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ORIGINAL ARTICLE

Survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in manure and manure-amended soil under tropical climatic conditions in Sub-Saharan Africa

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Abstract

Aims: To establish the fate of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in manure and manure-amended agricultural soils under tropical conditions in Sub-Saharan Africa.

Methods and Results: Survival of nonvirulent *E. coli* O157:H7 and *Salm.* Typhimurium at 4 and 7 log CFU g^{-1} in manure and manure-amended soil maintained at ≥80% r.h. or exposed to exclusive field or screen house conditions was determined in the Central Agro-Ecological Zone of Uganda. Maintaining the matrices at high moisture level promoted the persistence of high-density inocula and enhanced the decline of low-density inocula in the screen house, but moisture condition did not affect survival in the field. The large majority of the survival kinetics displayed complex patterns corresponding to the Double Weibull model. The two enteric bacteria survived longer in manure-amended soil than in manure. The 7 log CFU g^{-1} *E. coli* O157:H7 and *Salm.* Typhimurium survived for 49–84 and 63–98 days, while at 4 log CFU g^{-1}, persistence was 21–28 and 35–42 days, respectively.

Conclusions: Under tropical conditions, *E. coli* O157:H7 and *Salm.* Typhimurium persisted for 4 and 6 weeks at low inoculum density and for 12 and 14 weeks at high inoculum density, respectively.

Significance and Impact of the Study: Persistence in the tropics was (i) mostly shorter than previously observed in temperate regions thus suggesting that biophysical conditions in the tropics might be more detrimental to enteric bacteria than in temperate environments; (ii) inconsistent with published data isothermally determined previously hence indicating the irrelevance of single point isothermal data to estimate survival under dynamic temperature conditions.

Introduction

Integrated livestock–vegetable production is a typical peri-urban and small-holder system of agriculture in Sub-Saharan Africa. This production system offers opportunity for farmers to improve crop yields and maintain soil fertility through application of manure generated from livestock. Animal manure when used appropriately is a cheaper means of maintaining soil quality and fertility compared to commercial fertilizers. Farmers in Sub-Saharan African countries often apply noncomposted manure to soils to save time and labour costs associated with composting. However, the practice of incorporating noncomposted animal manure into agricultural soils can

compromise microbiological safety of fresh produce because animal manure is a well-recognized source of human enteric pathogens such as *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium (Pell 1997; Beuchat 1999; Brackett 1999; Jones 1999; Bach *et al.* 2002; Islam *et al.* 2004a; Hutchison *et al.* 2004).

Escherichia coli O157:H7 and *Salm*. Typhimurium are important food-borne pathogens that are known to be widely present in the gastrointestinal tract of ruminant animals (Wells *et al.* 1991; Zschöck *et al.* 2000; Wells *et al.* 2001; Laven *et al.* 2003) and are usually shed asymptomatically in faeces (Losinger *et al.* 1995; Faith *et al.* 1996; Omisakin *et al.* 2003; Cummings *et al.* 2009). Although enteric food-borne pathogenic bacteria such as *E. coli* O157:H7 and *Salm*. Typhimurium were thought to be primarily associated with animal products, an increasing association between fresh vegetables and food-borne infection outbreaks has become evident (Ackers *et al.* 1998; Tauxe *et al.* 1997; Hilborn *et al.* 1999; Beuchat 2002; Barak *et al.* 2005; Doyle and Erickson 2008). Concern about contamination of vegetables with faecal pathogenic bacteria in the agricultural environment, especially when noncomposted bovine manure is used to fertilize agricultural soils, has therefore been highlighted (Tauxe *et al.* 1997; Pell 1997). To reduce the risk of manure-borne pathogens contaminating vegetables grown in manure-fertilized soils, it has been suggested that appropriate time limits between the application of noncomposted manure and harvest of vegetables should be established (Natvig *et al.* 2002). In practice, this requires accurate information on the survival of the target pathogens in manure-amended soil under specific environmental conditions in which the vegetables are grown.

Several studies examined the survival and/or persistence of *Salm*. Typhimurium and *E. coli* O157:H7 in manure-amended soils and reported a variety of survival times (Natvig *et al.* 2002; Ingham *et al.* 2004; Islam *et al.* 2004a,b,c; Franz *et al.* 2005; Islam *et al.* 2005; Franz *et al.* 2007a). However, all these studies were performed in temperate climate or simulated temperate climatic conditions but there is no information on the survival of these two pathogens in manure-amended soils under tropical climatic conditions as found in Sub-Saharan Africa. Because soil quality, moisture conditions and temperature regime, factors which are important for survival of enteric bacteria (Mubiru *et al.* 2000; Garcia *et al.* 2010), are totally different in tropical countries compared to temperate countries, information that was generated in temperate climate cannot reliably be used to predict environmental survival of *E. coli* O157:H7 and *Salm*. Typhimurium in tropical climate. For instance, isothermal studies in the laboratory have shown that survival of enteric bacteria in manure and manure-amended soil decreases with increasing temperature (Kudva *et al.* 1998; Himathongkham *et al.* 1999; Semenov *et al.* 2007) thus indicating that *E. coli* O157:H7 and *Salm*. Typhimurium would decline faster under tropical conditions than in temperate conditions because of higher temperatures in the tropics. Moreover, it has been shown that the survival of *E. coli* O157:H7 and *Salm*. Typhimurium in bovine manure and in manure-amended soils is significantly affected by cattle diet (Franz *et al.* 2005). In Sub-Saharan Africa, cattle feeding regiment is basically forage based with almost no concentrate supplementation as opposed to livestock management practices in developed temperate countries. Considering the unique setting in terms of the occurrence of high moisture, higher temperatures, the degraded state of soils and low-quality cattle diet in tropical areas as opposed to temperate regions, it can be hypothesized that the survival of enteric bacteria in a tropical agricultural environment would be different from what have been determined in temperate regions. Therefore, it is necessary that the survival characteristics of *E. coli* O157:H7 and those of *Salm*. Typhimurium are determined under tropical climatic conditions. Furthermore, with a few exceptions, most studies on the survival of *E. coli* O157:H7 and *Salmonella* spp. in manure-amended soils have been conducted in controlled environmental conditions (Kudva *et al.* 1998; Bolton *et al.* 1999; Gagliardi and Karns 2002; Jiang *et al.* 2002; Natvig *et al.* 2002; Ritchie *et al.* 2003; Franz *et al.* 2005, 2007b, 2008; Semenov *et al.* 2008; Garcia *et al.* 2010). In practice, however, results obtained from climate-controlled conditions would be applicable in situations where vegetables are grown in climate-controlled environments, e.g. in green houses in temperate countries. However, in Sub-Saharan African countries, vegetable production takes place entirely in the field, exposed to fluctuating temperature and humidity conditions. Indeed, it is no doubt that freshly excreted manure has high moisture content, but in the field, manure or manure-amended soil matrix will be subjected to progressive desiccation over time during the dry season or subjected to a cycle of desiccation–rehydration during the rainy season depending on rainfall pattern. Up to date, the survival of *E. coli* O157:H7 and *Salm*. Typhimurium in manure and in manure-amended soils under these scenarios has not been investigated. Therefore, the objective of this study was to determine the survival of *E. coli* O157:H7 and *Salm*. Typhimurium in bovine manure and in manure-amended soil as affected by inoculum density and moisture under tropical field conditions, more specifically under the conditions encountered in the Central Agro-Ecological Zone (CAEZ) of Uganda (Wortman and Eledu 1999).

