

Fate of *Escherichia coli* originating from livestock faeces deposited directly onto pasture

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ABSTRACT

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Aims: To determine the fate of *Escherichia coli* deposited onto grassland via faeces, from naturally colonized cattle, sheep or pigs.

Methods and Results: Groups of cattle, sheep and pigs were penned outdoors on grass during November, and removed after 14 days. *Escherichia coli* populations in the ground declined over 134 days from initial average levels of 5.34, 4.31 and 4.96 log₁₀ CFU g⁻¹ in cattle, sheep and pig pens, respectively. The maximum *Escherichia coli* survival time was up to 162 days (190 days taking sampling interval and deposition time into account), but numbers varied significantly amongst the 20 replicates taken each day. *Escherichia coli* originating from cattle and sheep had average decimal reduction times (*D*-values) of 38 and 36 days, respectively; *E. coli* originating from pigs declined significantly faster (average *D*-value of 26 days).

Significance and Impact of the Study: *Escherichia coli* from livestock faeces can survive on grass for at least 5–6 months, affording opportunity for pathogenic biotypes to contaminate animals, plants or water.

Keywords: bacterial reduction, environment, manure, O157, Shiga toxin-producing *E. coli*, survival, verocytotoxigenic *E. coli*.

INTRODUCTION

Animal faeces can contain pathogenic bacteria including *Escherichia coli* O157, *Salmonella*, *Listeria monocytogenes* and *Campylobacter*. In recent studies, fresh cattle faeces naturally harboured an average of 43 *E. coli* O157 g⁻¹ (Fegan and Desmarchelier 2003). In addition, when fresh manures from cattle, sheep or pigs contained *E. coli* O157, and were collected from the houses where they were deposited, average levels were 2.89–3.59 log *E. coli* O157 g⁻¹ (Hutchison *et al.* 2002).

Livestock on farms can be housed indoors, when their waste is normally collected and then stored in an effort to reduce pathogen levels. Stored waste of variable ages is then usually disposed of by land-spreading. However, livestock can also be kept outside to graze on pasture. In that case, their faecal material is deposited directly onto the ground and is

clearly not treated by any intervention method to reduce the levels of any pathogenic bacteria that may be present.

The survival of pathogens originating from livestock faeces deposited on farm land during grazing is relevant for food safety. Pathogens may be re-cycled from animal-to-animal during grazing via external contamination of plant surfaces, or can potentially contaminate crops via internalization through root systems. Pathogens may also contaminate waterways via rainfall run-off.

Previous studies into the fate of bacterial pathogens in animal wastes have frequently been reliant on the organisms being inoculated into livestock wastes (Kudva *et al.* 1998; Bolton *et al.* 1999; Fukushima *et al.* 1999; Himathongkham *et al.* 1999; Jiang *et al.* 2002; Hutchison *et al.*, in press), due to the difficulty in obtaining animals that are naturally shedding pathogens in sufficiently high numbers for their decline to be followed. Laboratory-based studies have shown that *E. coli*, including serogroups O157, O111 and O26 survive for up to several months depending on the conditions, in experimentally inoculated animal faeces or

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wastes (Wang *et al.* 1996; Kudva *et al.* 1998; Bolton *et al.* 1999; Fukushima *et al.* 1999; Himathongkham *et al.* 1999). Jiang *et al.* (2002) found *E. coli* O157 survived up to 56 days in faeces : soil mixtures at 5°C during laboratory storage. On-farm, Bolton *et al.* (1999) inoculated *E. coli* O157 into cattle faeces spread on grass pasture, and recovered them from two of 10 soil replicates examined after 99 days. In addition, Kudva *et al.* (1998) inoculated sheep with *E. coli* O157 and recovered the organism from their faeces 1 year after their waste had been heaped on concrete. Few studies have, however, examined the survival of bacteria which occur naturally in high numbers in livestock faeces in the natural farm environment. Therefore, we examined the fate of naturally occurring *E. coli* after direct faecal deposition from cattle, sheep and pigs on open pasture land in order to determine their potential for survival.

