Inactivation of *Ascaris* eggs in source-separated urine and faeces by ammonia at ambient temperatures

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**ABSTRACT**

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 faeces to which urea had been added, in order to evaluate ammonia-based sanitation for production of safe fertilisers from human excreta. Urine was used concentrated or diluted 1:1 and 1:3 with tap-water at 4, 14, 24 and 34°C. Faecal material, with and without ash, was treated with 1% or 2% (w/w) urea at 24 and 34°C. At 34°C eggs were inactivated in less than 10 days in urine and in amended faeces. At 24°C only faeces with 2% (w/w) urea or 1% (w/w) 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 NH\(_3\)). At temperatures of ≥24°C, NH\(_3\) proved to be an efficient sanitising agent in urine and faeces at concentrations ≥60 mM. Treating faecal material at 34°C can give...
a 6 log_{10} egg inactivation within one month, whereas at 24°C 6 months of treatment are 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.

Keywords: Ammonia; Ascaris; faeces; pathogen inactivation; urine

INTRODUCTION

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 high 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 (28). 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 spp. 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 faecal matter use high temperature, high pH, or both. It is suggested that ammonia, which is toxic in its uncharged form NH$_3$ (26), contributes to pathogen inactivation by alkaline stabilisation (1, 15). Alkaline pH affects the equilibrium between NH$_4^+$ and NH$_3$ in aqueous solution in favour of the formation of NH$_3$. The microbicidal properties of NH$_3$ 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, 29).
The ovicidal effects of NH$_3$ 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% (v/v) NH$_3$ (pH 11.9) has been performed on eggs both from *A. suum* and *A. lumbricoides*, with eggs of *A. lumbricoides* harvested from worm uteri compared with eggs extracted from infected faeces. That study revealed that the inactivation was equal for *Ascaris* spp. eggs, independent of species or extraction method (8).

Separating faecal matter at source would minimise the occurrence of *Ascaris* eggs in other wastewater fractions, such as sewage sludge and waste water. However, it would increase the need for potent sanitation methods for the faecal matter, since this would not be subjected to dilution. Today, the most common treatment of source-separated faecal matter is low-temperature composting, *i.e.* long-term storage with little or no increase above ambient temperature (28). For source-separated urine, long-term storage based on the action of NH$_3$ is the only large-scale sanitation technique practised (14). Few studies have been performed on *Ascaris* egg inactivation in human faeces (22, 29), 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 faeces and urine, at temperatures corresponding to a range of ambient conditions, and to evaluate treatment methods for ensuring safe nutrient recycling of human-derived fertilisers.

**MATERIALS AND METHODS**
Material. Faecal material was collected during 3 months from a urine-diverting dry toilet used by a single household. After collection, excess toilet paper was removed and the faecal material was stored at 4°C. Before use in experiments, the dry matter (DM) content was adjusted to 17% (w/w) using tap-water (31). The ash used for treatment of faecal 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 homogenised 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 L⁻¹ urine and left for 24 h at 37°C.

Adult *A. suum* worms were collected from the intestine of slaughterhouse pigs, and the eggs were harvested by dissection of the two posterior centimetres of worm uterus. After washing according to 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% (w/v)) at 4°C until use.

Experimental set-up. Inactivation of *A. suum* in faeces was studied in two sets of material: untreated faecal matter and faeces amended with ash, simulating surface coverage after defecation commonly performed with dry toilets. The ash was added at a rate of 0.1 L ash per 100 g faeces with 17% (w/w) DM, representing an intermediate dosage in terms of actual practice. In ash-amended faeces the dry matter content was adjusted again to 17% (w/w) to avoid effects from the change in dry matter. Additions of urea were then made to both types of faecal material according to wet weight. Faecal material controls with no urea added were studied for both faeces and ash-amended faeces (Table 1). After homogenisation, the treated faeces were distributed (200 g) into soft containers (Grade Packaging...
After insertion of *A. suum* eggs (5 bags each) the containers were sealed, minimising headspace, and the contents were mixed manually for approximately 1 minute each. As a temperature control, single *A. suum* bags were incubated in 0.9% (w/v) NaCl solution. All containers were incubated in the dark at 24°C or 34°C for 35 days and egg bags were sampled according to Table 1.

Inactivation of *A. suum* eggs in stored urine was studied in 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 hours and then divided into 500 mL plastic flasks (1:1 dilution), or 50 mL plastic centrifuge tubes (1:0 and 1:3 dilution), filled up to minimise headspace. Four (4) bags with *A. suum* eggs were inserted into each container except for the 1:1 dilution at 4ºC and 14ºC, which had five bags each. The sealed containers were incubated in the dark for six months and sampled according to Table 1.

