

Inactivation of *Ascaris* Eggs in Source-Separated Urine and Feces by Ammonia at Ambient Temperatures[∇]

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Received 5 June 2008/Accepted 24 November 2008

Sustainable management of toilet waste must prevent disease transmission but allow reuse of plant nutrients. Inactivation of uterus-derived *Ascaris suum* eggs was studied in relation to ammonia in source-separated urine without additives and in human feces to which urea had been added, in order to evaluate ammonia-based sanitation for production of safe fertilizers from human excreta. Urine was used concentrated or diluted 1:1 and 1:3 with tap water at 4, 14, 24, and 34°C. Fecal material, with and without ash, was treated with 1% or 2% (wt/wt) urea at 24 and 34°C. At 34°C eggs were inactivated in less than 10 days in urine and in amended feces. At 24°C only feces with 2% (wt/wt) urea or 1% (wt/wt) urea at high pH (10) inactivated all eggs within 1 month, and no inactivation was observed after 75 days in urine diluted 1:3 (18 ± 11 mM NH₃). At temperatures of $\geq 24^\circ\text{C}$, NH₃ proved to be an efficient sanitizing agent in urine and feces at concentrations of ≥ 60 mM. Treating fecal material at 34°C can give a 6-log₁₀ egg inactivation within 1 month, whereas at 24°C 6 months of treatment is necessary for the same level of egg inactivation. At temperatures of 14°C and below, inactivation rates were low, with viable eggs after 6 months even in concentrated urine.

Today, approximately 2.6 billion people lack improved sanitation and human excreta are spread into the environment from untreated wastewater, which constitutes 70% of all wastewater (13), and by open defecation. The excreta are accompanied by large amounts of pathogens, polluting soil and water. Source-separating dry sanitation systems offer an alternative to meet the sanitation requirement, while plant nutrients and organic material from collected human excreta can be used for food production. However, reliable pathogen inactivation is crucial for safe reuse of human excreta (31). Reuse of wastewater and human excreta in agriculture has been related to infection with parasitic intestinal worms, of which *Ascaris lumbricoides* is the most widespread, with an estimated 1.4 billion people infected (3). The resistance of the eggs to many treatment factors and disinfectants makes *Ascaris* eggs a conservative indicator organism for environmental pollution and treatment efficiency (17).

For most biological treatments a thermophilic process temperature seems to be crucial for efficient sanitation (11, 25), and common methods for inactivating *Ascaris* eggs in sludge and fecal matter use high temperature, high pH, or both. It is suggested that ammonia, which is toxic in its uncharged form NH₃ (29), contributes to pathogen inactivation by alkaline stabilization (1, 15). Alkaline pH affects the equilibrium between NH₄⁺ and NH₃ in aqueous solution in favor of the formation of NH₃. The microbicidal properties of NH₃ have been observed both when ammonia is naturally occurring, as in urine, and when different biosolids have been treated with ammonia or ammonia-forming additives (7, 18, 19, 26).

The ovicidal effects of NH₃ on *Ascaris* eggs have been isolated

in aqueous solution (21, 23), and sewage sludge amended with ammonia has been shown to result in faster inactivation than untreated sludge (15, 20). *Ascaris suum*, which infects pigs, is often used as a model for *A. lumbricoides*, and in research uterus-derived eggs are used due to availability of large quantities of eggs and to the fact that the eggs are not larvated before experimental use. Alkaline ammonia treatment resulting in 0.6% (vol/vol) NH₃ (pH 11.9) has been performed with eggs from both *A. suum* and *A. lumbricoides*, with eggs of *A. lumbricoides* harvested from worm uteri compared with eggs extracted from infected feces. That study revealed that the inactivation was equal for *Ascaris* eggs, independent of the species or extraction method (8).

Separating fecal matter at the source would minimize the occurrence of *Ascaris* eggs in other wastewater fractions, such as sewage sludge and wastewater. However, it would increase the need for potent sanitation methods for the fecal matter, since this would not be subjected to dilution. Today, the most common treatment of source-separated fecal matter is low-temperature composting, i.e., long-term storage with little or no increase above ambient temperature (31). For source-separated urine, long-term storage based on the action of NH₃ is the only large-scale sanitation technique practiced (14). Few studies have been performed on *Ascaris* egg inactivation in human feces (22, 26), the source of *A. lumbricoides* eggs, and development of sanitation methods that are environmentally sound and reliable is an ongoing concern.