MATERIALS AND METHODS

Farm land and animal stocking

Groups of healthy adult livestock composed of eight cattle, 12 pigs and 20 sheep were obtained from local farms. During November 2002, each of the three livestock species was penned outdoors separately in 0.25 ha of fenced arable grassland (sandy-textured alluvial soil), producing heavy stocking densities. The pens were adjacent to each other and the soil temperature at 8 cm depth was recorded during the study [Tiny Talk dataloggers; Orion Components (Chichester) Ltd, Chichester, UK]. The animals had access to water *ad libitum* and were fed concentrates, while regular checks were made on their health. After 7 days, one sample consisting of up to 30 handfuls of fresh faeces was collected from the ground in each pen. After 14 days, the animals were removed, leaving heavily faecally contaminated ground in the pens.

Collection of faeces : soil material from the ground in the pens after faecal deposition

Within each pen, 20 sampling templates were laid on areas with the heaviest visible deposition of faecal material. Material within the templates was not disturbed by trampling, and was exposed to natural weather conditions during the course of the study (day 0 was 18 November; day 218 was 24 June). Random 5 cm deep soil cores ($n = 20$) were taken from within each sampling template on days 0 (the day the animals were removed from the pens), 2, 4, 6, 16, 23, 49, 63, 78 and thereafter at approximately 14-day intervals until day 218. The 20 random soil cores from each template were combined in a stomacher bag, producing one faeces : soil sample. The 20 faeces : soil samples were placed immediately in a cool box packed with ice and analysed within 24 h.

In addition, cores of control soils which had not contacted livestock faeces for the previous 2 years were taken at each sampling interval from four sampling templates laid adjacent to the livestock pens.

Determination of *E. coli* in faeces collected from the ground in the pens, faeces : soil samples or control soils

Numbers of *E. coli* were determined by homogenizing 25 g of material (faeces collected from the ground in the pens, faeces : soil samples or control soils) with 225 ml of Maximum Recovery Diluent (MRD; Oxoid, Basingstoke, UK) in a stomacher (Colworth 400; Seward Medical, London, UK) for 2 min. Serial decimal dilutions of each homogenate were prepared in MRD, and 1 ml volumes of appropriate dilutions were inoculated into pour plates of Violet Red Bile Agar (VRB; Oxoid). Plates were overlaid with VRB before being incubated at 44°C for 24 h. The limit of detection was 10 CFU g⁻¹. Typical purple colonies with a precipitate were enumerated as *E. coli*.

Analysis of results

Raw *E. coli* counts were normalized by transforming to log₁₀ CFU g⁻¹. Means, associated standard deviations and trendlines from the linear parts of each survival curve were calculated from each set of 20 replicates using Excel (Microsoft Excel 97, Microsoft Corporation, 1997). One-way analysis of variance was performed on datasets obtained at each sampling time, and overall means were assessed using the general linear model with repeated measures (SPSS 11.5, SPSS Inc., Chicago, IL, USA).

RESULTS

After 14 days, the condition of the soil was different for each livestock species. The ground on which the pigs had been penned was substantially trampled, with no remaining intact grass, and very few individual faecal droppings visible, as they had mostly become incorporated into the soil. Although the grass in the cattle pen was also largely depleted, some intact faecal deposits were visible on the surface of this pen. The ground in the sheep pen still contained visible grass, with a root system and topsoil, and the sheep faeces appeared mostly intact.