Materials and methods

Bacterial strains and inoculum preparation

Rifampicin-resistant derivatives of *Salmonella enterica* serovar Typhimurium LT2A (*Salm*. Typhimurium-Rifr) and *E. coli* O157:H7 ATCC 43888 (*E. coli* O157:H7-Rifr) obtained by spontaneous mutations (Ongeng *et al.* 2011) were used. *Salmonella* Typhimurium LT2A is a spontaneous virulence-attenuated variant of the virulent strain *Salm*. Typhimurium LT2V. Virulence attenuation in *Salm*. Typhimurium LT2A was shown to be due to a mutation in the *rpoS* gene (Swords *et al.* 1997). Wilmes-Riesenberg *et al.* (1997) showed that the mutated *rpoS* allele in *Salm*. Typhimurium LT2A did not affect survival of the organism in J774 cells and bone marrow-derived macrophages and that *Salm*. Typhimurium LT2A survived stationary phase and oxidative stresses as well as strains containing a wild-type *rpoS* allele. Moreover, *Salm*. Typhimurium LT2A endophytically colonized barley roots to the same degree as virulent strain *Salm*. Typhimurium DT104 (Kutter *et al.* 2006). *Escherichia coli* O157:H7 ATCC 43888 does not contain the *stx*1 and *stx*2 genes. Kudva *et al.* (1998) showed that the shiga toxin type 1 and 2 genes in *E. coli* O157:H7 did not influence bacterial survival in manure. To prepare inocula, *E. coli* O157:H7-Rifr and *Salm*. Typhimurium-Rifr were grown in 200 ml of LB broth containing 100 μg ml^{-1} rifampicin for 18 h at 37°C with agitation (150 rev min^{-1}). The cells were sedimented by centrifugation and washed three times in 0·9% saline and finally suspended in 0·9% saline. Cell density for each strain was adjusted with 0·9% saline to an optical density (OD_{650}) of 0·7 to give a population size of approx. 9 log CFU ml^{-1}. This was confirmed by plating 0·1 ml of an appropriate dilution on LB and on CT-SMAC (Cefixime Tellurite-Sorbitol MacConkey agar; Merck, Darmstadt, Germany) containing 100 μg ml^{-1} rifampicin, 50 μg ml^{-1} nystatin and 50 μg ml^{-1} cycloheximide (CT-SMAC-Rif100-Ny50-Cy50) for *E. coli* O157:H7-Rifr; XLT4 (Xylose Lysine Tergitol 4 agar; Merck) supplemented with 100 μg ml^{-1} rifampicin, 50 μg ml^{-1} nystatin and 50 μg ml^{-1} cycloheximide (XLT4-Rif100-Ny50-Cy50) for *Salm*. Typhimurium-Rifr.

Manure and soil

Fresh manure was obtained from grazing Nganda cattle at the Animal production unit of the National Crop Resources Research Institute, Namulonge (NaCRRI), while fresh loamy soil was obtained from an experimental field at NaCRRI. The animals grazed on a mixture of fresh grass and browse legumes. No indigenous organisms from manure and soil were detected on CT-SMAC-Rif100-Ny50-Cy50 and XLT4-Rif100-Ny50-Cy50 plates. Soil and manure samples were analysed for physical and chemical properties at the Laboratory of Soil Science, National Agricultural Research Laboratories, Kawanda, using methods previously described by Okalebo *et al.* (1993). These properties are shown in Table 1.

Experimental set-up

Contaminated manure was prepared by inoculating separately, *E. coli* O157:H7-Rifr and *Salm*. Typhimurium-Rifr at a rate of 7 or 4 log CFU g^{-1} in 100 g of manure which was mixed thoroughly by kneading in a plastic bag. Contaminated manure-amended soil was prepared by first inoculating either *E. coli* O157:H7-Rifr or *Salm*. Typhimurium-Rifr at a rate of 8 or 5 log CFU g^{-1} in 100 g of manure as mentioned earlier. Afterwards, 100 g of the inoculated manure was mixed with 900 g of soil to give approx. 7 or 4 log CFU g^{-1} of each test organism in manure-amended soil. The inoculated matrices were dispensed in 2-l plastic pots, divided into two sets and randomly placed in the screen house (an environment protected from rain but with perforated walls to allow light penetration and air exchange). One set received normal screen house conditions, and the other set was treated to maintain a high moisture level above 80% r.h. throughout the incubation period by periodic weighing (after every other day) and addition of sterilized distilled water when needed. Each treatment was replicated three

Table 1 Characteristics of manure and soil used in this study

	Characteristics										
Matrix	*Moisture content (%)	Organic matter (%)	pH	Sand (%)	Silt (%)	Clay (%)	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})
Soil	46	2·8	6·4	58·4	10·28	24·5	ND	0·041	0·21	1·3	0·41
Manure	85	ND	6·8	ND	ND	ND	5·2	2·7	1·8	2·1	1·3

ND, not determined.
*Determined on wet basis.

times. For field experiments, matrices were also prepared in pots and inoculated with the test organisms as described for the screen house experiment. The pots were divided into two sets and placed in an open space in the field. One set of pots experienced exclusive field conditions, and the other set was watered occasionally to maintain high moisture content above 80%. EL-USB-2 data loggers (Lascar Electronics Ltd, Wiltshire, UK) were buried in the middle of a few randomly selected matrices to register temperature and relative humidity in the matrices. In all cases, the matrices were sampled immediately after preparation and then weekly for up to 105 days for microbial analysis.

Enumeration of organisms

Two samples of 1 g were removed from the centre of each pot and placed in sterile preweighed 10-ml Falcon tubes containing 9 ml of 0·9% saline. The samples were vortexed twice for 2 min, and tenfold dilution series were made in 0·9% saline. Hundred microlitres of appropriate dilutions was used in surface spread plate counting to determine CFU numbers. *Salmonella* Typhimurium-Rifr was enumerated on XLT4-Rif100-Ny50-Cy50, while *E. coli* O157:H7-Rifr was enumerated on CT-SMAC-Rif100-Ny50-Cy50 following 24 h of incubation at 37°C. Plating on CT-SMAC-Rif100-Ny50-Cy50 and XLT4-Rif100-Ny50-Cy50 was previously shown to result into 100% recovery and selective enumeration of *E. coli* O157:H7-Rifr and *Salm*. Typhimurium-Rifr, respectively, in a nonsterile manure-amended soil background (Ongeng *et al.* 2011). When the populations reached the detection limit of 2 log CFU g^{-1}, an enrichment procedure was used to detect the presence of residual viable cells. For *E. coli* O157:H7-Rifr, 1 g sample was aseptically added to 10 ml of modified EC broth containing novobiocin (Merck), vortexed and incubated at 37°C for 24 h. Hundred microlitre aliquots of the enrichment broth was streaked on CT-SMAC-Rif100-Ny50-Cy50 plates and incubated for 24 h at 37°C. Samples were considered positive when nonsorbitol fermenting colonies typical of *E. coli* O157:H7 developed on the selection plates. For *Salm*. Typhimurium-Rifr, the samples were treated as in the case of *E. coli* O157:H7-Rifr except that enrichment was carried out in selenite cystine broth (Merck) followed by plating on XLT4-Rif100-Ny50-Cy50 plates. Samples were considered positive when black or black-centred colonies typical of *Salmonella* spp. appeared on the selection plates. The maximum and minimum readings for temperature and relative humidity between each sampling interval were derived from the data loggers.

Statistical analysis and modelling of bacterial survival

Data on microbial counts (CFU g^{-1}) were log-transformed (log CFU g^{-1}). The log-transformed data were then fitted making use of GInaFiT (Geeraerd *et al.* 2005). This freeware tool enables the generation of statistical measures and parameter values of the survivor curves. The goodness-of-fit of the survivor curves was assessed using the root mean sum of squared error (RMSE). The RMSE has been considered as the most simple and most informative measure of the goodness-of-fit, for both linear and nonlinear models (Ratkowsky 2003). Only data for high-density inocula were analysed with GInaFiT, while data for low-density inocula could not reliably be fitted because of the limited number of data points showing CFU numbers above the detection limit of the plate count technique. For each organism, the three replicate data sets were each fitted separately, and only data at or above the detection limit were used.