The objective of this study was to evaluate the effects of ammonia-based sanitation on *A. suum* eggs in source-separated human feces and urine, at temperatures corresponding to a range of ambient conditions, and to evaluate treatment methods for ensuring safe nutrient recycling of human-derived fertilizers.

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[∇] Published ahead of print on 5 December 2008.

MATERIALS AND METHODS

Material. Fecal material was collected during 3 months from a urine-diverting dry toilet used by a single household. After collection, excess toilet paper was

TABLE 1. Experimental setup for the excreta fractions

Material	Treatment		Temp (°C) ^b	Mass or vol	No. of replicates	Treatment time
	Primary	Secondary ^a				
Feces	0.1 liter ash 100 g ⁻¹ feces	0% urea (A), 1% urea (AU ₁)	24 and 34	200 g	3	35 days ^d
	None	0% urea (U ₀), 1% urea (U ₁), 2% urea (U ₂)	24 and 34	200 g	3	35 days ^d
Urine	1:1 dilution	Storage	4, 14, 24, and 34	500 ml	2	6 mo ^e
	1:0 and 1:3 dilution	Storage	4, 14, 24, and 34	50 ml	1 ^c	6 mo ^e

^a Letters in parentheses brackets for the treatment of fecal fraction indicate the presence of ash (A) and urea (U₀ to U₂) additions.

^b The deviation from the set temperature was at most ± 2 °C.

^c Setup with independent samples over time.

^d Sampling was on days 4, 9, 15, and 22 at 24°C and on days 3, 9, 13, 19, and 31 at 34°C.

^e At 4°C sampling was on days 7, 83, 150, and 190 (1:0); days 8, 19, 33, 92, and 182 (1:1); and days 7, 38, 100, and 190 (1:0). At 14°C sampling was on days 7, 8, 150, and 190 (1:0); days 8, 15, 26, 92, and 182 (1:1); and days 7, 38, 100 and 190 (1:0). At 24°C sampling was on days 7, 10, 20, and 73 (1:0); days 5, 12, 22, and 40 (1:1); and days 7, 14, 38, and 73 (1:0). At 34°C sampling was on days 1, 2, 3, and 7 (1:0); days 2, 5, 8, and 13 (1:1); and days 1, 2, 7, and 10 (1:0).

removed and the fecal material was stored at 4°C. Before use in experiments, the dry matter (DM) content was adjusted to 17% (wt/wt) using tap water (28). The ash used for treatment of fecal matter was from a mix of hard and soft wood combusted in a heating furnace. The ash was identified as being responsible for some variations in DM content and pH during the study at 24°C, and subsequently at 34°C the ash was homogenized by sieving (mesh, 7 mm).

The urine was collected both from a source-separating, low-flush sanitation system, resulting in a dilution of approximately 1:1, and directly from persons in a single household. The latter was used concentrated or diluted 1:3 after urea had been degraded with urease (Merck KGaA, Darmstadt, Germany) added at a rate of 50 mg liter⁻¹ urine and left for 24 h at 37°C.

Adult *A. suum* worms were collected from the intestines of slaughterhouse pigs, and the eggs were harvested by dissection of the posterior 2 cm of worm uterus. After washing as described by Eriksen (6), except for use of sodium hypochlorite, approximately 10⁴ eggs were placed in permeable nylon bags (2 · 25 cm²; mesh, 35 µm) and stored in physiological saline solution (0.9% [wt/vol]) at 4°C until use.

