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Survival of *E. coli* O157:H7 in organic wastes destined for land application

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ABSTRACT

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Aim: To determine the persistence of *Escherichia coli* O157 in contrasting organic wastes spread to land and to assess the potential environmental risk associated with the disposal of these wastes to land.

Methods and Results: Twenty-seven organic wastes originating from slaughterhouses, wastewater treatment plants (raw and treated sewage), creameries and farms (bovine slurry), were inoculated with *E. coli* O157:H7 and incubated at 10°C. Although pathogen numbers gradually declined in all the wastes, albeit at different rates even in the same waste type, *E. coli* O157:H7 was still viable in 77% of organic wastes tested after 2 months.

Conclusions: Long-term storage of organic wastes led to a significant and gradual decline in *E. coli* O157:H7 numbers. Consequently, storage may be a useful means of reducing the pathogen load of wastes destined for land application. However, in most cases, long-term storage cannot be expected to completely eliminate *E. coli* O157:H7 from waste.

Significance and Impact of the Study: Our results indicate that current legislation may be insufficient to protect the environment from *E. coli* O157:H7 contamination from untreated wastes spread to land.

Keywords: abattoir waste, cattle, *Escherichia coli* O157, landspreading, sewage sludge, survival, wastewater.

INTRODUCTION

Verocytotoxin producing *Escherichia coli* O157 (VTEC) was first recognized as being a virulent human pathogen and serious threat to public health in 1982 (Riley *et al.* 1983). Clinical symptoms of the disease range from mild diarrhoea and haemorrhagic colitis through to complications arising from infection such as haemolytic uraemic syndrome (HUS) and thombocytopaenic purpura (Su and Brandt 1995). Typically, 50% of HUS patients suffer from acute renal failure while the global mortality rate from *E. coli* O157 infection is approximately 5% (Waters *et al.* 1994; Siegler 1995; Alleberger *et al.* 1997).

In most countries, cattle are believed to be the predominant reservoir of VTEC within the environment, although its presence is thought to be increasing in other domestic (e.g. sheep, pigs) and wild animal (e.g. deer, birds) populations (Chapman *et al.* 1997; Chapman 2000). Within cattle, the pathogen appears to sporadically colonize the gut of healthy animals and is subsequently shed in the faeces.

Escherichia coli O157 food poisoning in the human population is primarily associated with the consumption of undercooked contaminated beef products (Anon. 1997a). However, outbreaks have also occurred because of the consumption of contaminated vegetables, milk and drinking water and a range of other food products (Park *et al.* 1999). Fresh fruit and vegetables have been responsible for a number of outbreaks in the USA of which most are thought to be directly attributable to cross contamination by meat within food processing environments (Anon. 1997b). In some outbreaks, however, there was evidence that either direct or indirect contact with animal wastes had caused human infection (Morgan *et al.* 1988; Cieslak *et al.* 1993; Cody *et al.* 1999). For example, a number of environment-associated outbreaks have been reported, usually where direct human

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contact with animal faeces has occurred, such as on campsites (Ogden *et al.* 2002), through visits to educational farm facilities (Milne *et al.* 1999) and through drinking of faecally contaminated freshwater (Ackman *et al.* 1997). Locking *et al.* (2001) concluded that contact (or likely contact) with animal faeces is a strong risk factor for VTEC infection.

It is common practice in many countries to apply effluents from farming, sewage treatment works, abattoirs and food industries to agricultural land as fertilizer, although application of untreated sewage to land is not permitted in Europe or the USA. On both grassland and arable land, wastes can either be routinely spread onto the soil surface or deep injected into the soil at 15–30 cm below the surface. Application of these untreated and potentially contaminated wastes to land may pose a significant health threat in terms of farm animal infection and dispersal into the wider environment. There is an obvious risk of VTEC infection arising from contamination of fruit and vegetable crops grown in soil to which abattoir waste, slurry or untreated sewage sludge has been applied, especially where the food products (e.g. salad vegetables) are consumed with minimal processing. In addition, dissemination of the organism throughout the environment via birds (Wallace *et al.* 1997), runoff to surface water and leaching into groundwater is also of concern (Ogden *et al.* 2001). Furthermore, there are few reports upon the extent to which re-growth of *E. coli* O157:H7 could occur in previously treated wastes.

