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Survival of *Ascaris suum* and *Ascaridia galli* eggs in liquid manure at different ammonia concentrations and temperatures

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ABSTRACT

Eggs of *Ascaris suum* from pigs are highly resistant and commonly used as a conservative indicator of pathogen inactivation during slurry storage. Eggs of *Ascaridia galli*, the poultry ascarid, are also known to be highly resistant but the suitability as an indicator of pathogen inactivation has never been tested. Pig slurry has to be stored for several months to inactivate pathogens but chemical treatment of slurry may reduce this time. The suitability of *A. galli* as an indicator of slurry sanitation was tested by comparing the survival of eggs of *A. suum* and *A. galli* in pig slurry. In addition, the effect of urea treatment on inactivation of ascarid eggs in relation to storage time was also tested. Nylon bags with 10,000 eggs of either species were placed in 200 ml plastic bottles containing either urea-treated (2%) or untreated pig slurry for up to 120 days at 20 °C, 6 days at 30 °C, 36 h at 40 °C or 2 h at 50 °C. At all the temperatures in both slurry types, *A. galli* eggs were inactivated at a significantly faster rate ($P < 0.05$) compared to *A. suum* eggs. For each 10 °C raise in temperature from 20 °C, T_{50} (time needed to inactivate 50% of eggs) for both types of eggs was reduced markedly. At all temperatures, viability of eggs of both species was significantly higher ($P < 0.05$) in untreated slurry compared to urea-treated slurry except *A. galli* eggs at 20 °C where no significant difference was detected. In untreated slurry, the levels of pH (6.33–9.08) and ammonia (0.01–1.74 mM) were lower ($P < 0.0001$) compared to that of urea-treated slurry (pH: 8.33–9.28 and ammonia 1–13 mM). The study demonstrated that *A. galli* eggs are more sensitive to unfavourable conditions compared to *A. suum* eggs. The use of *A. galli* eggs as hygiene indicator may thus be suitable to assess inactivation of pathogens that are more sensitive than *A. galli* eggs. Addition of urea may markedly reduce the storage time of slurry needed to inactivate *A. suum* and *A. galli* eggs.

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1. Introduction

Eggs of ascarid helminths (nematodes) are known to be highly resistant and can withstand highly unfavourable environmental conditions (Barrett, 1976; Wharton, 1983). The pig ascarid *Ascaris suum* is ubiquitous and the eggs can be easily processed and enumerated in the laboratory. The viability of these eggs is therefore often used as an indicator for assessing whether animal manure and human waste have been sufficiently sanitized under mesophilic conditions (Holmqvist and Stenström, 2001). At temperatures >50 °C, *A. suum* eggs die off rapidly (Burden and Ginnivan,

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1978; Barnard et al., 1987) and may not be suitable to use as hygiene indicators compared to other indicators like bacteriophages. The eggs of the chicken ascarid, *Ascaridia galli* are also considered to be very resistant to adverse conditions. The infection is non-zoonotic and eggs are easily obtainable. *A. galli* could thus be of use in areas where *A. suum* eggs are not available. Though *A. galli* eggs have been used in one sanitation study (Tønner-Klank et al., 2007), their suitability as a hygiene indicator has not been fully investigated.

Intensive pig production results in large quantities of slurry and solid manure which are commonly used as soil fertilizers after a period of storage. The manure may contain a wide variety of pathogens including those of zoonotic potential (Bradford et al., 2013). Application of stored slurry and solid manure to agricultural and horticultural crops can therefore lead to contamination of the produce as well as water sources through surface run-off (Mawdsley et al., 1995). Human consumption of contaminated produce, e.g. berries, lettuce and other crops consumed raw, may subsequently result in disease (Hanning et al., 2009). Similarly, poultry manure may also contain pathogens like viruses, bacteria and parasites (Terzich et al., 2000) and need to be treated before being used as a fertilizer.

On an average, storage capacity of slurry storage tanks in Europe is sufficient for 6 months (Menzi, 2002) indicating the maximum storage time. During storage of farm waste the pathogens may be inactivated due to exposure to unfavourable conditions, e.g. high temperatures due to composting processes (Grewal et al., 2006). Inactivation time during storage also depends upon factors like pH, aeration, ammonia, carbonate ions and volatile fatty acids (VFA) concentrations, dry matter content and pathogen type, and different studies have reported different storage times (Ghiglietti et al., 1997; Gantzer et al., 2001; Brewster et al., 2003; Nordin et al., 2009; Katakam et al., 2013) for inactivation of *A. suum* eggs.