Experimental setup. Inactivation of *A. suum* in feces was studied in two sets of material: untreated fecal matter and feces amended with ash, simulating surface coverage after defecation commonly performed with dry toilets. The ash was added at a rate of 0.1 liter ash per 100 g feces with 17% (wt/wt) DM, representing an intermediate dosage in terms of actual practice. In ash-amended feces the DM content was adjusted again to 17% (wt/wt) to avoid effects from the change in DM. Additions of urea were then made to both types of fecal material according to wet weight. Fecal material controls with no urea added were studied for both feces and ash-amended feces (Table 1). After homogenization, the treated feces were distributed (200 g) into soft containers (Grade Packaging Ltd., Coalville, United Kingdom). After insertion of *A. suum* eggs (five bags each), the containers were sealed, minimizing headspace, and the contents were mixed manually for approximately 1 min each. As a temperature control, single *A. suum* bags were incubated in 0.9% (wt/vol) NaCl solution. All containers were incubated in the dark at 24°C or 34°C for 35 days, and egg bags were sampled as described in Table 1.

Inactivation of *A. suum* eggs in stored urine was studied at three concentrations, in duplicate for the 1:1 dilution and as single independent treatments for concentrated and 1:3 diluted urine (Table 1). The urine was allowed to reach the respective target temperature for 16 h and then divided into 500-ml plastic flasks (1:1 dilution) or 50-ml plastic centrifuge tubes (1:0 and 1:3 dilutions), which were filled up to minimize headspace. Four bags with *A. suum* eggs were inserted into each container, except for the 1:1 dilutions at 4°C and 14°C, which had five bags each. The sealed containers were incubated in the dark for 6 months and sampled as described in Table 1.

Sampling and analysis. At sampling, an egg bag from each replicate was collected and rinsed in 0.9% (wt/vol) NaCl solution, followed by incubation at room temperature in 0.1 N sulfuric acid for 28 to 35 days to allow larval development (2). Viability counts were performed under the microscope. Unfertilized eggs, identified by their incomplete egg shells, were excluded from further counting. Eggs developing to the larval stage were considered viable, while prelarval stages were not.

For quantification of total ammonia (NH_{tot} = NH₃ + NH₄⁺) in the solute, 10 g of feces or 1 ml of urine from each replicate was analyzed spectrophotometrically on a Thermo Aquamate (Thermo Electron Ltd., Cambridge, United Kingdom) using the indolphenol blue method (Merck, Whitehouse Station, NJ).

The concentration of NH₃ (aq) was calculated from the measured NH_{tot} concentration, pH, and temperature using the dissociation constant $K_a = 10^{-(2.729.92/T + 0.09018)}$, where T is the temperature in degrees kelvin (4). For fecal treatments, sampling of ammonia was carried out on days 4, 9, and 19 at 34°C and on day 43 at 24°C. In urine, ammonia was measured initially and at the end of the study (day 182) for the 1:1 dilution and on day 150 for the 1:0 and 1:3 dilutions.

For analysis of pH, 10 g of feces or 3 ml of urine was removed from each replicate. The fecal samples were diluted in deionized water (1:9), and the urine samples were measured undiluted, both at room temperature, using an Inolab 720 pH meter (WTW, Germany). The fecal pH was measured on days 4, 9, and 19 at 34°C and on days 0, 1, 4, 8, 18, and 35 at 24°C. The pH of the 1:1 dilution was measured for all temperatures on days 1 and 111 and 182 (end of study). The pHs of the other two urine dilutions (1:0 and 1:3) were analyzed on day 150 only.

When initial phases with no inactivation were observed in the data, the data values were divided into two sets where a breakpoint was calculated from the intercept of the two functions or was otherwise set equal to the last sampling with no significant inactivation. Linear regression was performed (Minitab 15; Minitab Ltd., United Kingdom) on the latter data set in cases of lag phases and for the whole data set when no lag phase was observed, and time for 99% egg inactivation (t_{99}) (mean value and upper 95% confidence limit) was derived from the regression functions.

RESULTS

Ammonia and pH. The measured total ammonia concentration in the fecal treatments was somewhat low (15 to 84%) compared with theoretical concentrations estimated from urea additions and intrinsic ammonia in fecal batches (214 ± 4 and 247 ± 9 mM NH_{tot}). Ammonia recovery in urine (1:1) after 182 days ranged from 92 to 105% compared with day 1, with significantly lower concentrations after 6 months noted only in urine stored at 34°C (Table 2).