The public health risk associated with VTEC in wastes spread to land can be expected to be dependent both upon its ability to survive in wastes prior to landspreading and within the soil/vegetation after application. Both introduced pathogenic and non-pathogenic *E. coli* strains have been shown to persist in some soils and animal wastes for considerable periods of time (Kudva *et al.* 1998; Bolton *et al.* 1999; Fukushima *et al.* 1999; Maule 1999; Lau and Ingham 2001; Hepburn *et al.* 2002). However, previous studies have tended to concentrate on a waste from a single location and consequently the inherent variability of *E. coli* O157 to persist in organic wastes is unknown. Information on this subject will improve the quality of environmental risk assessments. Further, long-term *E. coli* O157 persistence in other common organic wastes has not been studied (e.g. abattoir waste, sewage). The aim of this study was therefore to compare the survival of *E. coli* O157 over a 2-month period in a diverse range of wastes commonly applied to agricultural land.

MATERIALS AND METHODS

Bacterial strain

An environmental isolate of *E. coli* O157:H7 (strain 3704) was used throughout this study (Campbell *et al.* 2001). The strain was maintained on semi-solid LB agar (Sigma Chemical Co., Poole, UK) and prior to the start of experimental work, liquid cultures were grown up overnight in 100 ml of LB broth (Sigma Chemical Co.) at 37°C on an orbital shaker at 150 rev min^{-1}. Colony forming units of *E. coli* O157 were quantified on sorbitol MacConkey agar (SMAC; Oxoid Ltd, Basingstoke, UK) containing 0·05 mg l^{-1} cefixime and 2·5 mg l^{-1} tellurite (CT supplement; Dynal Biotech, Bromborough, UK) after incubation at 37°C for 15 h.

Organic waste samples

Characteristics of the organic waste samples used in the experiments are shown in Table 1. All wastes were obtained from large commercial ovine abattoirs (AW, $n = 5$), municipal water treatment works [untreated sewage (USW) $n = 3$; treated sewage (TSW) $n = 5$; sewage sludge (SSW) $n = 5$], commercial cattle farms [bovine slurry (SW) $n = 5$] and commercial creameries (CW, $n = 4$) in North Wales. All the treated sewage samples had received primary treatment. The creameries produce both milk and cheese products. All samples were collected within a week of commencement of the experimental work and were stored at 4°C prior to use.

Characterization of wastes

Electrical conductivity (EC; Jenway 4010 EC meter, Jenway, Dunmow, UK) and pH (Orion 410A pH meter, Thermo, Waltham, MA, USA) were measured directly in liquid wastes, while for solid wastes (sewage sludges and thick slurries) EC was measured after a 1 : 1 (v/v) dilution of the waste with distilled water. Waste moisture content was determined by measuring weight loss after drying at 80°C overnight. Total C and N in each waste was quantified using a CHN2000 elemental analyser (Leco Corp., St Joseph, MI, USA).

For further analyses, liquid wastes were centrifuged at 12 000 × *g* for 5 min and the supernatant retained for chemical analysis. In the case of solid or semi-solid wastes, samples (10 g) were shaken with 30 ml of deionized water for 1 h on a reciprocating shaker (200 rev min^{-1}) and the supernatant recovered for analysis as described above. Phosphate in the supernatant solution was determined colorimetrically using a modification of the method described by Anderson and Ing (1993) using a VersaMax microplate reader (Molecular Devices Ltd, Wokingham, UK). Nitrate and ammonium concentrations were determined using a segmented-flow San-plus autoanalyser (Skalar Ltd, York, UK). Unless otherwise stated, all data for solid and semi-solid wastes are presented on a wet weight basis.

Background microbiology of samples

Microflora of the unspiked wastes were characterized by performing serial dilutions of each waste followed by plating

Table 1 Chemical and microbiological characteristics of the organic wastes used in the study. Inoculum recovery is the amount of *Escherichia coli* O157 recovered from the microcosms immediately after pathogen addition