Pig production and the associated application of slurry on fields may cause leakage of nitrogen and eutrophication of recipient water bodies. The European Commission (IPPC Directive 96/61/EC) recommends therefore that the best available practices (e.g. low protein diets, housing design) should be used to reduce such environmental pollution. It has been attempted to reduce the ammonia emissions from slurry by modifying dietary composition e.g. by adding fermentable carbohydrates (Canh et al., 1998b). However, slurry with reduced ammonia levels may require longer storage time to ensure effective inactivation of pathogens and this may be undesirable for economic and logistic reasons. Faster reduction of pathogens in sludge, slurry or faeces and thus more efficient sanitation may be achieved at higher temperatures, pH or ammonia concentrations (Eriksen et al., 1996; Pecson et al., 2007; Nordin et al., 2009; Bolton et al., 2013), and knowledge on how to manipulate these parameters may help reduce effective storage time.

The primary source of ammonia in slurry is rapid degradation of urea to ammonium and carbonate ions by urease secreted by faecal bacteria (Mobley and Hausinger, 1989). A minor source of ammonia is slow degradation of undigested proteins in the slurry during storage (Zeeman, 1991). In the aqueous phase, ammonium (NH_4^+) and ammonia (NH_3) are in an equilibrium which is influenced by temperature and pH. Though high temperatures shift the equilibrium towards NH_3, pH has a much stronger influence on equilibrium; at pH below 7 the total ammonia nitrogen (TAN) is mostly in NH_4^+ form and above 11 it is mostly in NH_3 form (Philippe et al., 2011). Though the exact mechanism of action is not known, ammonia (in NH_3 form) has been shown to have toxic effect on many microbes (Ghiglietti et al., 1997; Pecson et al., 2007; Bolton et al., 2013).

The objective of the present study was to compare the survival of eggs of ascarids (*A. suum* and *A. galli*) in pig slurry at different levels of ammonia and temperatures and to investigate how egg survival can be reduced by adding urea.

2. Materials and methods

2.1. Experimental design

A. suum and *A. galli* eggs were incubated in triplicate in water (control), urea-treated or untreated pig slurry at 20 °C, 30 °C, 40 °C and 50 °C under laboratory conditions. Samples stored at 20 °C, 30 °C and 40 °C were kept in incubators whereas those stored at 50 °C were kept in a water bath. pH, aqueous ammonia ($NH_{3(aq)}$), ascarid egg development and viability were monitored at regular time intervals depending on incubation temperature and expected inactivation time (Table 1).

2.2. Ascarid eggs

Faeces were collected from the large intestines of pigs at a Danish slaughter house and fresh chicken faeces were collected from the floor at a Danish poultry farm. Both *A. suum* eggs and *A. galli* eggs were isolated from the faeces by sequential sieving and washing through a series of sieves with mesh sizes 500 μm, 212 μm, and 90 μm followed by collection on a 36 μm sieve. Eggs were isolated from the material retained on the 36 μm mesh size sieve by flotation as described by Larsen and Roepstorff (1999). Nylon bags (size 2.5 × 2.5 cm^2) with a mesh size of 20 μm (Sefar AG, Heiden, Switzerland) were prepared each containing 10,000 fresh un-embryonated *A. suum* or *A. galli* eggs and were sealed with a glue gun.

2.3. Pig slurry

Fresh pig slurry was collected from an organic pig farm, where pigs were mainly fed cereals (barley), and transported to the laboratory within 4 h and stored at 5 °C for 5 days before setting up the experiment. Before the start of experiment the slurry was mixed thoroughly for 2 min and divided into two portions. Urea (Urea Technical grade, Applichem GmbH) was added to one of the portions to ensure a final urea concentration of 2% w/w. Approximately 150 g of slurry with or without urea was transferred to 200 ml plastic bottles. Two nylon bags containing either *A. suum* or *A. galli* eggs were then submerged in the slurry of each bottle. The bottle was only partially closed with a lid,

Table 1
Experimental design.

