331
$V1_{\text{with air}}$	117	254	154	240	219	184	94	223	52	205	29	177	8	121
$V2_{\text{lime3kg}}$	156	249	256	626	238	348	157	321	78	197	33	188	18	130
$V2_{\text{lime3kg air}}$	132	255	138	570	169	338	127	252	48	217	41	241	18	199
$V3_{\text{lime riceh}}$	146	224	29	284	155	231	49	208	25	101	64	242	31	171
$V3_{\text{lime riceh air}}$	100	194	20	282	138	164	119	176	25	133	25	176	10	96
$V4_{\text{lime10kg}}$	75	197	72	327	84	194	49	182	16	112	26	148	7	123
$V4_{\text{lime10kg air}}$	87	131	15	250	46	214	56	165	33	137	8	119	5	98

$V4_{\text{lime10kg air}}$ 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 $V4_{\text{lime10kg}}$ and $V4_{\text{lime10kg air}}$ (with 10% lime per total weight). However, vault option $V4_{\text{lime10kg air}}$ 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 $_{\text{lime10kg air}}$ 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

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.

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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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