	Inoculum recovery (% of total added)	T_{99} (days)	Total dry solids (%)	Total C (%)	Total N (%)	EC (mS cm^{-1})	pH	PO_4^{3-} (mg cm^{-3})	NO_3^- (mg N cm^{-3})	NH_4^+ (mg N cm^{-3})	Background counts (CFU cm^{-3})
Slurry waste											
SW1	14·7	3	11	4·6	0·33	13·3	7·29	30	0·8	1362	$7·2 \times 10^5$
SW2	21·5	16	15	5·5	0·46	11·2	6·90	97	0·4	1409	$2·1 \times 10^7$
SW3	39·2	22	12	3·1	0·22	6·8	8·17	2	1·5	42	$1·6 \times 10^7$
SW4	17·3	18	9	3·5	0·18	7·1	7·92	3	1·3	195	$1·6 \times 10^7$
SW5	13·1	1	0·1	0·2	<0·01	0·9	6·56	25	0·4	48	$4·5 \times 10^3$
Abattoir waste											
AW1	20·5	29	17	9·2	2·91	15·7	6·91	201	6·1	1332	$1·0 \times 10^7$
AW2	17·2	27	5	1·4	0·90	11·7	7·21	1372	0·1	569	$3·0 \times 10^7$
AW3	11·2	4	8	4·2	0·51	14·9	4·44	697	147	44	$1·1 \times 10^7$
AW4	19·4	34	8	2·9	0·67	17·7	6·66	704	0·5	1913	$6·5 \times 10^6$
AW5	4·6	36	1	0·9	0·11	8·9	6·94	341	<0·1	939	$2·2 \times 10^6$
Sewage waste											
USW1	14·1	1	0·1	0·1	<0·01	1·1	7·03	2	<0·1	13	$9·0 \times 10^5$
USW2	36·8	6	0·1	0·1	<0·01	1·2	6·66	5	0·2	24	$9·5 \times 10^6$
USW3	10·1	3	0·3	0·2	0·03	0·7	6·43	12	<0·1	12	$8·0 \times 10^5$
TSW1	9·5	1	0·1	0·1	<0·01	1·6	7·26	3	<0·1	20	$1·4 \times 10^6$
TSW2	15·8	5	0·3	0·1	<0·01	2·1	6·55	28	<0·1	24	$1·2 \times 10^6$
TSW3	39·0	29	0·3	0·1	0·06	1·1	6·86	12	1·0	20	$2·2 \times 10^5$
TSW4	2·0	15	0·1	0·1	<0·01	0·6	6·69	6	<0·1	19	$1·6 \times 10^4$
TSW5	63·0	22	0·1	0·1	<0·01	1·3	6·68	4	0·2	43	$1·3 \times 10^7$
Sewage sludge waste											
SSW1	14·1	3	18	6·9	0·84	3·8	8·15	42	0·3	873	$1·4 \times 10^7$
SSW2	28·2	27	3	1·1	0·21	3·3	6·14	33	1·2	14	$2·3 \times 10^6$
SSW3	0·0	0	27	8·2	0·60	5·5	11·97	1	1·8	16	$9·0 \times 10^2$
SSW4	11·2	56	3	1·1	0·16	5·5	7·12	37	0·9	255	$9·0 \times 10^2$
SSW5	13·3	37	2	0·3	0·02	2·1	5·62	33	1·3	208	$3·0 \times 10^7$
Creamery waste											
CW1	11·3	11	0·3	0·1	<0·01	1·4	7·17	17	<0·1	10	$1·0 \times 10^8$
CW2	9·6	0	0·1	ND	ND	9·8	12·42	10	11·6	6	$1·4 \times 10^1$
CW3	24·0	>64	2·4	1·8	0·27	3·1	5·95	1	1·4	31	$6·0 \times 10^8$
CW4	33·0	>64	0·3	0·1	0·04	4·0	5·80	21	69·0	13	$1·0 \times 10^7$

Values represent means.

EC, electrical conductivity; T_{99}, time taken for *E. coli* O157:H7 to decline by two $\log_{10}$ units; ND, not determined.

of the dilutions in duplicate onto R2A Agar (Oxoid Ltd) to quantify the total number of aerobic heterotrophic bacteria. Similarly, dilutions were also plated out onto CT-SMAC agar to enumerate presumptive *E. coli* O157 colonies. R2A plates were incubated at 20°C for 48 h and CT-SMAC plates at 37°C for 15 h before enumeration.

Preparation and inoculation of microcosms

Waste (5 cm^3) was placed inside individual 31 ml sterile plastic bottles to give 42 replicate microcosms for each waste type. Filled microcosms were loosely capped to allow aeration and placed in an incubator at 10°C overnight to equilibrate. Bacterial inoculum was prepared by adding 100 μl of an overnight culture of *E. coli* O157:H7 to a flask containing 100 ml fresh LB broth followed by incubation at 37°C on an orbital shaker (150 rev min^{-1}) for 18 h. Liquid culture was then centrifuged at 10 000 × *g*, washed three times in 1/4-strength Ringer's solution (Oxoid Ltd) and diluted 10-fold in 1/4-strength Ringer's solution. Aliquots (500 μl) were then added to each microcosm and mixed by shaking gently by hand. To quantify the amount of *E. coli* O157 added to each microcosm, the inoculum was serially diluted and plated onto duplicate plates of CT-SMAC agar. An incubation temperature of 10°C was chosen to reflect the mean annual air and soil temperature in North Wales and

the average storage temperature at the majority of locations from which organic wastes were collected.