Temperature (°C)	Storage time units	Storage time	
		Slurry with urea	Slurry without urea
20	days	30,60,75,90,105,120,150	30,60,75,90,105,120,135,150
30	days	1,2,3,4,5,6	2,4,6,12,18, 24
40	hours	2,4,6,8,10,12,36	2,6,12,18,24,30,36
50	minutes	20,40,60,80,100,120	20,40,60,80,100,120

allowing some ventilation as the bottle would else burst due to generation of gases from the slurry during storage.

2.4. Egg development and viability

At each sampling, the two nylon bags were recovered from a given bottle and rinsed thoroughly with tap water. Each bag was gently pulled apart and the eggs washed off into separate tubes and washed three times with 0.1 M H_2SO_4 buffer by centrifugation at 253 × *g* for 7 min and discarding the supernatant. After the final wash, the supernatant was discarded and H_2SO_4 was added to a total volume of 5 ml. The eggs were then embryonated at 22 °C for six weeks (Oksanen et al., 1990). Eggs were aerated every week by opening the lids for 2–3 min. A minimum of 100 eggs were examined microscopically before and after embryonation to determine the developmental stage of the eggs. After embryonation, eggs containing a fully developed larva were considered to be viable whereas all other stages of development were considered to be non-viable. Initial viability of eggs was measured by embryonating eggs from 10 nylon bags for each species in H_2SO_4 buffer (pH 1) for 6 weeks at 22 °C at the start of the experiment.

2.5. Measurements of pH and aqueous ammonia ($NH_{3(aq)}$)

The pH was measured in all slurry samples after mixing with de-ionized water at a ratio of 1:5 (w:w) (Jørgensen and Jensen, 2009). Slurry samples had initially been stored at −20 °C. Total ammonia nitrogen (TAN) was then later extracted from 5 g of thawed and homogenized slurry suspended in 1 M KCl to a total volume of 100 ml and subjected to end-over-end shaking for 45 min. The extracts were then filtered through filter papers (Advantec TM No. 5A, Advantec MFS, Inc., Dublin, CA, USA) and once more frozen at 20 °C. TAN was later measured in the thawed extracts by a flow injector analyser system (Lachat Instruments Division, Milwaukee, WI, USA). $NH_{3(aq)}$ concentrations were calculated using the TAN, pH and dissociation constant (p*K*a) values as described by Armstrong et al. (1978).

2.6. Statistical analysis

For each temperature, the effect of slurry type (+/− urea), time and their interaction on pH and $NH_{3(aq)}$ concentration was analysed using a linear model with slurry type as a categorical variable and time as a continuous variable (Proc GLM, SAS 9.2, SAS institute, Inc; Cary, NC). The percentage of viable eggs in slurry and water controls stored at each temperature were compared by fitting the following Boltzmann sigmoidal function equation using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). This model was selected as it has fixed top and bottom constants and a variable slope and the time needed to inactivate 50% of the eggs (T_{50}) can be calculated to compare the egg viability curves.

$$Y_T = b + \frac{a - b}{1 + \exp\left((T_{50} - t)/\text{slope}\right)}$$

where Y_t = percent viable eggs time t, a = initial viability percent (top constant), b = zero viability percent (bottom constant), T_{50} = time needed for reduction in 50% viability and t = time.

Two estimates of T_{50} were considered significantly different ($P < 0.05$) if their respective 95% confidence intervals did not overlap. This is a cautious approach, as (smaller) overlaps of confidence intervals can be found even if two estimates are significantly different (Knezevic, 2008).

3. Results

3.1. pH and aqueous ammonia ($NH_{3(aq)}$)

The pH of untreated slurry was lower compared to that of urea-treated slurry at all temperatures ($P < 0.0001$) (Fig. 1). The pH of both slurry types at 20 °C and 30 °C was slightly lower than that at 40 °C and 50 °C. The pH of untreated slurry stored at 20 °C, 30 °C and 40 °C steadily increased with storage time ($P < 0.001$) whereas at 50 °C, it did not change over time. pH of urea-treated slurry did not show any significant change over time at any of the temperatures.

The initial $NH_{3(aq)}$ concentrations in the urea-treated slurry were higher at higher temperatures (from 5.8 mM to 13 mM), and levels decreased at varying degrees over time at 30–50 °C ($P = 0.03$) but remained fairly stable at 20 °C (Fig. 2). In untreated slurry, the $NH_{3(aq)}$ concentration remained very low (below 0.1 mM) throughout the study period at 30–50 °C and slightly increased over time at 20 °C ($P = 0.03$). The $NH_{3(aq)}$ concentration in untreated slurry thus remained much lower than in the urea-treated slurry at all temperatures.