The pH of the unamended fecal material was 8.0 to 8.3. Addition of 1% (wt/wt) or 2% (wt/wt) urea increased the pH to just below and above 9, respectively, and during the 1-month study the pH decreased by 0.2 unit for both treatment concentrations. At 34°C, the addition of sieved ash (in treatments A and AU₁) increased the pH to 12.8, after which the pH decreased by 0.1 unit by day 20. The nonsieved ash used at 24°C gave pHs of 10.5 and 10.0 for A and AU₁, respectively (Table 2), which by day 19 had decreased to 9.7 and 9.6, respectively. The urine pH was in the range of 8.7 to 9.1 considering all

TABLE 2. pH range, total ammonia concentration, and ammonia recovery^a

Material	Treatment	34°C			24°C			14°C			4°C		
		pH range ^a	NH ₃ ^{tot} (mM) ^b	NH ₃ ^{recovery} (%) ^c	pH range	NH ₃ ^{tot} (mM)	NH ₃ ^{recovery} (%)	pH range	NH ₃ ^{tot} (mM)	NH ₃ ^{recovery} (%)	pH range	NH ₃ ^{tot} (mM)	NH ₃ ^{recovery} (%)
Feces	U ₂	9.0–8.8	862 ± 72	83 ± 7	9.1–8.9	675 ± 68	67 ± 7						
	U ₁	8.8–8.7	541 ± 8	84 ± 1	8.9–8.7	441 ± 29	73 ± 5						
	AU ₁	12.8–12.7	72 ± 6	15 ± 1	10.0–9.6	261 ± 31	51 ± 6						
	A	12.8–12.7	71 ± 6	78 ± 6	10.5–9.7	60 ± 6	65 ± 6						
	U ₀	8.3–8.1	247 ± 9		8.3–8.0	214 ± 4							
Urine	1:0	9.0	439 ± 16		9.0	415 ± 30		9.1	419 ± 32		9.1	432 ± 12	
	1:1	8.9–8.8	203 ± 3	92 ± 6	8.9–8.7	219 ± 5	97 ± 3	8.9–8.8	205 ± 3	101 ± 6	8.9–8.8	207 ± 2	105 ± 2
	1:3	8.7	131 ± 1 ^a		8.7	107 ± 9		9.0	103 ± 7		9.1	105 ± 8	

^a Initial pH (maximum) to final pH (minimum) in fecal material.

^b Total ammonia concentration (NH₃ + NH₄⁺) in fecal material measured on day 35 (24°C) and day 3 (34°C) and in urine on day 182 (dilution 1:1) and day 150 (dilutions 1:0 and 1:3). Values are means ± standard deviations.

^c For fecal matter, recovery of ammonia is measured ammonia compared with the expected amount according to additions of urea, while for urine the fraction is ammonia measured at day 182 (1:1) compared with day 1. Values are means ± standard deviations.

dilutions, with the somewhat lower pH observed in diluted urine, especially at temperatures of ≥24°C (Table 2).

Ascaris egg inactivation. The eggs used in fecal treatment at 24 and 34°C originated from two batches with initial viabilities of 62% ± 0.1% and 80% ± 4.5%, respectively. At 34°C no viable eggs were found from day 4 in feces with ash plus 1% (wt/wt) urea (AU₁) or 2% (wt/wt) urea (U₂). With only ash (A), two viable eggs were found on day 4. This inactivation was confirmed on day 10, with >2,000 eggs counted for each treatment. In feces treated with 1% (wt/wt) urea (U₁) also, no viable eggs were found on day 10. After day 31, no viable eggs

(of 1,629 eggs) were detected in unamended feces (U₀). In the 0.9% NaCl temperature control, only 7% of the eggs were inactivated by day 35. Unamended feces (U₀) at 34°C initially caused a peak, with higher egg viability on days 3 and 8 compared with the initial viability (Fig. 1a).