Escherichia coli O157:H7 survival in microcosms

Six replicate microcosms for each waste type were harvested within 2 h of inoculation, then on days 2, 4, 8 16, 32 and 64 after inoculation. Sterile 1/4-strength Ringer's solution (20 ml) was added to each microcosm which was then vortexed briefly, shaken at 150 rev min^{-1} on a reciprocating shaker for 15 min and then allowed to stand for 5 min following a second brief vortex. Serial dilutions of these solutions were plated in duplicate onto CT-SMAC agar and incubated at 37°C for 18 h. Colonies of typical *E. coli* O157 morphology were then counted. When counts were approaching the detection limit by this method, enrichment was used to screen for low numbers of viable cells.

Enrichment was carried out by adding 1 ml of the Ringer's solution/spiked waste mix from three replicate microcosms to 15 ml of modified tryptone soya broth (Oxoid Ltd) and shaking (150 rev min^{-1}) at 37°C for 6 h. Aliquots of the broth were then spread onto CT-SMAC agar and incubated as for quantitative plates. Presence or absence of colonies with typical *E. coli* O157 morphology were recorded. The detection limit of the CT-SMAC plate count procedure was 25 CFU cm^{-3} of waste and the detection limit of the enrichment technique was 5 CFU cm^{-3} of waste.

Statistical analyses

Wastes were assigned to categories of 'waste type' as given in Table 1. Data from *E. coli* O157:H7 plate counts were log_{10}-transformed and subjected to ANOVA with microcosm incubation time and waste type or waste name (Table 1) as factors using Genstat 5·4·1 (Rothamsted Research, Harpenden, UK). Least significant differences for the interaction terms were used to identify significant differences. A linear optimization routine in Sigmaplot 8·0 (SPSS Inc., Chicago, IL, USA) was used to calculate the time taken for a 2-log reduction in mean CFU (T_{99}) and these data were subjected to a one-way ANOVA with waste type (Table 1) as factors. Where *E. coli* O157 colonies were detected by enrichment only, a value equal to half the detection limit of the plate counts was used in the statistical analysis (12·5 CFU cm^{-3}).

RESULTS

Background microbiology of wastes

The number of aerobic heterotrophic bacteria in the wastes ranged widely from $1{\cdot}4 \times 10^1$ (creamery waste CW2) to $6{\cdot}0 \times 10^8$ CFU cm^{-3} (creamery waste CW3) when quantified with R2A agar (Table 1). Although presumptive *E. coli* O157 colonies were present in some of the wastes (e.g. three of the five slurries), the numbers were generally low and were generally equivalent to <0·5% of the *E. coli* O157 colonies recovered from spiked samples within 2 h of inoculation.

Inoculum and recovery of cells

The *E. coli* O157 inoculum added to each microcosm yielded an initial contamination level of 2×10^7 CFU cm^{-3} waste, based upon viable counts on CT-SMAC agar. The immediate recovery of *E. coli* O157 from the wastes ranged widely from 0 to 63% of the initial added inoculum (mean ± S.E.M. = 19 ± 3%; Table 1). When considering all the wastes, no significant relationship existed between the amount of inoculum immediately recovered and the amount of solids or background microflora in the wastes ($P > 0{\cdot}05$). Generally, the recovery of viable *E. coli* O157 cells from the wastes decreased with increasing incubation time ($P < 0{\cdot}001$). Although some fluctuation in pathogen numbers occurred over time (waste × time interaction, $P < 0{\cdot}001$), the greatest recovery of *E. coli* O157 over the whole experimental period was observed in creamery waste 4 (CW4; 2×10^7 CFU cm^{-3}) and was least in sewage sludge SSW3 and creamery waste CW2. In the latter two cases, no colonies were detected presumably because of their extreme alkalinity. There was no significant effect of waste type on pathogen decline as measured by T_{99} values which ranged from 0 day (SSW3; CW2) to over 64 days (CW3 and CW4; Table 1). When all of the wastes were considered together, none of the measured chemical characteristics could account significantly for differences in the T_{99} value, although there was evidence of a trend towards decreasing T_{99} values with increasing pH. In addition, background microflora counts made with R2A agar were also not significantly correlated with pathogen decline as measured by T_{99} values ($P > 0{\cdot}05$).