After testing several models available in GInaFiT and using RMSE as a selection criterion, the majority of the experimental data at high inoculum density turned out best fitted to the Double Weibull Model (Coroller *et al.* 2006; eqn (1)), except for *Salm*. Typhimurium-Rifr in manure samples that experienced exclusive field conditions where the classical log-linear model (Bigelow and Esty 1920; eqn (2)) was more suitable.

$$N_{(t)} = \frac{N_0}{1+10^{\alpha}}\left[10^{-\left(\frac{t}{\delta_1}\right)^p+\alpha} + 10^{-\left(\frac{t}{\delta_2}\right)^p}\right] \quad (1)$$

$$\log N = \log N_0 - \frac{k_{\max}\cdot t}{2{\cdot}303} \quad (2)$$

Equation (1) is based on the assumption that the overall cell density consists of two subpopulations, i.e. one subpopulation that is more sensitive to environmental stresses than a second subpopulation. In eqn (1), N is the number of survivors (CFU g^{-1}), N_0 is the initial inoculum size (CFU g^{-1}), t is the time (days), p is the shape parameter (dimensionless), δ_1 is the time for the first decimal reduction of subpopulation 1 (days), δ_2 is the time for first decimal reduction of the second subpopulation (days) and α is the $\log_{10}$ of the ratio of the fraction of more sensitive subpopulation to the fraction of less sensitive subpopulation at time zero. In eqn (2), N, N_0 and t have the same meaning as in eqn (1), while k_{max} is the first-order inactivation rate constant (day^{-1}). As two different models are being used, the time for 4D reduction (t_{4D}) is also calculated (Buchanan *et al.* 1993). This parameter allows comparison of the overall survival

kinetics, irrespective of the underlying trend, at a certain level of reduction, in this case, at 4 log reduction. One-way ANOVA was used to compare the parameter values, and the t_{4D} of the fitted models among treatments and the means were separated using Tukey HSD test at 5% level of significance. The classical 95% confidence interval and prediction interval for the fitted curves were calculated via the *lsqnonlin* procedure of the MATLAB Optimization Toolbox (Version 2007b; The Mathworks Inc., www.mathworks.com), which provides an estimate for the Jacobian matrix, and, hence, an approximation for the parameter variance–covariance matrix.

To check whether the population size of the organisms increased significantly at some points during survival, paired Student's *t*-test was used to compare mean CFU counts between sampling points at 5% level of significance. All statistical analyses were performed using GENSTAT (GENSTAT Discovery Edition 3; www.vsni.co.uk/software/genstat-discovery/).

Results

Environmental conditions

During the course of the study, the organisms experienced fluctuating conditions of temperature between a minimum of 16 and a maximum of 42°C. For matrices that were maintained at high moisture level, humidity remained above 80% throughout the experimental period. Humidity in matrices that were subjected exclusively to screen house conditions decreased progressively with time. For matrices that experienced exclusively field conditions, humidity fluctuated according to rainfall pattern. Figure 1 shows data for a few selected cases.

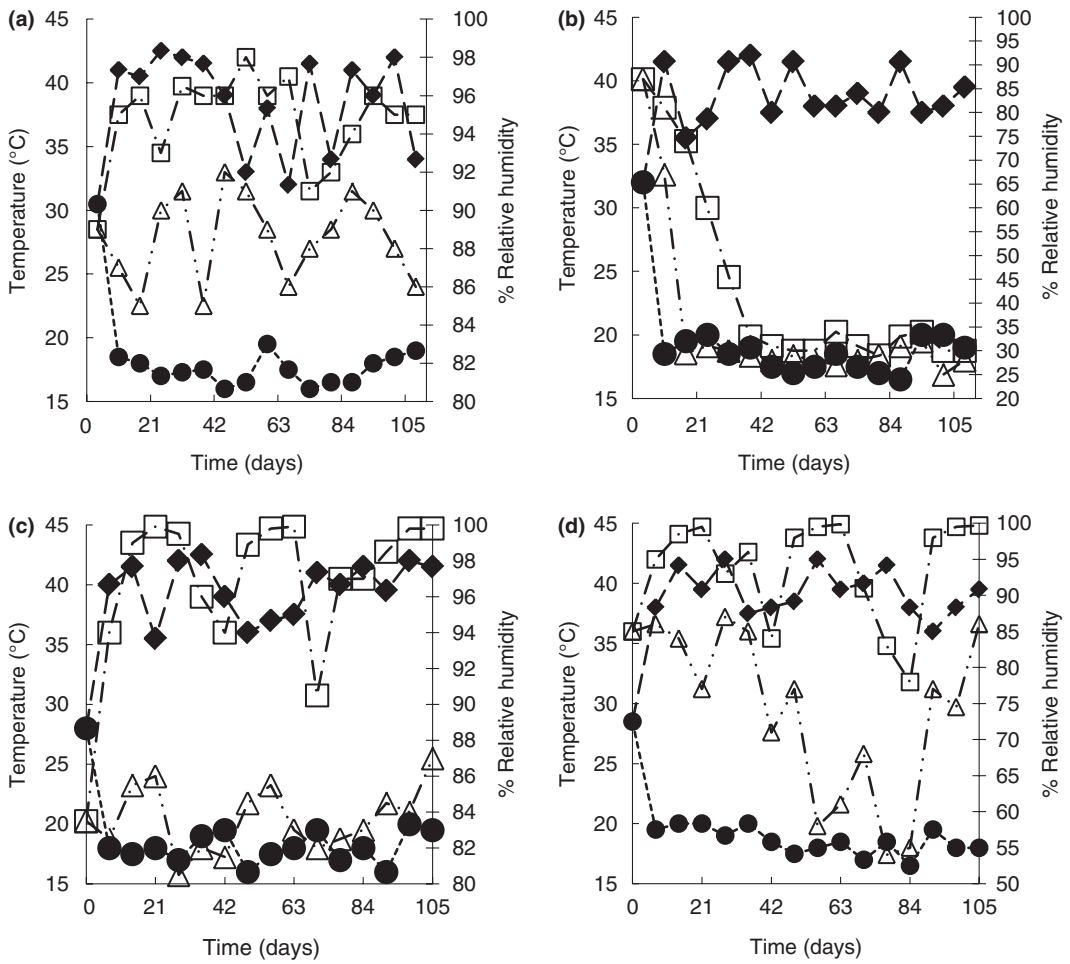

Figure 1 Average weekly maximum and minimum temperature and humidity values in selected matrices used in this study. (a) Manure-amended soil maintained at high moisture level in the screen house. (b) Manure exclusively exposed to screen house conditions. (c) Manure-amended soil matrix maintained at high moisture level in the field. (d) Manure-amended soil exclusively exposed to field conditions. (◆) Maximum temperature; (●) minimum temperature; (□) maximum humidity and (Δ) minimum humidity.

Survival of *Escherichia coli* O157:H7-Rifr under screen house conditions

At high initial inoculum density (approx. 7 log CFU g^{-1}) and in matrices that were held at high moisture level, the mean population size of *E. coli* O157:H7-Rifr increased by 1·2 and 0·6 log CFU g^{-1} in manure and manure-amended soil, respectively, 1 week postinoculation, as confirmed by the paired Student's *t*-test (data not shown). Thereafter, cell counts of the organism decreased gradually till the detection limit of the plating method 70 and 56 days postinoculation in manure-amended soil and in manure, respectively (Fig. 2a). Using the enrichment procedure, *E. coli* O157:H7-Rifr remained detectable in manure-amended soil and in manure for up to 77 and 63 days postinoculation, respectively. In matrices that experienced exclusive screen house conditions, the population size of *E. coli* O157:H7-Rifr started to decrease 1 week postinoculation, and the trend continued till the detection limit of the plating method was reached 42 and 35 days postinoculation in manure-amended soil and manure, respectively (Fig. 2a). The organism remained detectable by enrichment till day 56 and 49 postinoculation in manure-amended soil and in manure, respectively. Statistical measures of the fits and parameter values of the fitted curves for the survival of *E. coli* O157:H7-Rifr in manure and manure-amended soil in the screen house when inoculated at 7 log CFU g^{-1} according to the Double Weibull model are presented in Table 2. The t_{4D} for *E. coli* O157:H7-Rifr in the screen house was significantly shorter (approx. 6 days) in manure samples than in manure-amended soil irrespective of moisture conditions. However, maintaining the matrices at high moisture level in the screen house significantly increased the t_{4D} for the organism by about 12 and 18 days in manure and manure-amended soil, respectively (Table 2). The δ_1 parameter values for the decline functions of *E. coli* O157:H7-Rifr in the screen house were significantly higher in matrices maintained at high moisture level than in matrices exposed to exclusive screen house conditions, but matrix type had no significant effect on δ_1 value of the organism ($P > 0{\cdot}05$). The δ_2 parameter also followed the same trend as in the case of δ_1 although δ_2 was significantly shorter (about 13 days) in manure maintained at high moisture level than in manure-amended soil with the same moisture condition. There was no significant difference among treatments for the shape parameter (p) of the Double Weibull model describing the survival of *E. coli* O157:H7-Rifr except between manure and manure-amended soil maintained at high moisture level in which the shape parameter was significantly larger (about a factor of 2) in the former than in the latter matrix (Table 2). Overall, it is known that in the Double Weibull model, a significant correlation exists between the rate parameters, δ_1, δ_2, and the shape parameter, p (Coroller *et al.* 2006). Therefore, comparison of these parameters between different conditions should be considered together. By doing so, it is clear that all four matrix conditions resulted into significantly different survival curves (as in Fig. 2a) and as also confirmed by the four different t_{4D} values. The time to reach the detection limit (*ttd*) of the plate count method according to the identified model (Table 3) was significantly shorter in manure than in manure-amended soil irrespective of moisture condition. In addition, the model-derived *ttd* was significantly shorter in matrices that experienced exclusive screen