At 24°C no viable *Ascaris* eggs were found in AU₁ by day 22 (of 750 eggs), and only one viable egg was found in U₂ by day 35 (of 806 eggs). At the end of the study (day 35), there were no significant differences in inactivation between A and U₁, with 90% ± 4.5% and 78% ± 6.5% inactivation of viable eggs, respectively. At the same time in the unamended feces (U₀), 50% ± 16% of the

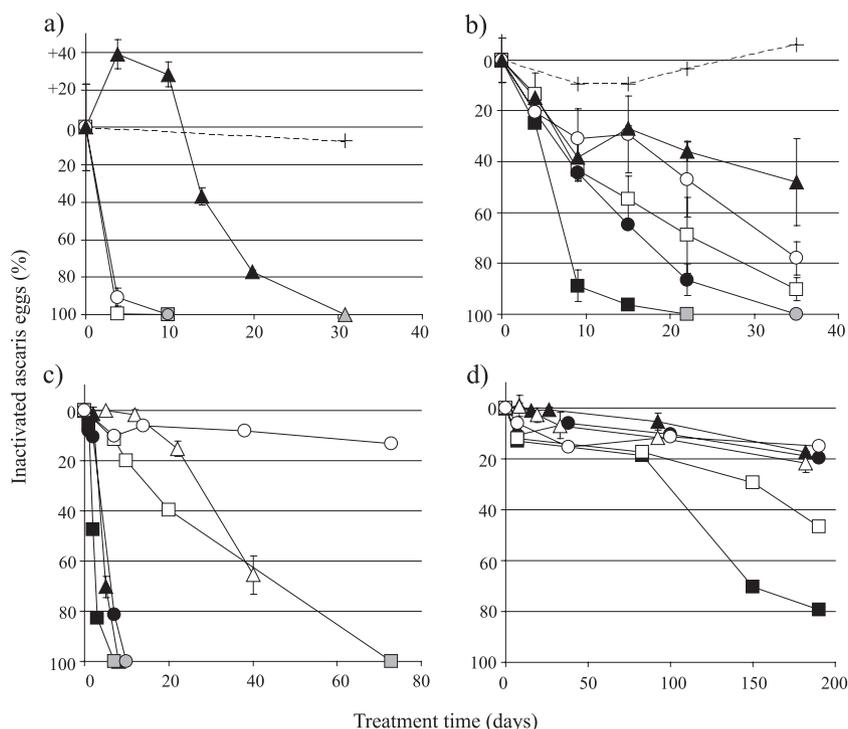


FIG. 1. Inactivation of viable *Ascaris suum* eggs (percent inactivation ± standard deviation) as a function of time (days) in feces at 34°C (a) and 24°C (b) treated with 2% (wt/wt) urea (●), 1% (wt/wt) urea (○), 1% (wt/wt) urea plus ash (■), or ash (□) or unamended (▲) and in urine stored at 24/34°C (c) and at 4/14°C (d) which was diluted 1:0 (■), 1:1 (▲), and 1:3 (●) with water. The lower temperature (4 and 24°C) in panels c and d is indicated with open symbols. Shaded symbols indicate no viable larvae at sampling, and broken lines represent viability of controls held in 0.09% NaCl.

TABLE 3. Concentrations of NH₃ (aq) in solution calculated from maximum pH, temperature, and total ammonia, together with time 99% inactivation (t₉₉) of viable *A. suum* eggs

Material	Treatment	Maximum pH	34°C				24°C				14°C				4°C			
			NH ₃ (aq)		t ₉₉ (days) ^a		NH ₃ (aq)		t ₉₉ (days)		NH ₃ (aq)		t ₉₉ (days)		NH ₃ (aq)		t ₉₉ (days)	
			mM		Mean	95%	mM		Mean	95%	mM		Mean	95%	mM		Mean	95%
			(mean ± SD)	%			(mean ± SD)	%			(mean ± SD)	%			(mean ± SD)	%		
Feces	U ₂	9.0	440 ± 40	51	3.8	6.4	230 ± 20	35	28	33								
	U ₁	8.9	250 ± 4	46	4.1	5.9	130 ± 9	30	47 ^c	59								
	AU ₁	12.8 vs 10	72 ± 6	100	3.8	6.4	220 ± 26	84	13	18								
	A	12.8 vs 10.5	71 ± 6	100	3.7	4.1	57 ± 6	94	35 ^c	50								
	U ₀	8.3	43 ± 2	17	21 (8)	27	20 ± 0.4	10	74 ^c	114								
Urine	1:0	9.0, 9.1 ^b	236 ± 8	54	3.4 (≥1)	4.0	141 ± 11	34	48	62	94 ± 3	22	240 ^c	380	57 ± 6	13	480 ^c	770
	1:1	8.9, 8.9	100 ± 8	46	6.3 (≥2)	7.3	66 ± 2	30	56 (17) ^c	70	39 ± 10	17	1,060 ^c	1,250	16 ± 0.3	8	840 ^c	1,050
	1:3	8.7, 9.1	40 ± 31	44	8.5 (≥2)	14	18 ± 11	22	— ^d	—	20 ± 6	20	— ^d	—	14 ± 1	14	— ^d	—