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

For quantification of total ammonia (\(\text{NH}_{\text{tot}} = \text{NH}_3 + \text{NH}_4^+\)) in solute, 10 g of faeces or 1 mL of urine from each replicate were analysed spectrophotometrically on a Thermo Aquamate (Thermo Electron Ltd.; Cambridge, UK) using the indolphenol blue method (Merck; Whitehouse Station, NJ). Concentration of NH\(_3\) (aq) was calculated from the measured NH\(_{\text{tot}}\) concentration, pH and temperature using the dissociation constant, \(K_a=10^{-(2729.92/T+0.09018)}\), where T is the temperature in degrees Kelvin (4).

In faecal 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 faeces or 3 mL of urine were removed from each replicate. The faecal samples were diluted in de-ionised water (1:9) and the urine samples were measured undiluted, both at room temperature, using an Inolab 720 pH meter (WTW, Germany). The faecal 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 pH of the other two urine dilutions (1:0 and 1:3) was analysed 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., UK) on the latter data set in cases of lag phases, for the whole data set when no lag phase was observed, and time for 99% egg inactivation \( t_{99} \), mean value and the upper 95% confidence limit was derived from the regression functions.

RESULTS

Ammonia and pH. The measured total ammonia concentration in the faecal treatments was somewhat low (15-84%) compared with theoretical concentrations estimated from urea additions and intrinsic ammonia in faecal batches (214±4 and 247±9 mM NH\(_{tot}\)). Ammonia recovery in urine (1:1) after 182 days ranged from 92-105% 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 faecal material was 8.0-8.3. Addition of 1% (w/w) or 2% (w/w) urea increased the pH to just below and above 9, respectively, and during the one-month study the pH decreased by 0.2 units for both treatment concentrations. At 34°C, the addition of sieved ash (in treatments A and AU\(_1\)) increased the pH to 12.8, after which the pH decreased by 0.1 units by day 20.
The non-sieved ash used at 24°C gave a pH 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 8.7-9.1 considering all dilutions, with the somewhat lower pH observed in diluted urine, especially at temperatures ≥24°C (Table 2).

**Ascaris egg inactivation.** The eggs used in faecal treatment at 24 and 34°C originated from two batches with an initial viability of 62±0.1% and 80±4.5%, respectively. At 34°C no viable eggs were found from day 4 in faeces with ash plus 1% (w/w) urea (AU₁) or 2% (w/w) urea (U₂). With only ash (A) 2 viable eggs were found on day 4. This inactivation was confirmed on day 10, with >2000 eggs counted for each treatment. In faeces treated with 1% (w/w) urea (U₁) too, no viable eggs were found on day 10. After day 31 no viable eggs (of 1629 eggs) were detected in unamended faeces (U₀). In the 0.9% NaCl temperature control, only 7% of the eggs were inactivated by day 35. Unamended faeces (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 1 viable egg 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 faeces (U₀), 50±16% of the *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 440-1446 eggs) from day 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 4-24°C, the urine and faecal 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 \( t_{90} \) values of more than one year considering the upper 95% confidence interval (Table 3).

When the *Ascaris* eggs in faecal 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 lower extent. On day 4 at 24°C the two ash treatments (A and AU\(_1\)) had 38±6% covered eggs, while urea treatments and unamended faeces 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 modelling 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 too low sampling frequency. Similar lag phases have been observed by Ghigletti *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 time for total
inactivation was derived and approximated to represent time for a 99% inactivation ($t_{99}$) (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.

**NH$_3$ and egg inactivation.** At 24°C there was a linear correlation between NH$_3$ (aq) and inactivation time ($R^2 = 0.94$) except for the 1:3 urine dilution, which had no inactivation, and treatments with ash (A and AU$_1$), which had a faster inactivation in relation to NH$_3$ compared with other treatments. At 34°C it was not possible to relate the egg inactivation rates to NH$_3$ concentrations (71-440 mM), due to 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 NH$_3$ concentrations (1:3 diluted urine and unamended faeces), but these were not explained by the material (Table 3).

The results indicate that at NH$_3$ concentrations ~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 NH$_3$ concentration when studying *Ascaris* egg inactivation in both water and sludge amended with aqueous ammonia at 22°C. With an NH$_3$ concentration of 23 mM NH$_3$ (aq, pH 12.3) they observed no inactivation during 90 days, whereas with 46 mM NH$_3$ (aq, pH 12.5) no viable eggs were found at day 40. However in the present study, there was significant inactivation at 20 mM NH$_3$ for unamended faeces but not for 1:3 diluted urine with 18 mM NH$_3$ (Table 3). In general the egg inactivation in relation to NH$_3$ corresponds to findings by Ghiglietti *et al.* (7) and Pecson *et al.* (20), who also amended sewage sludge with ammonia (1000 and 5000 mg L$^{-1}$ NH$_3$–N at pH 12) even though some discrepancies in inactivation rates were found, especially at high and low NH$_3$ 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 inactivation time five-fold at equal
NH₃ concentration and pH (U₁ at 34°C and U₂ at 24°C) (Table 3). Pecson et al. (20) report 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-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 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 up to 94mM, 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 (27). 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 unhydrolysed urine, i.e. the nitrogen was still present as urea (data not shown). During a one-week study, no significant inactivation was detected, indicating high tolerance to extreme pH. The inactivation of Ascaris eggs during less than four days of treatment at pH 12.8 (A and AU₁ at 34°C) is thereby 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 pH ≥12 (DM ~6 or 20% (w/w)) with low NH₃ concentrations, 14-15 mM (21) or not reported (5), lower inactivation rates of A. suum were reported compared with the present rates in ash treatments, confirming the persistence of the Ascaris eggs to high pH alone.