3.2. Egg development and viability

None of the *A. suum* and *A. galli* eggs showed any sign of development during incubation in the slurry irrespective of type and temperature. None of the control eggs in water developed at 40 °C and 50 °C whereas control eggs kept at 30 °C showed different stages of development

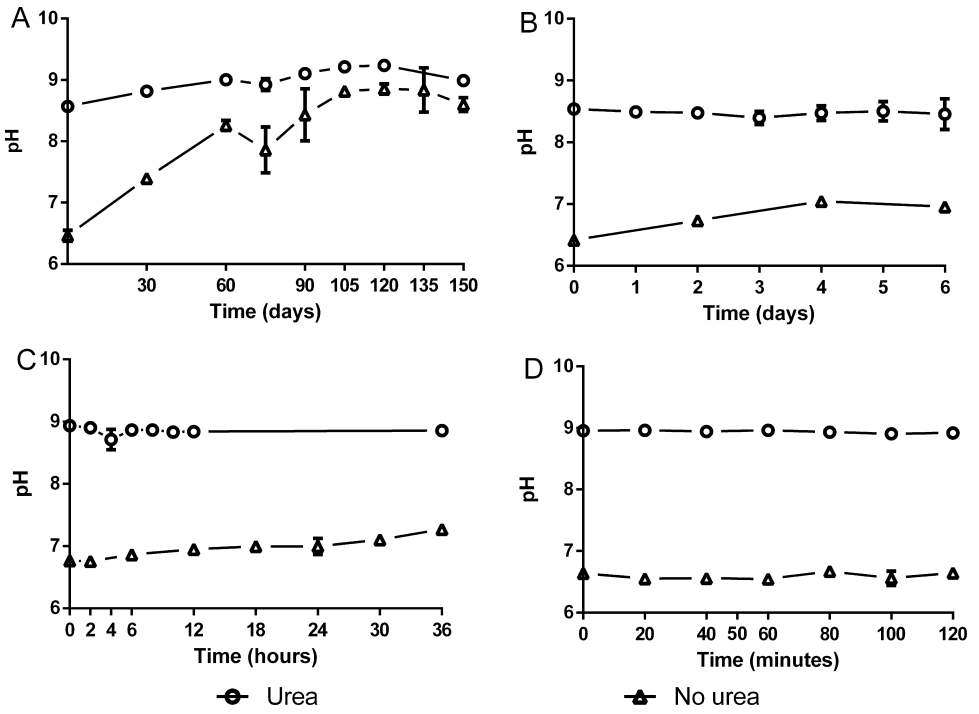

Fig. 1. Changes in mean (±SD) pH values during storage of urea-treated and untreated pig slurry at 20 °C (A), 30 °C (B), 40 °C (C) and 50 °C (D). All measurements were means of three replicates. Please note that horizontal axes have different scales.