^a Predicted by linear regression of data sets with significant inactivation and given as mean and upper 95th percentile. The lag phase, when present, is given in parentheses with the predicted time (days) for t₉₉.

^b The first value is for 34 and 24°C, and the second is for 14 and 4°C.

^c t₉₉ not confirmed with zero viable larvae.

^d Regression coefficient not significantly (P > 0.05) different from zero.

A. suum eggs were inactivated, while in the NaCl temperature control, no egg inactivation was observed (Fig. 1b).

The initial viability of the *A. suum* eggs used in urine was 93% ± 3%. At 34°C, no viable eggs were detected (of 440 to 1,446 eggs) from days 7, 8, and 10 for urine diluted 1:0, 1:1, and 1:3, respectively. At 24°C, no viable eggs were found from day 73 (of 288 eggs) in the 1:0 dilution. At the last sampling at 24°C, on day 40 in the 1:1 dilution and day 73 in the 1:3 dilution, 66% ± 7.5% and 13% of the eggs were inactivated, respectively (Fig. 1c). After 6 months at 4 and 14°C, the eggs in diluted urine (1:1 and 1:3) were inactivated by at most 22% ± 0.0%, whereas the concentrated urine resulted in 47 and 79% inactivation, respectively (Fig. 1).

Except for the 1:3 diluted urine at 4 to 24°C, the urine and fecal treatments resulted in significant (P < 0.05) egg inactivation. However, the inactivation trend in some treatments (urine at 4 and 14°C) was low, which resulted in t₉₉ values of more than 1 year, considering the upper 95% confidence interval (Table 3).

When the *Ascaris* eggs in fecal material were treated, formation of a brown, ridged covering layer on the eggs was observed in treatments at 24°C, with a larger proportion of covered eggs observed in treatments amended with ash. At 34°C this was observed to a lesser extent. On day 4 at 24°C, the two ash treatments (A and AU₁) had 38% ± 6% covered eggs, while urea treatments and unamended feces had less than 4% ± 3% and 12% ± 5% covered eggs, respectively. The number of covered eggs decreased progressively over time, and by day 35 only 1% ± 1% were covered in any treatment. The covered eggs showed a tendency for somewhat higher viability than the uncovered eggs. However, the results and inactivation figures in the current study include both covered and uncovered eggs.

DISCUSSION

Lag phases and data interpretation. In modeling microbial inactivation rates, the hypothesis of a time-dependent exponential decay is often applied. However, initial and terminal lag phases commonly occur. In the present study initial lag phases with no significant inactivation were observed for some

treatments (Fig. 1c). However, undetected lag phases may be explained by a too-low sampling frequency. Similar lag phases have been observed by Ghiglietti et al. (7, 8) and Pecson et al. (20), who introduced a model for shouldered inactivation based on exponential decay. When that model was used in the current study, time estimates for total egg inactivation were in some cases double the times actually observed. Linear regression gave a better fit to whole or divided sets of data, and the time for total inactivation was derived and approximated to represent time for a 99% inactivation (t₉₉) (Table 3). The presence of lag phases has an impact on how to interpret egg inactivation rates. When evaluating treatment methods, inactivation should be studied for time spans relevant for treatment when little can be said about the actual inactivation beyond the point of the study.