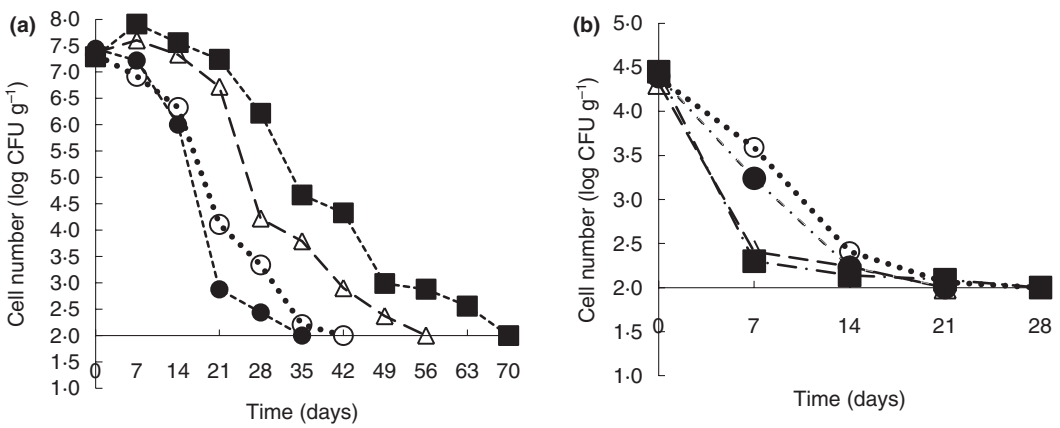

Figure 2 Survival of *Escherichia coli* O157:H7-Rifr in manure and manure-amended soil in the screen house. (a) High inoculum density; (b) low inoculum density. (■) Manure-amended soil maintained at high moisture level; (Δ) manure maintained at high moisture level; (○) manure-amended soil exposed to exclusive screen house conditions; (●) manure exposed to exclusive screen house conditions. Data points are averages of three replicates. Error bars are not shown for clarity of illustration.

Table 2 Statistical measures and parameter values of the fitted models describing the survival of *Escherichia coli* O157:H7-Rifr and *Salmonella* Typhimurium-Rifr in manure and manure-amended soil in the screen house according to the Double Weibull model

Matrix type	TMT	RMSE	AdjR^2	t_{4D}	N_0	α	δ_1	δ_2	p
E. coli O157: H7-Rifr									
Manure	*	0·36	0·97	32 ± 0·12^{a}	7·5 ± 0·02^{a}	3·9 ± 0·11^{ab}	21 ± 0·12^{a}	48 ± 1·61^{a}	4·5 ± 0·76^{a}
Manure	†	0·18	0·99	20 ± 0·12^{b}	7·4 ± 0·03^{a}	4·6 ± 0·14^{a}	12 ± 0·19^{b}	37 ± 1·47^{b}	3·1 ± 0·07^{a}
Manure-amended soil	*	0·43	0·97	44 ± 0·44^{c}	7·7 ± 0·06^{a}	4·0 ± 0·23^{ab}	24 ± 0·64^{a}	61 ± 2·16^{c}	2·4 ± 0·16^{b}
Manure-amended soil	†	0·33	0·98	26 ± 0·11^{d}	7·3 ± 0·08^{a}	3·5 ± 0·16^{b}	14 ± 0·88^{b}	33 ± 2·29^{b}	2·8 ± 0·39^{ab}
Salm. Typhimurium-Rifr									
Manure	*	0·27	0·98	39 ± 0·44^{a}	7·3 ± 0·07^{a}	3·9 ± 0·09^{ab}	22 ± 0·65^{a}	63 ± 2·51^{a}	2·8 ± 0·14^{a}
Manure	†	0·29	0·98	25 ± 0·16^{b}	7·5 ± 0·04^{a}	4·3 ± 0·02^{a}	10 ± 0·82^{b}	41 ± 3·90^{b}	1·7 ± 0·16^{b}
Manure-amended soil	*	0·33	0·97	57 ± 0·51^{c}	7·4 ± 0·15^{a}	3·4 ± 0·12^{b}	28 ± 1·21^{c}	67 ± 1·90^{a}	2·7 ± 0·35^{a}
Manure-amended soil	†	0·24	0·97	29 ± 0·81^{b}	7·3 ± 0·02^{a}	3·7 ± 0·06^{ab}	14 ± 0·25^{d}	43 ± 0·76^{b}	2·5 ± 0·09^{a}

Reported values are means ± SE of three replicates. For each organism, values in the same column followed by the same superscripts are not significantly different ($P > 0·05$).

TMT, treatment; RMSE, root mean sum of squared error; AdjR^2, adjusted R^2; t_{4D}, time (days) to attain a 4 log reduction; N_0, initial cell count (log CFU g^{-1}); α, parameter that relates the fraction of the first subpopulation to the second subpopulation; δ_1, time (days) for first decimal reduction of subpopulation 1; δ_2, time (days) for first decimal reduction of subpopulation 2; p, shape parameter.

*Matrix maintained at high moisture level.

†Matrix experienced exclusively screen house conditions.

Table 3 Time to reach the detection limit (*ttd*) of the plate count method (2 log CFU g^{-1}) for *Escherichia coli* O157:H7-Rifr and *Salmonella* Typhimurium-Rifr in manure and manure-amended soil inoculated at 7 log CFU g^{-1} in the screen house according to the Double Weibull model

		Time to reach detection limit (*ttd*) of the plate count method (days)	
Matrix type	Treatment	*E. coli* O157:H7-Rifr	*Salm.* Typhimurium-Rifr
Manure	*	54 ± 0·33^{a}	73 ± 0·33^{a}
Manure	†	35 ± 0·00^{b}	47 ± 0·33^{b}
Manure-amended soil	*	75 ± 0·58^{c}	87 ± 0·56^{c}
Manure-amended soil	†	41 ± 0·33^{d}	53 ± 0·67^{b}

Reported values are means ± SE of three replicates. For each organism, values in the same column followed by the same superscripts are not significantly different ($P > 0·05$).

*Matrix maintained at high moisture level.

†Matrix exposed to exclusive screen house conditions.

house conditions than in matrices that were maintained at high moisture level.

The survival pattern of *E. coli* O157:H7-Rifr introduced at low initial inoculum density (approx. 4 log CFU g^{-1}) under screen house conditions is shown in Fig. 2b. In general, the mean population size of *E. coli* O157:H7-Rifr declined with time and, within the sampling moments considered, there was no evidence of proliferation of the organism in all the matrices as was observed in the case of high initial inoculum density in high moisture matrices. Cell counts of *E. coli* O157:H7-Rifr introduced at low initial inoculum density fell by 50% in matrices maintained at high moisture level was recorded 7 days postinoculation and thereafter declined gradually to the detection limit of the plating method 28 and 21 days postinoculation in manure-amended soil and in manure, respectively. In matrices that were exposed to exclusive screen house conditions, the population size of *E. coli* O157:H7-Rifr introduced at a low initial inoculum density declined gradually but took similar time periods as in the case of cells in matrices that were maintained at high moisture level to reach the detection limit of the plate count method. The organism was, for all conditions, thereafter not detectable by enrichment.