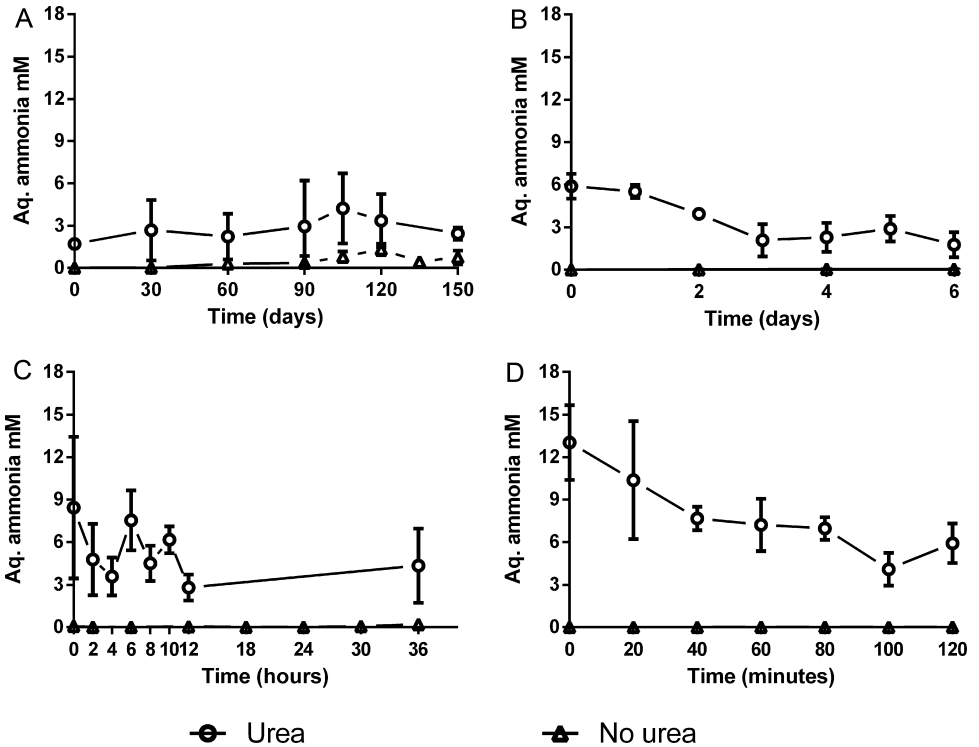

Fig. 2. Changes in mean (±SD) aqueous ammonia ($NH_{3(aq)}$) concentrations during storage of urea-treated and untreated pig slurry at 20 °C (A), 30 °C (B), 40 °C (C) and 50 °C (D). All measurements were means of three replicates. Please note that horizontal axes have different scales.

at days 1 to 6. Fully developed control eggs were observed at 20 °C after day 30.

Temporal changes in viability of *A. suum* and *A. galli* eggs at the different temperatures are shown in Fig. 3. Mean initial viability of *A. suum* and *A. galli* eggs was estimated to be 98% and 91%, respectively. At all temperatures, viability of both egg species was significantly higher ($P < 0.05$) in untreated slurry compared to urea-treated slurry except for *A. galli* eggs stored at 20 °C for which no significant difference was detected. At 40 °C, there was no decline in viability of *A. suum* eggs in untreated slurry within the study period of 36 h and hence T_{50} could not be calculated (Table 2). The viability of *A. suum* control eggs kept at 20 °C, 30 °C and 40 °C remained consistently high throughout the study period whereas those stored at 50 °C showed decreased viability after 60 min. The viability of *A. galli* control eggs kept at 20 °C and 30 °C was also constantly high throughout the study period, but *A. galli* eggs kept at 40 °C and 50 °C showed decreased viability before or at a rate similar to those in untreated slurry (Fig. 3).

In urea-treated slurry kept at 20 °C, total inactivation of *A. suum* and *A. galli* eggs occurred after 90 days and 105 days, respectively. At this time 95% and 6% of *A. suum* and *A. galli* eggs were still viable in untreated slurry (Fig. 3). At 30 °C, after day 6, the viability of *A. suum* and *A. galli* eggs in urea-treated slurry was 76% and 5%, respectively, whereas it was 91% and 19% for *A. suum* and *A. galli* eggs in untreated slurry. At 40 °C, after 36 h, all *A. suum* and *A. galli* eggs were dead while 96% of *A. suum* eggs and 6% of *A. galli* eggs were still viable in untreated slurry. At 50 °C, 1% and 0% of *A. suum* and *A. galli* eggs were viable, respectively, after 120 min, a time when 43% and 1% of *A. suum* and *A. galli* eggs, respectively, were viable in untreated slurry (Fig. 3).

Overall in the present study, higher $NH_{3(aq)}$ concentrations were associated with lower viability of eggs. For both types of eggs and in both slurry types, the estimated T_{50} decreased drastically as the temperature increased from 20 °C to 50 °C. Estimates of T_{50} were lower in urea-treated slurry compared to that for untreated slurry with the exception of *A. galli* eggs at 20 °C. The estimates of T_{50} for *A. suum* were higher compared to that of *A. galli* eggs (Table 2).

4. Discussion

The present study compared the survival of *A. galli* eggs with those from *A. suum* with the aim to assess whether the former could be used as an alternative to *A. suum* as a conservative hygiene indicator for slurry. The results revealed that *A. galli* eggs are less suitable as indicators for the hygienic quality of treated pig slurry as they died faster than *A. suum* eggs.