NH₃ and egg inactivation. At 24°C there was a linear correlation between NH₃ (aq) and inactivation time (r² = 0.94), except for the 1:3 urine dilution, which had no inactivation, and treatments with ash (A and AU₁), which had a faster inactivation in relation to NH₃ than other treatments. At 34°C it was not possible to relate the egg inactivation rates to NH₃ concentrations (71 to 440 mM), due to the low frequency of sampling in relation to fast inactivation. At both 24°C and 34°C there were some inconsistencies in inactivation rates between treatments with similar NH₃ concentrations (1:3 diluted urine and unamended feces), but these were not explained by the material (Table 3).

The results indicate that at NH₃ concentrations of ~40 mM, factors other than those monitored here contributed to the inactivation rates and that ~20 mM might be a threshold concentration for egg inactivation (Table 3). Ghiglietti et al. (7) found a similar threshold NH₃ concentration when studying *Ascaris* egg inactivation in both water and sludge amended with aqueous ammonia at 22°C. With an NH₃ concentration of 23 mM NH₃ (aq, pH 12.3), they observed no inactivation during 90 days, whereas with 46 mM NH₃ (aq, pH 12.5), no viable eggs were found at day 40. However in the present study, there was significant inactivation at 20 mM NH₃ for unamended feces but not for 1:3 diluted urine with 18 mM NH₃ (Table 3). In general the egg inactivation in relation to NH₃

corresponds to findings by Ghiglietti et al. (7) and Pecson et al. (20), who also amended sewage sludge with ammonia (1,000 and 5,000 mg liter⁻¹ NH₃-N at pH 12), even though some discrepancies in inactivation rates were found, especially at high and low NH₃ concentrations.

Importance of temperature. Temperature is important for ammonia-based inactivation of *Ascaris* eggs, and in the present study an increase from 24 to 34°C reduced the inactivation time fivefold at equal NH₃ concentration and pH (U₁ at 34°C and U₂ at 24°C) (Table 3). Pecson et al. (20) reported a similar reduction in inactivation time with a temperature increase from 20°C to 30°C at 309 mM NH₃. In the ammonia-free temperature controls (0.9% NaCl) in the present study, viability was stable at both 24 and 34°C for the month studied (Fig. 1a and b). Other studies in which the effects of temperature were isolated from those of other factors support these findings and indicate a threshold value for thermal inactivation of between 30 and 40°C (2, 21). At 14°C and 4°C, the inactivation in the present study was in general low during the 6-month study period, even with NH₃ concentrations of up to 94 mM, and was insignificant for the 1:3 urine dilutions. The temperature-dependent inactivation by NH₃ may be explained by increased permeability of the shell lipid layer with increased temperature (30). The lipid layer is impermeable to most substances except gases and lipid solvents, e.g., NH₃.

pH and egg inactivation. Studies were also carried out to monitor inactivation of uterus-derived *Ascaris suum* eggs at high pH (>13) at 24°C in unhydrolyzed urine, i.e., where the nitrogen was still present as urea (data not shown). During a 1-week study, no significant inactivation was detected, indicating high tolerance to extreme pH. The inactivation of *Ascaris* eggs during less than 4 days of treatment at pH 12.8 (A and AU₁ at 34°C) is therefore not solely attributable to the pH. The high resistance to alkali can partly be explained by the fact that charged molecules such as OH⁻ require active transport over membranes, resulting in a higher resistance to pH alone.

In studies of alkaline treatment of sewage sludge to pHs of ≥12 (DM, ~6 or 20% [wt/wt]) with low NH₃ concentrations, 14 to 15 mM (21), or not reported (5), *A. suum* inactivation rates lower than the present rates in ash treatments were reported, confirming the persistence of the *Ascaris* eggs with high pH alone.

Ammonia and ash as chemical disinfectants. The results of ammonia measurements indicate some losses in the fecal treatments during the study. For the urine, losses during the 6-month study were recorded only when samples were stored at 34°C. The small losses in the urine indicate that repeated sampling, which required more time with open containers for the fecal material, together with a high surface-to-volume ratio and alkaline pH, was a major cause of the losses from fecal material.