Sewage waste

In comparison with the other waste types, the measured chemical and physical characteristics of the treated (TSW) and untreated (USW) liquid sewage samples were similar in most respects (Table 1). In contrast, the sewage sludges had lower moisture contents and higher nutrient concentrations than the liquid sewages. This was particularly evident with respect to their available N and P content. Figure 1 shows the persistence of *E. coli* O157 cells in treated and untreated liquid sewage. It is clear that although pathogen persistence between independent replicates of the same waste were similar, persistence varied markedly between wastes of a similar nature. Generally, the decline of *E. coli* O157 cells in

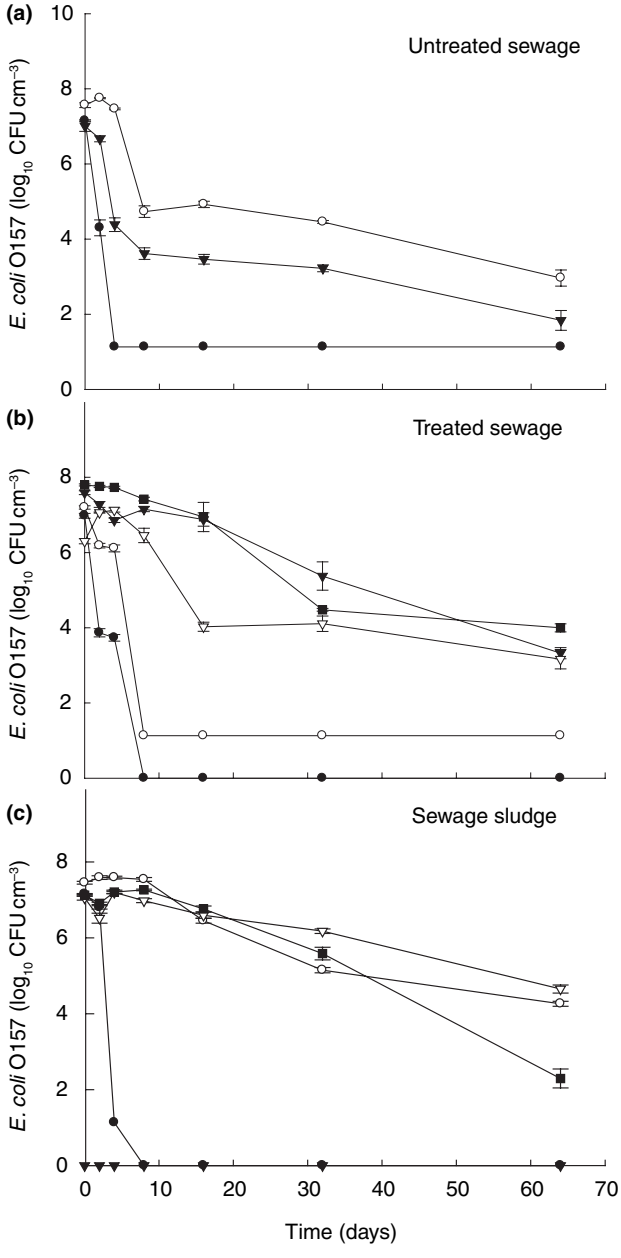

Fig. 1 Survival of *Escherichia coli* O157:H7 in sewage. (a) *E. coli* O157:H7 persistence in three untreated liquid sewage samples [influent sewage; (●) USW1, (○) USW2, (▽) USW3]. (b) *E. coli* O157:H7 persistence in five treated liquid sewage samples [effluent; (●) TSW1, (○) TSW2, (▽) TSW3, (▼) TSW4, (■) TSW5]. (c) *E. coli* O157:H7 persistence in five sewage sludges [(●) SSW1, (○) SSW3, (▼) SSW4, (■) SSW5]. The data shown are $\log_{10}$ (y + 1) transformed means of plate counts (CFU cm^{-3}). Values represent mean ± S.E.M. (n = 6)

liquid sewage which had received no treatment (i.e. wastewater entering the treatment works) was greater than in treated sewage (i.e. wastewater leaving the treatment works; $P < 0{\cdot}001$). This trend was reflected in the T_{99} values (Table 1). Low numbers of cells could still be

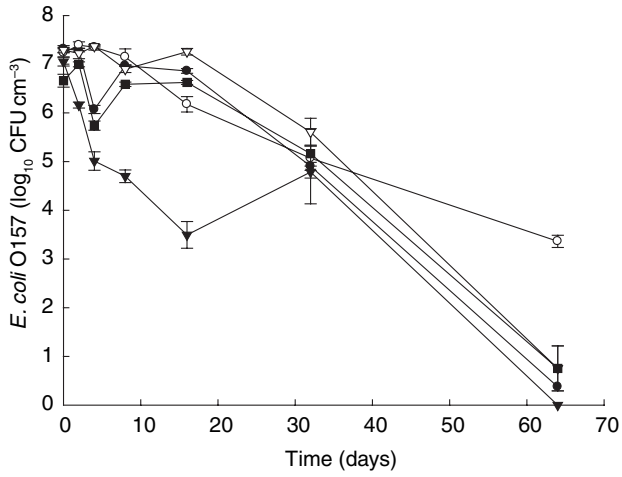

Fig. 2 Survival of *Escherichia coli* O157:H7 in five different abattoir wastes (AW1 to AW5). The data shown are $\log_{10}$ (y + 1) transformed means of plate counts (CFU cm^{-3}). Values represent mean ± S.E.M. (n = 6). (●) AW1, (○) AW2, (▽) AW3, (▼) AW4, (■) AW5

detected following enrichment throughout the course of the 2-month experiment in seven of the eight liquid sewages.