The difference in inactivation times between two species might be due to the structural differences of the egg shells. The *A. suum* shell contains four layers, namely an outer proteinaceous layer (uterine-derived), a vitelline layer, a chitinous layer and an innermost highly impermeable lipid layer (ascaroside layer) (Wharton, 1983). The *A. galli* shell contains only three layers, namely an outer proteinaceous layer, a middle chitinous layer and an inner lipoid layer (Ackert, 1931; Christenson et al., 1942). The presence of an extra layer may make *A. suum* eggs more heat tolerant and resistant to other unfavourable conditions (e.g. high $NH_{3(aq)}$ levels) compared to *A. galli* eggs. However, it remains to be further investigated exactly how this as well as the other layers protects against environmental stress.

Survival of specific microbial indicators is generally used to estimate the effectiveness of a treatment method to inactivate pathogens in slurry, sewage sludge or other types of organic wastes. Helminth eggs are well-known for their high resistance to environmental stress, e.g. they may survive for years in agricultural soil (Kransnonos, 1978). Several studies have evaluated different viral, bacterial and parasitic species as hygienic indicators (Placha et al., 2001; Ottoson et al., 2008; Bøtner and Belsham, 2012; Katakam et al., 2013) depending upon purpose of the study. To consider an organism as a good hygiene indicator, isolation and quantification methods should be simple, reliable, definitive, cost-effective and the indicator should show higher survival rates as compared to other pathogens (Böhm et al., 1999). Bacterial, e.g. *Escherichia coli* and enterococci, and phages, e.g. *E. coli* phage, are used as indicators of bacterial and viral pathogens, respectively, but these indicators show a much faster die-off than parasites, in particular helminth parasites. Enterococci may under certain conditions even show regrowth (Christensen et al., 2002). Thus, helminth eggs are excellent hygienic indicators as their slower die-off ensures that both bacterial and viral pathogens are fully inactivated.

The initial pH (6.46) of the slurry used in the present study was relatively low compared to most previous studies citing pH values of fresh pig slurry from 6.31 to 8.65 (Kirchmann and Witter, 1992; Canh et al., 1998a; Sutton et al., 1999; Le et al., 2009; Katakam et al., 2013). The pH value of fresh slurry may depend upon the feed ingredients, and high protein diets generally result in higher pH and ammonia levels (Canh et al., 1998a; Le et al., 2007) whereas high fermentable carbohydrate diets result in high levels of volatile fatty acids (VFA), thereby reducing the pH (Paul and Beauchamp, 1989; Canh et al., 1998b). In the present study, slurry was obtained from a farm where the pigs were mainly fed on cereals (about 70%) which might have resulted in low pH levels. In the present study, addition of urea to the fresh slurry raised the pH by 2 units, which may have been due to enzymatic conversion of the added urea to ammonium (NH_4^+) by urease secreted by faecal bacteria present in the slurry (Muck and Steenhuis, 1981).

Variation of pH levels is likely a main factor behind the observed differences in $NH_{3(aq)}$ levels between the two slurry types as at pH below 7, TAN will be mostly in NH_4^+ form whereas above pH 7, increase in pH dramatically increases the conversion NH_4^+ to NH_3 (Philippe et al., 2011). The increase in $NH_{3(aq)}$ levels with increase in storage temperature in urea-treated slurry might thus be due to a temperature mediated shift in the equilibrium between NH_4^+ and NH_3 towards NH_3 (Philippe et al., 2011). The decrease in level of $NH_{3(aq)}$ over time at 30–50 °C might be due to volatilization of NH_3 due to partial closure of bottles. The slight increase in $NH_{3(aq)}$ levels over time in untreated