Survival of *Salmonella* Typhimurium-Rifr under screen house conditions

Salmonella Typhimurium-Rifr introduced at high initial inoculum density under screen house conditions showed some peculiar differences with *E. coli* O157:H7-Rifr when inoculated at the same initial cell density (Fig. 3a). First, *Salm*. Typhimurium-Rifr survived longer than *E. coli* O157:H7-Rifr in all the systems. Secondly, *Salm*. Typhimurium-Rifr seemed not to grow in any of the matrices as was the case for *E. coli* O157:H7-Rifr in manure-amended soil and in manure, but its population remained stable for 2 weeks before any noticeable decline was recorded. This was confirmed by nonsignificant paired Student's *t*-test (data not shown). In matrices maintained at high moisture level and inoculated at high cell density, the population size of *Salm*. Typhimurium-Rifr declined

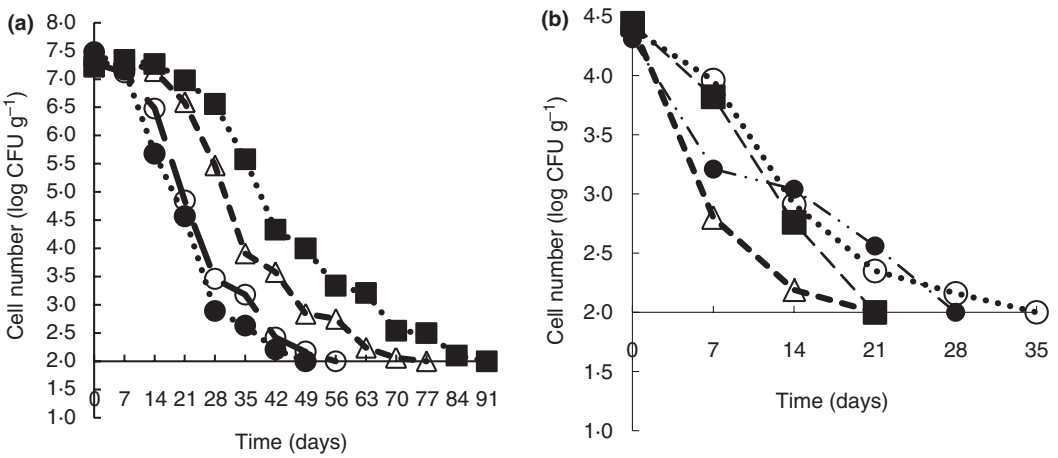

Figure 3 Survival of *Salmonella* Typhimurium-Rifr in manure and manure-amended soil in the screen house. (a) High inoculum density; (b) low inoculum density. (■) manure-amended soil maintained at high moisture level; (Δ) manure maintained at high moisture level; (○) manure-amended soil exposed to exclusive screen house conditions; (●) manure exposed to exclusive screen house conditions. Data points are averages of three replicates. Error bars are not shown for clarity of illustration.

gradually till the detection limit of the plate count technique was reached 91 and 77 days postinoculation in manure-amended soil and in manure, respectively. With enrichment, the organism remained detectable in manure-amended soil and manure till day 98 and 84, respectively. However, in matrices that were exposed to exclusive screen house conditions, CFU of *Salm*. Typhimurium-Rifr declined with time, and there was no evidence of a shoulder period (initial delay) as experienced in the systems where high moisture conditions were kept. The population size of *Salm*. Typhimurium-Rifr reached the detection limit of the plating method 56 and 49 days postinoculation in manure-amended soil and in manure, respectively. The organism remained detectable till day 70 and 63 in manure-amended soil and in manure, respectively, when using the enrichment procedure. Table 2 shows the statistical measures of the fits and parameter values of the fitted curves for survival of *Salm*. Typhimurium-Rifr inoculated at 7 log CFU g^{-1} in manure and manure-amended soil in the screen house according to the Double Weibull model. The effect of matrix type on the t_{4D} for the population of *Salm*. Typhimurium-Rifr under screen house conditions was dependent on moisture condition in the matrices (Table 2). For matrices maintained at high moisture level, t_{4D} was significantly shorter (18 days) in manure samples than in manure-amended soil. On the other hand, for matrices that experienced exclusive screen house conditions, matrix type had no effect on t_{4D}. In general, it was statistically evident that maintaining the matrices at high moisture level increased the t_{4D} for the population of *Salm*. Typhimurium-Rifr under screen house conditions with approx. 14 and 28 days for manure and manure-amended soil, respectively. The δ_1 parameter values were significantly lower (about 5 days) in manure than in manure-amended soil of similar moisture condition, while maintaining the matrices at high moisture level under screen house conditions approximately doubled the respective δ_1 value in both matrices. The δ_2 parameter was statistically similar for matrices with identical moisture conditions but shorter (approx. 20 days) in matrices that experienced exclusive screen house conditions than in matrices that were maintained at high moisture level. Moreover, in matrices that were maintained at high moisture level, the model-derived *ttd* (Table 3) was significantly shorter in manure than in manure-amended soil, but similar in manure and manure-amended soil samples that were exposed to exclusive screen house conditions.

CFU of *Salm*. Typhimurium-Rifr introduced at a low initial inoculum density and incubated under screen house conditions declined with time, and there was no evidence of growth or a shoulder period (Fig. 3b) irrespective of the matrix type or moisture level. In matrices that were maintained at high moisture level, the population size of *Salm*. Typhimurium-Rifr dropped to the detection limit of the plating method 21 days postinoculation in both manure-amended soil and manure and remained detectable by enrichment till day 35. In matrices that were exposed to exclusive screen house conditions, CFU of *Salm*. Typhimurium-Rifr reached the detection limit of the plating method 35 and 28 days postinoculation in manure-amended soil and in manure, respectively. However, by enrichment, the organism was detectable till day 42 in both manure-amended soil and manure.

Survival of *Escherichia coli* O157:H7-Rifr in the field

Survival curves for *E. coli* O157:H7-Rifr inoculated at high density under field conditions are shown in Fig. 4a. During the first week of field exposure, *E. coli* O157:H7-Rifr grew in all the matrices especially in manure-amended soil samples that experienced exclusive field conditions. This was confirmed by significant paired Student's *t*-test (data not shown). In matrices that were maintained at high moisture level, the population size of *E. coli* O157:H7-Rifr declined with time to the detection limit 77 and 63 days postinoculation in manure-amended soil and in manure, respectively. Using enrichment, the organism remained detectable till day 84 postinoculation in the manure-amended soil samples that were maintained at high moisture level, but could not be detected in manure at the same moisture condition beyond day 63. For matrices that experienced exclusive field conditions, *E. coli* O157:H7-Rifr declined gradually to the detection limit 63 and 84 days postinoculation in manure and manure-amended soil, respectively, and was not detected anymore thereafter. Table 4 shows statistical measures of the fits and parameter values of the fitted models for survival of *E. coli* O157:H7-Rifr inoculated 7 log CFU g^{-1} in manure and manure-amended soil in the field as described by the Double Weibull model. The t_{4D} for the survival of *E. coli* O157:H7-Rirf was significantly shorter (approx. 7 days) in manure than in manure-amended soil but there was insufficient evidence to demonstrate the effect of moisture condition on t_{4D}. The δ_1 and δ_2 parameters followed the same trend as of t_{4D}, while the shape parameter (p) was completely unaffected by matrix type or moisture conditions (Table 4). The model-derived *ttd* for *E. coli* O157:H7-Rifr in the field was significantly shorter in manure than in manure-amended soil but was not affected by moisture condition in the matrices (Table 5).

At low inoculum density, the population size of *E. coli* O157:H7-Rifr in all the matrices decreased by about 50% 7 days postexposure in the field. Thereafter, the cell density of the organism in all the matrices decreased to the detection limit of the plate count technique 21 days postexposure in the field (Fig. 4b). The enrichment culture method did not detect *E. coli* O157:H7-Rifr in all the matrices beyond day 21.