The recovery of *E. coli* O157:H7 from a range of contaminated sewage sludges over time is shown in Fig. 1. Apart from sewage sludge 3, which had been treated with $Ca(OH)_2$ and possessed an extreme pH, the initial recovery of the added pathogen was similar to that of liquid sewage samples ($P > 0{\cdot}05$). The gradual decline in pathogen numbers in the sewage sludges over the 64-day experimental period was also similar to that of the liquid sewage samples.

Abattoir wastes

Generally, abattoir wastes were characterized by high nutrient contents in comparison with most of the other waste types (Table 1). The initial recovery of the pathogen was similar in all the abattoir wastes tested ($0{\cdot}9 \times 10^6$ to $4{\cdot}2 \times 10^6$ CFU cm^{-3}; Fig. 2). Viable counts of *E. coli* O157 in four of five of the abattoir wastes decreased little (*ca* 1 $\log_{10}$ unit) until day 16 after which population numbers declined at a faster rate. After 64 days, cells were detected in all abattoir wastes with the exception of AW1. The T_{99} values reflected the initial persistence of the pathogen within the abattoir wastes with a mean T_{99} value of 26 ± 6 days.

Cattle slurry

The chemical characteristics of the five slurries were very different as shown in Table 1. In all the cattle slurries tested, the amount of *E. coli* O157 surviving declined steadily over time with the rate of pathogen decline differing markedly between slurry type (Fig. 3; $P < 0{\cdot}001$). After 64 days of

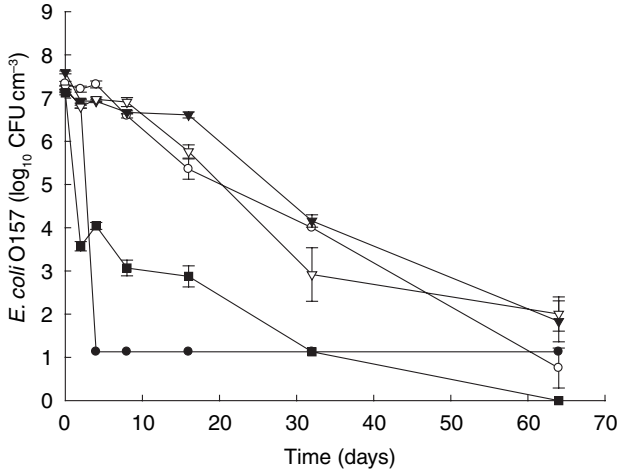

Fig. 3 Survival of *Escherichia coli* O157:H7 in five different slurry wastes (SW1 to SW5). The data shown are $\log_{10}$ (y + 1) transformed means of plate counts (CFU cm^{-3}). Values represent mean ± S.E.M. (n = 6). (●) SW1, (○) SW2, (▽) SW3, (▼) SW4, (■) SW5

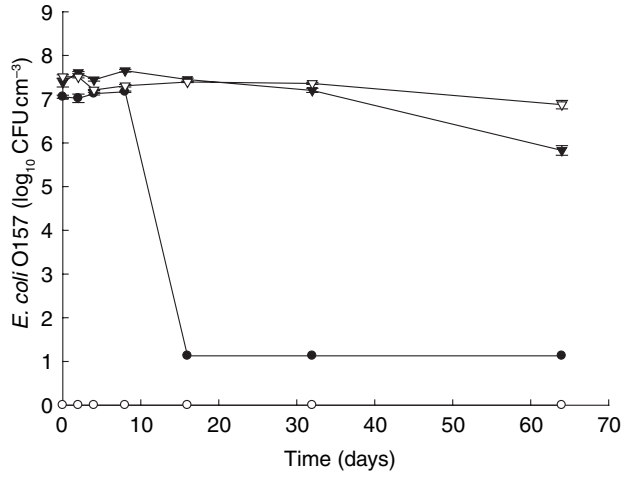

Fig. 4 Survival of *Escherichia coli* O157:H7 in four different creamery effluent wastes (CW1 to CW4). The data shown are $\log_{10}$ (y + 1) transformed means of plate counts (CFU cm^{-3}). Values represent mean ± S.E.M. (n = 6). (●) CW1, (○) CW2, (▽) CW3, (▼) CW4

incubation, *E. coli* O157 was still detectable in most of the slurries with maximal amounts recovered from SW4 ($1{\cdot}0 \times 10^2$ CFU cm^{-3}). Generally, the T_{99} values reflected the rapid decline in pathogen persistence in slurry with a mean (±S.E.M.) T_{99} value of 12 ± 4 days.