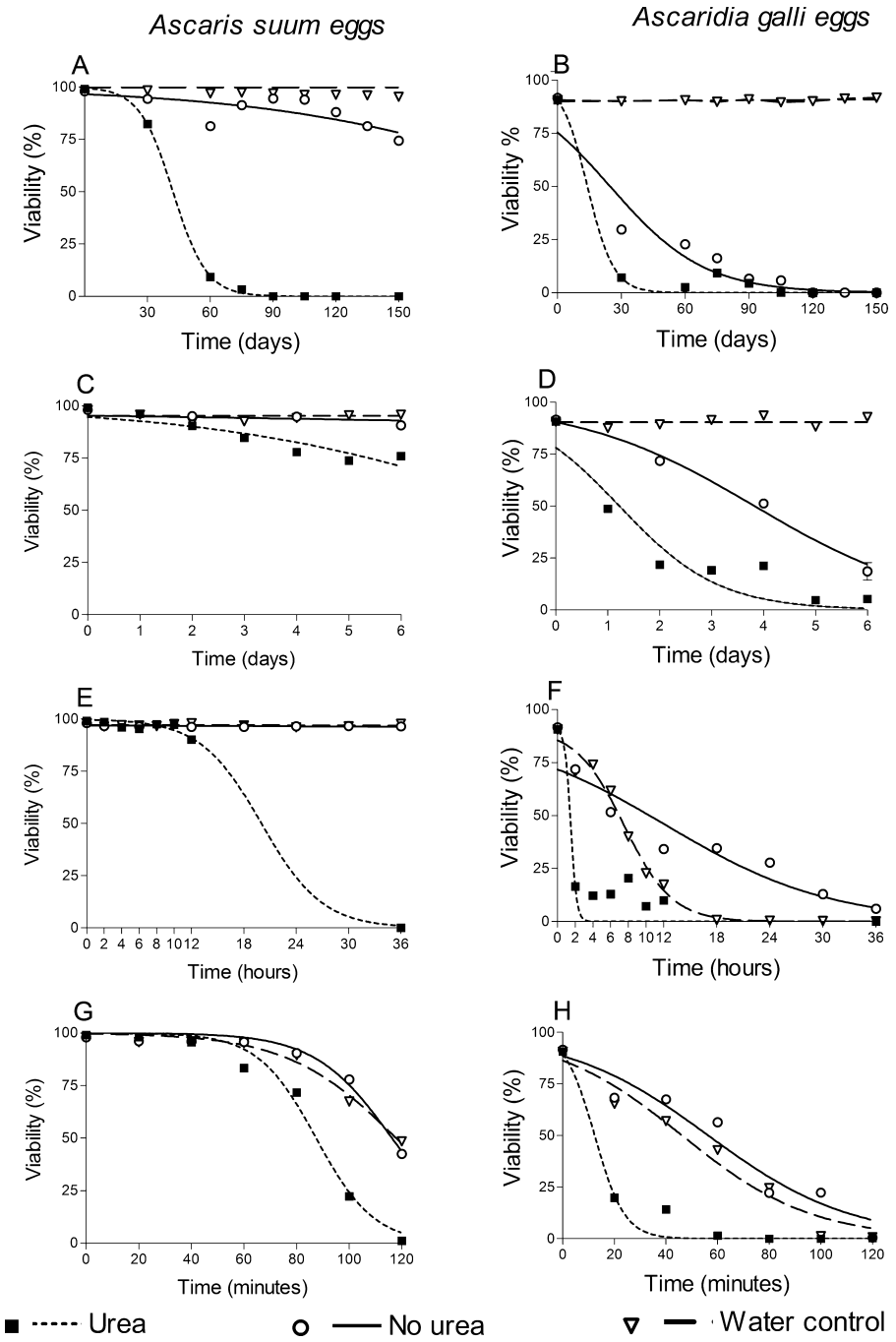

Fig. 3. Mean viability of *Ascaris suum* and *Ascaridia galli* eggs in pig slurry with and without urea and in water controls stored at 20 °C (A and B), 30 °C (C and D), 40 °C (E and F) and 50 °C (G and H) respectively. All measurements were means of three replicates. Please note that horizontal axes have different scales.

slurry at 20 °C might be due to slow degradation of organic nitrogen during storage (Zeeman, 1991).

The present results confirm earlier studies that increased temperature and $NH_{3(aq)}$ concentration may increase the inactivation of helminth eggs (Pecson et al., 2007; Nordin et al., 2009). At 50 °C, both *A. galli* and *A. suum* eggs were inactivated at similar rates in untreated slurry and water, but at a slower rate compared to urea-treated slurry. This indicates that temperature was the main contributing factor in egg mortality for the two former treatments, whereas addition of urea helped increase $NH_{3(aq)}$ concentrations and thereby further increased the mortality rate. The *A. galli* eggs in water controls at 40 °C and 50 °C were inactivated at a faster rate compared to

Table 2
Estimated T_{50} in days with 95% lower and upper confidence intervals of *Ascaris suum* and *Ascaridia galli* eggs at different temperatures in pig slurry with and without urea.