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

Treatments with ash at 34°C (A and AU1) resulted in indifferent 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 faecal 
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 compared with other 
studies (16) is also a factor of the lower dry matter content combined with mixing.

The lower inactivation rate in unamended faeces compared with urine with the same concentration of 
total ammonia was due to lower pH and thereby lower concentration of NH3. Amending faeces with 1% 
(w/w) and 2% (w/w) urea resulted in three-fold and five-fold higher total ammonia concentration, 
respectively, and in more than four-fold shorter inactivation time compared with unamended faeces. 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 fertiliser value.

Sanitation goals and health-based targets. In Nigeria, the average excretion rate within infected 
children has been shown to be 13000±18000 Ascaris eggs per gram faeces (9). To safeguard human 
health when excreta-based fertilisers 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 guidelines (28) to ensure safe reuse of faecal 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 (10, 19, 30). *Ascaris* eggs in urine originate from faecal contamination, which has been estimated at 9.1 mg L\(^{-1}\) based on the content of faecal sterols (24), resulting in a 10\(^5\)-fold dilution. The required reduction rate, and thereby required time of treatment, for diverted urine would be shorter than for faeces.

Considering the 95% confidence interval, the present study indicates that closed faecal storage at 24°C would require approximately 1 year to ensure a 6 \(\log_{10}\) inactivation in *Ascaris* eggs, which is consistent with current guidelines (28). At 34°C closed faecal storage was estimated to give a 6 \(\log_{10}\) inactivation within 3 months and with urea or ash amendment to pH >12 (i.e. 70 mM NH\(_3\)) treatment times could be reduced to 1 month. The lowest temperature at which faecal 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 sanitisation level in urine and the current results support the proposed guidelines (28) that a storage temperature above 20°C is needed for unrestricted use of urine as a fertiliser. At 24°C at least a 3 \(\log_{10}\) inactivation can be achieved by 6 months of storage with urine diluted not more than 1:1 (66 mM NH\(_3\)). At 34°C a 3 \(\log_{10}\) inactivation in *Ascaris* eggs can be achieved within 1.5 months of storage even with urine diluted 1:3 (40mM NH\(_3\)). 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 \(t_{99}\), where inactivation by treatments in many cases was confirmed with a total inactivation in 75% of that time and lag phases, when present, were overestimated. Therefore the treatments would result in assured *Ascaris* egg inactivation in relation to NH\(_3\) concentration, which should preferably be monitored.

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REFERENCES


FIGURE 1. Inactivation of viable Ascaris suum eggs (% inactivation ± S.D.) as a function of time (days) in: (a) faeces at 34°C and (b) faeces at 24°C treated with 2% (w/w) urea (●), 1% (w/w) urea (○), 1% (w/w) urea+ash (■), ash (□) or unamended (▲); (c) urine stored at 24/34°C and (d) urine stored at 4/14°C, which was diluted 1:0 (■), 1:1 (▲) and 1:3 (●) with water. The lower temperature (4 and 24°C) in diagrams c and d is marked with unfilled symbols. Shaded symbols indicate no viable larva at sampling and broken lines represent viability of controls held in 0.09% NaCl.

TABLE 1. The experimental set-up for the excreta fractions showing the primary and secondary treatments represented by the different regimes. Letters in brackets for the treatment of faecal fraction indicate the presence of ash (A) and urea additions (U0-2). The deviation was at most ±2°C from the temperature set