Survival of *Salmonella* Typhimurium-Rifr in the field

Survival patterns of *Salm*. Typhimurium-Rifr at high inoculum density in the field are shown in Fig. 5a. In general, irrespective of matrix type, CFU of *Salm*. Typhimurium-Rifr remained fairly stable in all the matrices till 7 days postexposure and thereafter started to decline. In matrices that were maintained at high moisture level, CFU of *Salm*. Typhimurium-Rifr in manure-amended soil and in manure fell till the detection limit of the direct plate count method 84 and 77 days postexposure, and the organism could not be detected by enrichment beyond 98 and 84 days in manure-amended soil and in manure, respectively. In matrices exclusively exposed to field conditions, CFU of *Salm*. Typhimurium-Rifr declined till the detection limit of the plating method 84 and 77 days postexposure in manure-amended soil and in manure, respectively, and the organism could not be detected anymore by the enrichment. However, *Salm*. Typhimurium-Rifr introduced at high initial inoculum

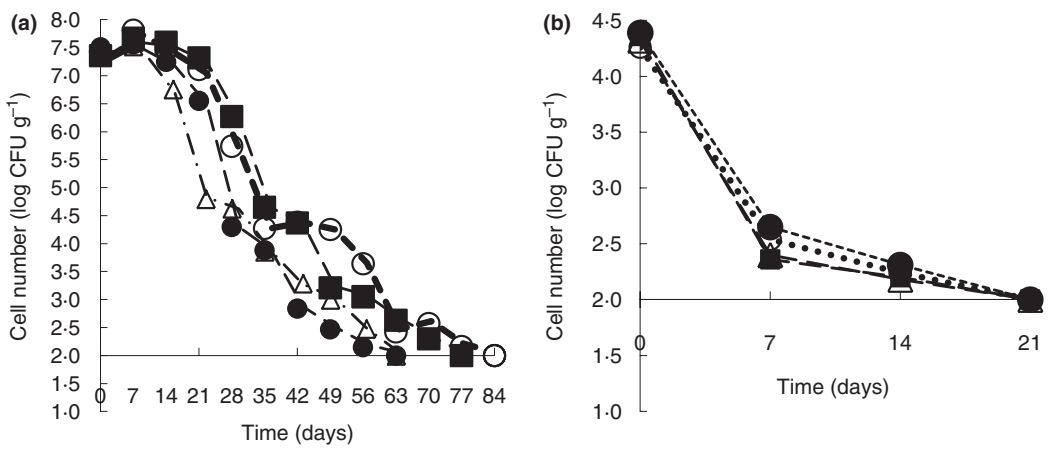

Figure 4 Survival of *Escherichia coli* O157:H7-Rifr in manure and manure-amended soil in the field. (a): high inoculum density; (b) low inoculum density. (■) Manure-amended soil maintained at high moisture level; (Δ) manure-amended soil maintained at high moisture level; (○) manure exposed to exclusive field conditions; (●) manure exposed to exclusive field conditions. Data points are averages of three replicates. Error bars are not shown for clarity of illustration.

Table 4 Statistical measures and parameter values of the fitted models describing the survival of *Escherichia coli* O157:H7-Rifr and *Salmonella* Typhimurium-Rifr in the field according to the Double Weibull and log-linear models

Matrix type	TMT	RMSE	AdjR^2	t_{4D}	N_0	α	δ_1	δ_2	p	k_{max}
E. coli O157: H7-Rifr										
*Manure	†	0·33	0·98	38 ± 1·59^a	7·5 ± 0·03^a	3·6 ± 0·07^a	24 ± 1·23^a	47 ± 1·75^a	4·3 ± 0·8^a	NA
*Manure	‡	0·19	0·99	40 ± 0·12^a	7·4 ± 0·04^a	4·6 ± 0·14^b	20 ± 0·17^b	50 ± 1·51^a	3·1 ± 0·08^a	NA
*Manure-amended soil	†	0·36	0·97	45 ± 0·68^b	7·7 ± 0·07^a	4·0 ± 0·29^{ab}	33 ± 0·68^c	62 ± 4·79^b	2·6 ± 0·16^a	NA
*Manure-amended soil	‡	0·33	0·98	46 ± 0·50^b	7·26 ± 0·07^a	3·5 ± 0·12^a	35 ± 0·98^c	64 ± 2·41^b	2·8 ± 0·46^a	NA
Salm. Typhimurium-Rifr										
*Manure	†	0·29	0·98	45 ± 1·54^a	7·6 ± 0·12^a	3·2 ± 0·07^a	19 ± 1·75^a	53 ± 6·1^a	2·0 ± 0·34^a	NA
§Manure	‡	0·35	0·96	49 ± 0·52^b	7·5 ± 0·11^a	NA	NA	NA	NA	¶0·19 ± 0·14
*Manure-amended soil	†	0·25	0·98	52 ± 0·89^b	7·6 ± 0·12^a	3·1 ± 0·03^a	22 ± 3·45^a	57 ± 9·34^a	1·9 ± 0·35^a	NA
*Manure-amended soil	‡	0·38	0·98	58 ± 0·28^c	7·2 ± 0·04^a	3·7 ± 0·01^a	26 ± 0·98^a	67 ± 3·8^a	1·8 ± 0·07^a	NA

Reported values are means ± SE of three replicates. For each organism, values in the same column followed by the same superscripts are not significantly different ($P > 0·05$).

TMT, treatment; RMSE, root mean sum of squared error; AdjR^2, adjusted R^2; t_{4D}, time (days) to attain 4 log reduction; N_0, initial cell count (log CFU g^{-1}); α, parameter that relates the fraction of the first subpopulation to the second subpopulation; δ_1, time (days) for first decimal reduction of subpopulation 1; δ_2, time (days) for first decimal reduction of subpopulation 2; p, shape parameter; k_{max}, inactivation rate constant for the log-linear model.

*According to Double Weibull.

†Matrix maintained at high moisture level.

‡Matrix experienced exclusively field conditions.

§According to log-linear model.

¶Not used in statistical comparison.

Table 5 Time to reach the detection limit (*ttd*) of the plate count method (2 log CFU g^{-1}) for *Escherichia coli* O157:H7-Rifr and *Salmonella* Typhimurium-Rifr in manure and manure-amended soil inoculated at 7 log CFU g^{-1} in the field according to the Double Weibull or log-linear model

Matrix type	Treatment	Model type	Time to reach detection limit (*ttd*) of the plate count method (days)	
			E. coli O157:H7-Rifr	*Salm*. Typhimurium-Rifr
Manure	*	DW	60 ± 0·00^a	68 ± 3·48^a
Manure	†	LL	62 ± 0·58^a	66 ± 0·57^a
Manure-amended soil	*	DW	75 ± 0·67^b	85 ± 2·00^b
Manure-amended soil	†	DW	80 ± 0·58^b	83 ± 0·33^b

Reported values are means ± SE of three replicates. For each organism, values in the same column followed by the same superscripts are not significantly different ($P > 0·05$).

DW, Double Weibull; LL, log-linear.

*Matrix maintained at high moisture level.

†Matrix exposed to exclusive field conditions.

density in manure-amended soil increased in size between day 21 and 28 and stagnated between day 56 and 63 post-exposure under exclusive field conditions. Statistical measures of the fits and parameter values of the fitted models for survival of *Salm*. Typhimurium-Rifr introduced at 7 log CFU g^{-1} in manure and manure-amended soil in the field based on the Double Weibull and the log-linear functions are presented in Table 4. The δ_1, δ_2 and p parameters of the Double Weibull model could only be compared between manure and manure-amended soil maintained at high moisture level or between manure-amended soil maintained at high moisture level and that exposed to exclusive field conditions. This is because the survival of *Salm*. Typhimurium-Rifr in manure samples exposed to exclusive field conditions followed the log-linear model. These Double Weibull parameters were not affected by matrix type neither by moisture condition in manure-amended soil samples. The t_{4D} for the survival of *Salm*. Typhimurium-Rifr in the field could be compared between matrices and between moisture condition in the matrices because GInaFiT provides the t_{4D} parameter for both the Double Weibull and log-linear model, and, more importantly, the t_{4D} value is a suitable parameter for comparing survival curves following different models

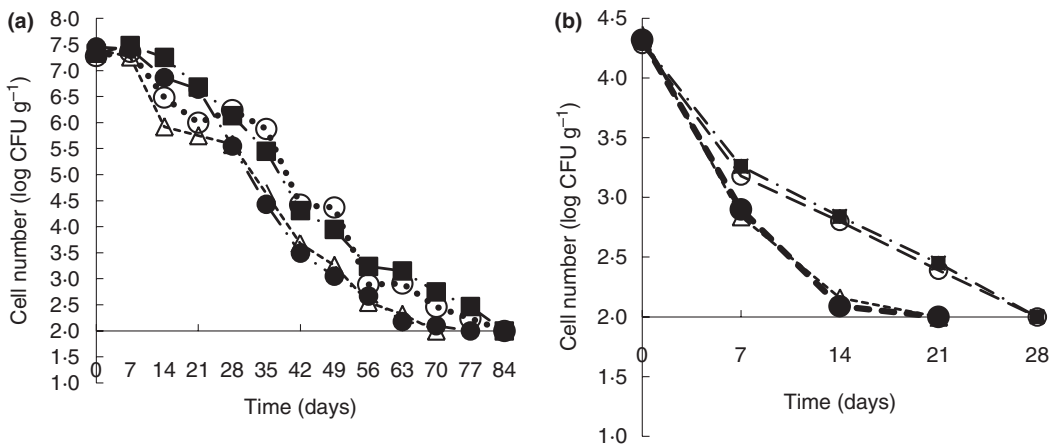

Figure 5 Survival of *Salmonella* Typhimurium-Rifr in manure and manure-amended soil in the field. (a) High inoculum density; (b): low inoculum density. (■) Manure-amended soil maintained at high moisture level; (Δ) manure maintained at high moisture level; (○) manure-amended soil exclusively exposed to field conditions; (●) manure exclusively exposed to field conditions. Data points are averages of three replicates. Error bars are not shown for clarity of illustration.