Temperature (°C)	Urea		No urea	
	A. suum	*A. galli*	*A. suum*	*A. galli*
20	42.3 (40.0–44.5)	14.1 (9.5–18.7)	242.6 (167.6–317.6)	24.8(16.7–32.9)
30	21.8 (17.2–26.4)	1.22 (0.86–1.58)	41.6 (29.4–53.8)	3.81 (3.61–4.01)
40	0.83 (0.67–0.99)	0.047(0.028–0.066)	–	0.42 (0.32–0.51)
50	0.06 (0.05–0.06)	0.009(0.007–0.010)	0.08 (0.07–0.08)	0.039(0.03–0.04)

the eggs in untreated slurry. It is possible that the organic matter in the slurry protected the eggs as speculated by Popat et al. (2010) who studied the thermal inactivation of *A. suum* eggs at 51–55 °C and concluded that anionic detergents and amino acids in sludge may provide substantial protection against thermal inactivation *A. suum* eggs.

At ≤40 °C and ≤30 °C *A. suum* and *A. galli* eggs, respectively, temperature alone did not affect survival of eggs in water. At each temperature, egg mortality increased with increased storage in both urea-treated and untreated slurry. Permeability of the egg shell of another *Ascaris* sp. (*A. lumbricoides*) increases markedly at temperatures above 44 °C (Barrett, 1976) and the observed faster inactivation of eggs at 50 °C might be due to increased permeability of eggs to $NH_{3(aq)}$. Slower inactivation at lower temperatures might be due to prolonged time needed to increase the permeability of the egg shell to $NH_{3(aq)}$ in combination with lower development of ammonia at lower temperatures. Several other studies have also showed the toxic effect of ammonia (in NH_3 form) on many other microbes (Ghiglietti et al., 1997; Pecson et al., 2007; Vinnerås, 2007; Ottoson et al., 2008; Nordin et al., 2009; Bolton et al., 2013) and the mechanism of inactivation of microbes by ammonia might be due to alteration of membrane potential, increase in cytoplasmic pH and/or loss of potassium ions (Kadam and Boone, 1996; Bujoczek, 2001).

Though there was a large increase in $NH_{3(aq)}$ levels in urea-treated slurry, the levels were apparently too low to inactivate the eggs at 20 °C. In the absence of other inhibiting factors, at 20 °C and at a pH 12, Pecson et al. (2007) observed 99% inactivation of *A. suum* eggs within 87 days at around 58 mM $NH_{3(aq)}$ and within 25 days at 294 mM. In the present study, at 20 °C, all the eggs were inactivated in 90 days though the $NH_{3(aq)}$ concentration ranged from 2 to 4 mM in urea-treated slurry. Inactivation of *A. suum* eggs even at seemingly very low levels of ammonia in the present study indicates that, along with ammonia, other factors not measured in the study may also have been responsible. They might be carbonate ions or volatile fatty acids (VFA) which are also known for pathogen inactivation. Diez-Gonzalez et al. (2000) and Park and Diez-Gonzalez (2003) reported the effect of carbonate ions in inactivation of food borne pathogens like *E. coli* and *Salmonella typhimurium* in cattle manure. Similarly Kunte et al. (2000, 2004) reported the effect of VFA inactivation of some enteric bacteria and viruses. Little is known about effect of carbonate ions and VFA on helminth eggs and further studies are needed.

The effect of urea-addition could have practical implications at normal storage temperatures. At 20 °C prolonged survival of *A. suum* was observed for the untreated slurry but inactivation within days was achieved with urea-addition. Animal slurry should be stored for a certain period before it is spread on the fields (Burton and Turner, 2003). In Europe, pig slurry is generally stored for around 6 months (Burton and Turner, 2003), but several studies have reported that *A. suum* eggs may survive in pig slurry beyond 6 months (Gaasenbeek and Borgsteede, 1998; Papajova et al., 2005; Katakam et al., 2013). This is confirmed by the present study which also demonstrated that addition of urea can alter the physico-chemical balance in the slurry so that all *A. galli* and *A. suum* eggs can be inactivated to maximum extent by day 60 and day 90, respectively, when stored at 20 °C.

In conclusion, the present study has demonstrated that *A. galli* eggs are more sensitive to unfavourable conditions compared to *A. suum* eggs, and hence their use as hygiene indicators may be limited to assess inactivation of other susceptible pathogens. Addition of 2% urea may markedly reduce (by 17–76% for *A. suum* eggs and 11–57% for *A. galli* eggs depending upon storage temperature) the storage time of slurry needed to inactivate *A. suum* and *A. galli* eggs.

Addition of urea may markedly reduce the storage time of slurry needed to inactivate *A. suum* and *A. galli* eggs.

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