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<th>Faeces</th>
<th>Urine</th>
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<td>Primary treatment</td>
<td>0.1 L ash 100 g\textsuperscript{-1} faeces</td>
<td>Dilution (1:1) Dilution (1:0) and (1:3) Storage</td>
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<tr>
<td>Secondary treatment</td>
<td>0% urea (A\textsubscript{0}) 0% urea (U\textsubscript{0})</td>
<td>1% urea (U\textsubscript{1}) 2% urea (U\textsubscript{2})</td>
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<tr>
<td>Temperature</td>
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<td>4, 14, 24 and 34°C</td>
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<tr>
<td>Volume</td>
<td>200 g</td>
<td>500 mL 50 mL</td>
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<td>2 1\textsuperscript{a}</td>
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<tr>
<td>Treatment time</td>
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<td>6 months\textsuperscript{c}</td>
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a) Set-up with independent samples over time

b) Sampled day 4, 9, 15 and 22 at 24°C and day 3, 9, 13, 19 and 31 at 34°C
c) At 4°C sampled day 7, 83, 150 and 190 (1:0); day 8, 19, 33, 92, 182 (1:1); and day 7, 38, 100 and 190 (1:0). At 14°C sampled day 7, 8, 150 and 190 (1:0); day 8, 15, 26, 92 and 182 (1:1); and day...
At 24°C sampled day 7, 10, 20 and 73 (1:0); day 5, 12, 22 and 40 (1:1) and day 7, 14, 38 and 73 (1:0). At 34°C sampled day 1, 2, 3 and 7 (1:0); day 2, 5, 8 and 13 (1:1) and day 1, 2, 7 and 10 (1:0).

### TABLE 2

The pH range from initial (max) to final (min) together with the total ammonia concentrations ($\text{NH}_{\text{tot}} = \text{NH}_3 + \text{NH}_4^+$) in faecal material at 24°C (day 35) and 34°C (day 3), and in urine at 4°C, 14°C, 24°C and 34°C on day 182 (dilution 1:1) and day 150 (dilutions 1:0 and 1:3). For faecal matter, recovery of ammonia (%) is measured ammonia compared with expected amount according to additions of urea, while for the urine the fraction is ammonia measured at day 182 (1:1) compared with day 1.

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<td>NH$_{\text{recovery}}$ (%)</td>
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<td>101±6</td>
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<tr>
<td>1:3</td>
<td>9.0</td>
<td>103±7</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$NH$_3$ concentrationat 14°C.
TABLE 3. Concentrations of NH$_3$ (aq) in solution calculated from max pH, temperature and total ammonia, expressed in mM±S.D and as percentage of total ammonia, together with time (days) for 99% inactivation ($t_{99}$) of viable A. suum eggs predicted by linear regression of data sets with significant inactivation given as mean and upper 95 percentile. Lag phase, when present, is given in brackets with the predicted time (days) for $t_{99}$.

<table>
<thead>
<tr>
<th>Material and treatment</th>
<th>Max pH</th>
<th>NH$_3$ (aq)</th>
<th>t$_{99}$</th>
<th>NH$_3$ (aq)</th>
<th>t$_{99}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>34°C</td>
<td>24°C</td>
<td>24°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(mM) (%)</td>
<td>mean (%)</td>
<td>(mM) (%)</td>
<td>mean (%)</td>
</tr>
<tr>
<td>Faeces</td>
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<td></td>
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<tr>
<td>U$_2$</td>
<td>9.0</td>
<td>440±40</td>
<td>51</td>
<td>3.8</td>
<td>6.4</td>
</tr>
<tr>
<td>U$_1$</td>
<td>8.9</td>
<td>250±4</td>
<td>46</td>
<td>4.1</td>
<td>5.9</td>
</tr>
<tr>
<td>AU$_1$</td>
<td>12.8 vs 10</td>
<td>72±6</td>
<td>100</td>
<td>3.8</td>
<td>6.4</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>12.8 vs 10.5</td>
<td>71±6</td>
<td>100</td>
<td>3.7</td>
<td>4.1</td>
</tr>
<tr>
<td>U$_0$</td>
<td>8.3</td>
<td>43±2</td>
<td>17</td>
<td>21 (8)</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td>34°C</td>
<td>24°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:0</td>
<td>9.0</td>
<td>236±8</td>
<td>54</td>
<td>3.4 (≥1)</td>
<td>4.0</td>
</tr>
<tr>
<td>1:1</td>
<td>8.9</td>
<td>100±8</td>
<td>46</td>
<td>6.3 (≥2)</td>
<td>7.3</td>
</tr>
<tr>
<td>1:3</td>
<td>8.7</td>
<td>40±31</td>
<td>44</td>
<td>8.5 (≥2)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14°C</td>
<td>4°C</td>
<td></td>
<td></td>
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<tr>
<td>1:0</td>
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<td>94±3</td>
<td>22</td>
<td>240$^a$</td>
<td>380</td>
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<td>39±10</td>
<td>17</td>
<td>1060$^a$</td>
<td>1250</td>
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<tr>
<td>1:3</td>
<td>9.1</td>
<td>20±6</td>
<td>20</td>
<td>b</td>
<td>14±1</td>
</tr>
</tbody>
</table>

a) $t_{99}$ not confirmed with zero viable larvae.

b) Regression coefficient not significantly (p>0.05) different from zero.
Inactivated ascaris eggs (%)

Treatment time (days)