(Buchanan *et al.* 1993). Statistical analysis showed that maintaining the matrices at high moisture level significantly decreased the t_{4D} for *Salm*. Typhimurium-Rifr in both manure and manure-amended soil by approx. 5 days under field conditions. The t_{4D} value was significantly lower (approx. 7 days) in manure than observed in manure-amended soil except in the case of manure exposed to exclusive field conditions and manure-amended soil maintained at high moisture level where t_{4D} value was statistically similar between them. The model-derived *ttd* for *Salm*. Typhimurium-Rifr in the field (Table 5) was significantly shorter in manure than in manure-amended soil but moisture condition had no effect.

Survival curves for *Salm*. Typhimurium-Rifr introduced at low initial inoculum density in the field are shown in Fig. 5b. Survival pattern of *Salm*. Typhimurium-Rifr in high moisture-manure-amended soil was similar to the pattern observed in manure-amended soil exclusively exposed to field conditions. This was also the case for manure samples. However, the organism survived 1 week longer in manure-amended soil (till day 35) than in manure samples (till day 28) as determined by enrichment.

Discussion

The results of this study demonstrate that the survival of *E. coli* O157:H7-Rifr and *Salm*. Typhimurium-Rifr in manure and in manure-amended soil in the CAEZ of Uganda depends on inoculum density and moisture condition in the matrix. High-density inocula survived longer in matrices maintained at high moisture level than in matrices that experienced exclusive screen house conditions, hence showing that desiccation is an important factor that affects the survival of *E. coli* O157:H7-Rifr and *Salm*. Typhimurium-Rifr at high density in manure and in manure-amended soil matrix. This is justified by the fact that humidity in matrices that experienced exclusive screen house conditions fell with time (Fig. 1b). Kudva *et al.* (1998) reported that *E. coli* O157:H7 survived longer in aerated manure than in nonaerated samples and suggested that the observed difference in survival times was probably due to dehydration which concurs with the findings of this study. At low inoculum density, *E. coli* O157:H7-Rifr and *Salm*. Typhimurium-Rifr survived better in matrices that experienced exclusive screen house conditions than in matrices maintained at high moisture level, respectively, thus demonstrating in this case that periodic addition of water to maintain high moisture conditions was detrimental to the survival of the organisms at low density instead. The observation that the effect of moisture condition on survival was dependent on inoculum size suggests that there was an interaction between moisture level and inoculum density although the nature of this interaction was not determined. It is well known that survival of any organism in a particular environment depends on the effects of biotic and abiotic factors and interactions between them (Sjogren 1994). At low inoculum density, the effects of biotic factors (e.g. antagonism, interspecies competition for nutrients, predation) on *E. coli* O157:H7-Rifr and *Salm*. Typhimurium-Rifr were probably more pronounced when moisture level in the matrices was high, suggesting that high moisture enhanced the capabilities of biotic factors to reduce the population of *E. coli* O157:H7-Rifr and *Salm*. Typhimurium-Rifr.

Salmonella Typhimurium-Rifr persisted much longer than *E. coli* O157:H7-Rifr in similar matrices in the

screen house, irrespective of inoculum density. This scenario has also been observed in other studies although under different experimental conditions (Himathongkham *et al.* 1999; Franz *et al.* 2005; Semenov *et al.* 2007), which suggests that *Salm*. Typhimurium is a more persistent organism than *E. coli* O157:H7. Proliferation of *E. coli* O157:H7-Rifr in high moisture-manure and manure-amended soil matrices after 1 week of incubation in the screen house contrasted sharply with the behaviour of *Salm*. Typhimurium-Rifr where cell counts of the organism remained stable instead. It is possible that *Salm*. Typhimurium-Rifr might have grown in some of the matrices maintained at high moisture level as *E. coli* O157:H7-Rifr did but sampling interval of 1 week might have been too wide to detect any appreciable growth of the organism. Typically, it was observed that the two organisms survived longer in manure-amended soil than in manure. This is consistent with the results of other studies (Jiang *et al.* 2002; You *et al.* 2006). Soil composition, pH, water activity, redox potential, presence of rhizosphere and microbial interaction are some of the factors postulated to influence survival or inactivation of pathogenic bacteria in the soil (Fenlon *et al.* 2000; van Overbeek *et al.* 2010). The fact that survival time was longer in manure-amended soil than in manure suggests that manure-amended soil provided more favourable conditions to the test organisms, despite the fact that manure is richer in nutrients than soil. A probable reason for this can be that indigenous organisms in manure, e.g. coliforms, were more antagonistic than indigenous soil flora to *E. coli* O157:H7-Rifr and *Salm*. Typhimurium-Rifr. This was also suggested by You *et al.* (2006) who showed that *S*. Newport persisted for 184, 332 and 405 days in manure, manure-amended nonsterilized soil and manure-amended sterilized soil, respectively. Franz *et al.* (2007a) showed that the overall survival time of *E. coli* O157:H7 in cattle manure was negatively related to the number of coliforms. Considering that coliforms normally occur in high numbers in cattle faeces (Cox *et al.* 2005) as opposed to soil, the faster decline of *E. coli* O157:H7-Rifr and *Salm*. Typhimurium-Rifr in manure compared to manure-amended soil can partly be attributed to the competitive activities of coliform in manure. Therefore, the longer survival time in manure-amended soil might have been because of the dilution effect of the soil fraction on the density of coliforms and other indigenous organisms in manure, notwithstanding the possible effects of other factors. This argument draws evidence from the findings of Jiang *et al.* (2002) which demonstrated that *E. coli* O157:H7 declined more rapidly in unautoclaved soils amended with manure at a ratio of one part manure to ten parts soil than in soil samples containing dilute amounts of manure. These findings show clearly that amendment of manure to soil can enhance the length of time that *E. coli* O157:H7, and *Salm*. Typhimurium remain viable in manure in the CAEZ of Uganda by 1 week under experimental conditions of this study.

Survival of *E. coli* O157:H7-Rifr and *Salm.* Typhimurium-Rifr in the field depended on inoculum density and matrix type, except for *E. coli* O157:H7-Rifr at low inoculum density whose survival time was independent of the matrix type within the time points sampled. It was apparent that cell density of *Salm*. Typhimurium-Rifr at high inoculum level surged in manure-amended soil matrices exposed to exclusive field conditions between the third and fourth week postexposure, but stagnated in decline between the eighth and ninth week postexposure (Fig. 5a). This trend of events could be a consequence of moisture fluctuations, but the increase in cell numbers between the third and fourth week could be due to resuscitation of the stressed fraction of the population as the organism did not exhibit any significant growth at the initial start of the experiment as demonstrated by nonsignificant paired Student's *t*-test (data not shown). There is no apparent explanation to account for the stagnation in cell counts of *Salm*. Typhimurium-Rifr observed between eighth and ninth week postexposure in the field. Both *E. coli* O157:H7-Rifr and *Salm*. Typhimurium-Rifr survived longer in manure-amended soil matrices than in manure matrices at high inoculum density. However, *Salm*. Typhimurium-Rifr survived longer than *E. coli* O157:H7-Rifr, thus confirming the observation from screen house experiment which suggested that *Salm*. Typhimurium-Rifr is a much more persistent organism than *E. coli* O157:H7-Rifr. At low inoculum density, the persistence time of *Salm*. Typhimurium-Rifr was shorter in manure samples than in manure-amended soil by 1 week in the field. However, matrix type had no effect on the survival time of *E. coli* O157:H7-Rifr at low inoculum density, which is in contrast with the findings from the screen house experiment. By contrast too, at low inoculum density, *Salm*. Typhimurium-Rifr persisted longer than *E. coli* O157:H7-Rifr in both matrices in the field as was the case for the screen house experiment.