Creamery waste

The creamery wastes differed significantly in their chemical and biological composition with CW2 in particular having a very low indigenous microbial population and extremely high pH (Table 1). This is likely to be the result of use of strongly alkaline chemicals for cleaning of creamery surfaces and equipment. The persistence of *E. coli* O157 in the creamery wastes is shown in Fig. 4. Cells could not be recovered from creamery waste CW2, within 2 h of inoculation or at any point thereafter. Survival characteristics in CW3 and CW4 are clearly different to those of CW1 and CW2 ($P < 0{\cdot}001$). There was very little decline in *E. coli* O157:H7 in creamery wastes CW3 and CW4 over the entire 64-day experimental period (*ca* 0·5–1·5 $\log_{10}$ unit drop in CFU).

DISCUSSION

The most important finding of our study is that *E. coli* O157:H7 was able to persist for more than 2 months in 21 of the 27 wastes tested. Another key finding of our work was that even wastes of a similar origin behave very differently with respect to their *E. coli* O157 persistence. Long-term persistence of this organism has been demonstrated previously in some organic wastes, notably slurries and manures (Kudva *et al.* 1998; Bolton *et al.* 1999; Fukushima *et al.* 1999), however, this study is the first to compare survival in a wide range of organic wastes from diverse sources. In some of the wastes tested, after an initial population decline, *E. coli* O157 numbers increased during the early stages of the experiment (up to 4 days). This is consistent with the short-term study of Hepburn *et al.* (2002) on animal blood which suggests that pathogens are capable of growing within contaminated organic wastes. Importantly, however, this growth phase was not prolonged, possibly because of a range of factors such as depletion of available resources, increased microbial competition and protozoal grazing, as well as changes in the chemical and physical nature of the waste (e.g. pH, O_2 status etc.).

Organic wastes provide a vehicle by which *E. coli* O157:H7 may enter and become disseminated throughout the agricultural environment, thus posing a potential health risk. All of the wastes employed in this study, with the exception of raw and primary-treated sewage, have recently been applied to land within Europe. Although legislation prevents the disposal of untreated sewage to land in Europe and the USA, in many less developed countries raw and treated sewage liquids are frequently used for the irrigation of crops (Scott *et al.* 2000). Disposal of organic wastes to land is likely to increase as a consequence of the increasing cost of landfill and a Europe-wide decline in soil quality which has been primarily caused by a loss of soil organic matter. Where possible, many organic wastes are disposed off quickly to land as storage is not viewed as a desirable option because of a variety of economic and environmental constraints (e.g. storage space and costs, increased risk of odours). Long-term storage of wastes, however, is often a

necessity when unsuitable land spreading conditions occur. For example, slurries are frequently stored over the winter months in on-farm slurry tanks and sewage sludge may be stored at the treatment site prior to disposal. Abattoirs seldom store waste on-site, however, sub-contractors who remove the waste may store the waste until disposal sites become available and appropriate weather conditions prevail (Anon. 2001). Storage has been suggested previously as a means of reducing pathogen numbers in contaminated animal waste (Jones 1999). Our results indicate that storage could be considered as a relatively cheap option for reducing, but not eliminating, *E. coli* O157 in wastes, particularly where other options for reducing pathogens are also likely to be expensive (e.g. anaerobic digestion), or may render the waste less useful (e.g. liming).

Following waste application to land, pathogen survival is likely to be affected by a range of factors including solar radiation, desiccation, temperature and contact with soil (Bolton *et al.* 1999). While it is dangerous to extrapolate our data to try to predict *E. coli* O157 survival after land application, higher pathogen numbers remaining in waste at the time of application would pose an increased risk of the organisms surviving in sufficient numbers to cause infection. Thus the long-term survival of *E. coli* O157 in these wastes has implications for the subsequent transmission to crops and adjacent watercourses and also to direct infection of humans and animals in contact with the contaminated land (Maule 2000).