Treatments with ash at 34°C (A and AU₁) resulted in similar ammonia recovery, pH, and inactivation rate (Table 2). The probable reason was the high pH (12.8) inhibiting the enzymatic hydrolysis of urea (12). At 24°C the ammonia recovery in AU₁ showed that urea was at least partly degraded, indicating a breakpoint above pH 10 where no hydrolysis can occur. Therefore fecal material collected with ash amendments resulting in a pH well above 10 might not be suitable for further treatment with urea. A higher egg inactivation effect from ash

in this study than in other studies (16) is also a factor resulting from the lower DM content combined with mixing.

The lower inactivation rate in unamended feces than in urine with the same concentration of total ammonia was due to a lower pH and therefore a lower concentration of NH₃. Amending feces with 1% (wt/wt) and 2% (wt/wt) urea resulted in threefold- and fivefold-higher total ammonia concentrations, respectively, and in more than a fourfold-shorter inactivation time compared with unamended feces. As NH₃ is the inactivation agent, losses have to be prevented during the treatment. This is easily achieved by enclosed treatment in containers, barrels, or tanks, depending on the volume treated. The advantage of enclosed treatment is preservation of ammonia in the material, both enhancing pathogen inactivation and increasing the fertilizer value.

Sanitation goals and health-based targets. In Nigeria, the average excretion rate in infected children has been shown to be 13,000 ± 18,000 *Ascaris* eggs per gram of feces (10). To safeguard human health when excreta-based fertilizers are used for crop production, a sufficient pathogen inactivation needs to be achieved, and a health-based target of a 6-log₁₀ reduction in pathogen concentration has been set by the WHO (31) to ensure safe reuse of fecal matter. A 6-log₁₀ inactivation of *Ascaris* eggs is a high target inactivation and would most probably give a higher inactivation of many other enteric pathogens (9, 19, 27). *Ascaris* eggs in urine originate from fecal contamination, which has been estimated at 9.1 mg liter⁻¹ based on the content of fecal sterols (24), resulting in a 10⁵-fold dilution. The required reduction rate, and therefore the required time of treatment, for diverted urine would be shorter than that for feces.

Considering the 95% confidence interval, the present study indicates that closed fecal storage at 24°C would require approximately 1 year to ensure a 6-log₁₀ inactivation of *Ascaris* eggs, which is consistent with current guidelines (31). At 34°C closed fecal storage was estimated to give a 6-log₁₀ inactivation within 3 months and with urea or ash amendment to pHs of >12 (i.e., 70 mM NH₃), treatment times could be reduced to 1 month. The lowest temperature at which fecal treatment was investigated was 24°C, but results from the urine at 4 and 14°C indicate that long duration in combination with intense treatment factors might be necessary to inactivate *Ascaris* eggs at these temperatures.

Ascaris eggs have been used as a conservative indicator of the sanitization level in urine, and the current results support the proposed guidelines (31) that a storage temperature of above 20°C is needed for unrestricted use of urine as a fertilizer. At 24°C at least a 3-log₁₀ inactivation can be achieved by 6 months of storage with urine diluted not more than 1:1 (66 mM NH₃). At 34°C a 3-log₁₀ inactivation of *Ascaris* eggs can be achieved within 1.5 months of storage even with urine diluted 1:3 (40 mM NH₃). Less-diluted urine gives faster inactivation and a required storage time of less than 1 month. For inactivation at 14°C and below, the urine needs to be concentrated, but treatment times will still exceed 6 months. Urine must not be diluted more than 1:1 to achieve inactivation at temperatures of 24°C and below. These guidelines are based on the 95 percentile t₉₉, where inactivation by treatments in many cases was confirmed with a total inactivation in 75% of that time and where lag phases, when present, were overestimated. There-

fore, the treatments would result in assured *Ascaris* egg inactivation in relation to NH_3 concentration, which should preferably be monitored.

ACKNOWLEDGMENTS

This study was financed by the Swedish Research Council Formas, the Swedish International Development Agency Research Council SAREC, the program EcoSanRes, and the Swedish University for Agricultural Sciences.

We are grateful to Andrea Farias Asmus for valuable assistance in the laboratory.

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