Various survival times have been reported for *E. coli* O157:H7 and *Salm*. Typhimurium in manure and in manure-amended soil matrices under temperate conditions. It was initially expected that survival times of the two enteric bacteria in tropical agricultural environment would be different from values observed in temperate climate because of unique differences in soil quality, moisture conditions, temperature regime and cattle feeding regiment between the two geographical regions. The results of this study revealed that persistence times of the organisms under tropical conditions were in most cases

much shorter than earlier observed under temperate climatic conditions (21 months: Kudva *et al.* 1998; 120 days: Hutchison *et al.* 2004; 154–217 days: Islam *et al.* 2004a; 203–231 days: Islam *et al.* 2004b; 161–231 days: Islam *et al.* 2004c; 154–196 days: Islam *et al.* 2005; 300 days: Nicholson *et al.* 2005) although results of a few studies suggest otherwise (16–64 days: Hutchison *et al.* 2004; 16–63 days: Hutchison *et al.* 2005). This finding suggests that biophysical conditions in the tropics might be more detrimental to *E. coli* O157:H7 and *Salmonella* spp. than is the case in temperate environment. Furthermore, the persistence times of *E. coli* O157:H7 and *Salm*. Typhimurium observed in this study are not consistent with survival times that were determined under isothermal conditions (Kudva *et al.* 1998; Bolton *et al.* 1999; Jiang *et al.* 2002; Franz *et al.* 2005; You *et al.* 2006; Franz *et al.* 2007a,b; Garcia *et al.* 2010), thus indicating that survival under dynamic field conditions cannot be estimated from survival time determined at one particular isothermal condition. However, it is rather difficult to compare results of survival times from other studies with the findings from this study because of differences in experimental set-ups as well as differences in properties and composition of the soil and manure used.

Modelling the survival of enteric food-borne pathogens in an agricultural environment has been rarely performed despite the fact that microbial safety problems associated with fresh vegetables can start from the field. Fairly recent studies that incorporated modelling aspects have been only carried out based on experiments performed under controlled conditions (You *et al.* 2006; Franz *et al.* 2005, 2007a,b; Semenov *et al.* 2009; Semenov *et al.* 2010). Data on survival of the 7 log CFU g^{-1} *E. coli* O157:H7-Rifr and *Salm*. Typhimurium-Rifr obtained under realistic scenarios in the field and in the screen house were fitted using the GInaFiT software (Geeraerd *et al.* 2005). It was observed that the best fit was produced by the Double Weibull Model (Coroller *et al.* 2006) except in one case where the classical log-linear model (Bigelow and Esty 1920) was preferable based on the RMSE criterion. It has been reported that the shape of survivor curves for nonthermal inactivation of micro-organisms as caused by unfavourable environmental stress displays more pronounced heterogeneity according to the intensity of the stress (Coroller *et al.* 2006). The pattern of survival curves has been shown to vary with the physiological state of the cells and on the adaptation phenomena prior to stress experience (Greenacre *et al.* 2003; Lee *et al.* 1994; Phan-Thanh *et al.* 2000). In this study, inocula at the same initial physiological state were used, and therefore, the observed variability in some of the parameters of the Double Weibull model between high-moisture and desiccated-matrices can be attributed to the stress that the organisms experienced in the matrices, not withstanding the possible errors due to matrix heterogeneity. There is no apparent explanation to account for why the survival of *Salm*. Typhimurium-Rifr in manure samples exposed to exclusive field conditions followed the classical log-linear model as opposed to the Double Weibull model. Even under isothermal conditions, survival of enteric pathogens in manure or manure-amended soils has been shown to follow various survival patterns depending on the experimental set-up. At 24°C, the survival of *S*. Newport in manure and manure-amended soils was shown to follow the log-linear model (You *et al.* 2006). In other studies, survival of *E. coli* O157:H7 and *Salm*. Typhimurium in manure held at 20°C was fitted by logistic regression in one case (Franz *et al.* 2005) and modelled using a biphasic model in another case (Franz *et al.* 2007b). But nevertheless, the models used in this study were able to describe survival patterns of the organisms in the matrices investigated and the parameters provided logical meaning consistent with practical realities. This was clearly shown by the fact that the observed *ttd* values (Figs 2a, 3a, 4a and 5a) were similar to those determined according to the identified model (Table 3 and 5). Physicochemical conditions in the matrices and inherent biotic factors might explain the decline phenomena and subsequent differences in parameter values of the model among treatments.

Inclusion of high moisture matrices in the experimental design enabled the determination of how desiccation or desiccation–rehydration phenomenon would affect the survival of *E. coli* O157:H7-Rifr and *Salm*. Typhimurium-Rifr. Inoculum densities (4 and 7 log CFU g^{-1}) used in this study have been previously applied in studies on the survival of *E. coli* O157:H7 and *Salmonella* spp. in manure and manure-amended soil in temperate climatic conditions (Hutchison *et al.* 2005; Franz *et al.* 2005; Semenov *et al.* 2007; Williams *et al.* 2007). Inoculum concentration of 4 and 7 log CFU g^{-1} was used to simulate a realistic contamination level in bovine manure and a worst case scenario, respectively, although some authors believe that high-density inocula in the order of 7 log CFU g^{-1} and more is unrealistic (Franz *et al.* 2005). This contention is supported by data that show that infected cattle excreted between 2 and 5 log CFU g^{-1} *E. coli* O157:H7 in their faeces (Nicholson *et al.* 2000). However, Fukushima and Seki (2004) showed that *E. coli* O157:H7 was recovered up to a concentration of 8 log CFU g^{-1} from bovine faeces in Japan. On the other hand, the 7 log CFU g^{-1} inoculum density was used for *Salm*. Typhimurium-Rifr to compare its behaviour with that of *E. coli* O157:H7-Rifr. Fegan *et al.* (2004) reported that the cell number of *Salmonella* spp. in faeces originating from cattle presented for slaughter was between <3 and $2{\cdot}8 \times 10^3$ MPN g^{-1} faeces which is just 1 log lower than

the concentration associated with a low-dose inoculum used in this study.

Conclusions

The survival of *E. coli* O157:H7-Rifr and *Salm*. Typhimurium-Rifr in cattle manure and in manure-amended soil was dependent on moisture conditions in the matrices and inoculum density. While both organisms survived longer in manure-amended soil than in manure samples, *Salm*. Typhimurium-Rifr was more persistent than *E. coli* O157:H7-Rifr. The results of this study provides for the first time information on the survival of *E. coli* O157:H7 and *Salm*. Typhimurium under tropical climatic conditions. In particular, this study demonstrates that: (i) the persistence time of *E. coli* O157:H7 and *Salm*. Typhimurium at low inoculum density under tropical conditions in the CAEZ of Uganda does not exceed 4 and 6 weeks, respectively; and (ii) *E. coli* O157:H7 and *Salmonella* spp. at high inoculum density can persist only up to 12 and 14 weeks, respectively. This suggests that in real fields, their persistent presence in the soil beyond the time limits demonstrated in this study would be due to continuous introduction of fresh contaminants by potential reservoirs. The persistence times determined in this study: (i) were in most cases shorter than those earlier observed in temperate regions thus suggesting that biophysical conditions in the tropics might be more detrimental to *E. coli* O157:H7 and *Salmonella* spp. than is the case in the temperate environment and therefore indicating that the survival of *E. coli* O157:H7 and *Salm*. Typhimurium under tropical conditions cannot be predicted from data obtained in temperate environments; (ii) are inconsistent with survival times that have been obtained under isothermal conditions hence indicating that survival under dynamic conditions cannot be estimated from survival time determined at one particular isothermal condition. A major limitation of this study was that use of single strains precluded the effect of variability that may exist among different strains.

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