Direct contact with animal faeces has been implicated in human *E. coli* O157 infection, and consequently the disposal of pathogen contaminated animal slurries and manures poses a significant problem. Kudva *et al.* (1998) found that cells inoculated into bovine slurry at 10^7 CFU cm^{-3} were undetectable within 5 days at 23 and 37°C, although counts in the region of 10^5 CFU cm^{-3} were evident after 1 month in bovine slurry incubated at 4°C. Our study supports the work of Kudva *et al.* (1998) in that viable cells were still detectable in all but one of the slurries 2 months after inoculation at an incubation temperature of 10°C (*ca* 10^2 CFU cm^{-3} at 64 days). In most countries there is no statutory guidance on the length of time that animals should be excluded from land following abattoir and slurry waste application. Guidance from UK regulatory authorities (DEFRA and Environment Agency) advises that animals should be kept off the land for 42 days following application. Currently, even after legal compliance, it is still possible that sufficient numbers of cells could survive in wastes spread to land to cause infection in humans, re-infection of animals and dissemination of the organism through the environment. In order to determine absolute survival times for *E. coli* O157 in organic wastes used in this study, it would be necessary to repeat the experiment with a longer period of incubation. Use of immunomagnetic separation techniques towards the end of the storage period could also improve recovery of the pathogen. In addition, it would also be desirable to quantify the relative number of viable and viable but nonculturable *E. coli* O157.

As *E. coli* O157 is located primarily within the animal gut, the handling and disposal of the intestinal contents upon animal slaughter poses a potential environmental hazard. The only previous study on *E. coli* O157 survival in slaughterhouse waste was carried out by Hepburn *et al.* (2002) in which the authors measured the short-term (up to 48 h) survival in ovine and bovine blood and gut contents. Although conducted at a different temperature, the short-term survival rate of *E. coli* O157 in gut contents in our experiment was similar to that observed by Hepburn *et al.* (2002). However, importantly we have also demonstrated significant long-term persistence in abattoir waste (over 2 months). We conclude that persistence of *E. coli* O157 in abattoir waste handling equipment, holding tanks and landspreading machinery is possible and that cross contamination of wastes batches may occur. Furthermore, as liquid abattoir waste has in the past been routinely spread to agricultural land in the UK, it is possible that the disposal of contaminated waste may have exacerbated the spread of *E. coli* O157 within domesticated and wild animal populations.

Although sewage has been widely implicated in the coliform contamination of freshwater, groundwater, marine environments and foodstuffs (Jones 1999; Wachtel *et al.* 2002), this is the first study to report on survival of *E. coli* O157 in sewage. Decline of *E. coli* O157 was generally more rapid in sewage wastes than in creamery and abattoir wastes and slurries, although cells persisted throughout the experiment in seven of eight liquid sewages and in three of the five sludges. In combination with studies on *E. coli* O157 survival in soil and on vegetation, we conclude that application of contaminated sewage to crops is likely to pose a significant health risk (Jones 1999; Maule 1999). Unfortunately, because of the health and safety constraints imposed on field experimentation with *E. coli* O157, it has not been possible to monitor the persistence of *E. coli* O157 through the various stages of processing at a sewage treatment works, however, this warrants further attention. As the relative persistence of *E. coli* O157 in environmental matrices in comparison with other nontoxigenic *E. coli* strains remains unknown, it is difficult to extrapolate with certainty from existing research on non-VTEC *E. coli* strains. Further work is therefore required to compare the environmental persistence of VTEC *E. coli* with other non-VTEC *E. coli*.

In a few of the organic wastes examined here, *E. coli* O157 was found to die off extremely quickly (e.g. SSW1, SSW3, TSW1, CW4). In most wastes, this was associated with a high degree of alkalinity, in some cases because of the known addition of $Ca(OH)_2$ (SSW3) or in other cases because of the

probable addition of NaOCl (CW4) during industrial wastewater processing. This clearly shows that *E. coli* O157 can be effectively eradicated by raising the pH of organic wastes (Bujoczek *et al.* 2001). However, it also highlights the variable nature of wastewater. Wastes sampled daily from CW4 over a period of 7 days showed substantial variation in pH (6–12) which appears to be a consequence of processes used within the industry (data not shown). Furthermore, recovery of the initial inoculum of *E. coli* O157:H7 varied between both similar and different waste types, potentially influencing detection of the organism. This highlights the need to examine a wide range of samples before recommendations about persistence in different environmental matrices can be made.

In general, the long-term storage of organic wastes led to a significant and gradual decline in *E. coli* O157:H7 numbers. Consequently, storage may be a useful means of reducing the pathogen load of wastes destined for land application. However, in most cases, long-term storage cannot be expected to completely eliminate pathogens from waste and consequently it is likely that application of untreated contaminated waste to land will exacerbate the environmental spread of *E. coli* O157. With regard to risk assessment, pathogen persistence even in waste types of the same origin was found to be highly variable. Consequently, further work is required to determine the critical factors that regulate pathogen survival in waste with an aim to devise effective management strategies to minimize pathogen load.

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