Fresh cattle, sheep and pig faeces collected 7 days after the animals had been introduced to the pens contained, on average, *E. coli* levels of 7.70, 7.59 and 7.48 log₁₀ CFU g⁻¹, respectively. However, by the time the animals were removed from the pens, *E. coli* levels in the soils averaged 5.34, 4.31 and 4.96 log₁₀ CFU g⁻¹, respectively, in material from the cattle, sheep and pig pens. The average *E. coli*

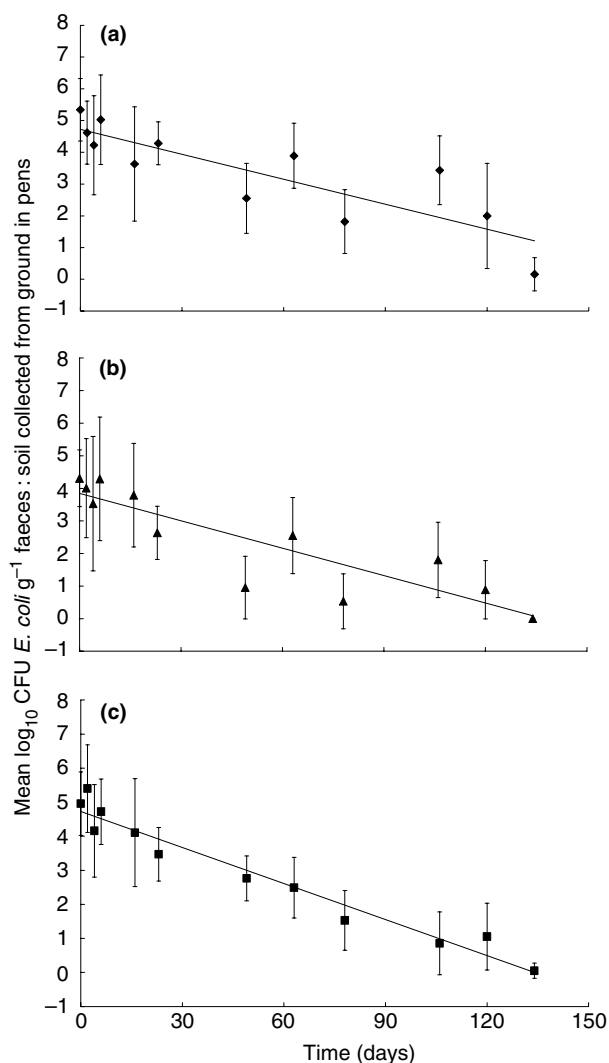


Fig. 1 Decline in *Escherichia coli* populations in faeces : soil mixtures taken from the ground after housing (a) cattle, (b) sheep and (c) pigs. Each point represents the average of 20 replicates. Error bars indicate standard deviations at each sampling time

populations declined over 134 days (Fig. 1) to 0.50 log CFU g⁻¹ or less, which was similar to levels found in control soils. The maximum survival time observed for *E. coli* in any of the replicate samples was 162 days (Table 1). There were statistically significant differences in the number of *E. coli* originating from the three livestock species on some individual sampling days, although these differences were not consistent. For example, on day 49, significantly more *E. coli* were found in material from the cattle and pig pens compared with material from the sheep pen (data not shown; $P < 0.001$), while on day 63, significantly greater numbers of *E. coli* were found in material from the cattle pen, compared with those found in material originating from the sheep or pig pens (data not shown;

Table 1 Survival data for *Escherichia coli* originating from livestock faeces

Livestock species	<i>D</i> -values (days)	Survival time (days)*
Cattle	38	162
Sheep	36	120
Pig	28	162

*Survival time: the length of time before *E. coli* was not detected in any replicate.

$P < 0.001$). Overall, the average declines measured were first-order kinetics, with no clear evidence of shoulders or tails, enabling decimal reduction times (*D*-values) to be calculated from the linear parts of the survivor curves (Table 1). The *E. coli* that originated from pig faeces declined significantly faster (1-log drop over 28 days) than those derived from either cattle or sheep faeces (1-log drops over 38 or 36 days, respectively; $P < 0.05$).

The variability in *E. coli* counts for each livestock species was very high (Fig. 1). For example, on day 4, the minimum and maximum recorded *E. coli* levels found amongst the 20 replicates from the cattle pen were 0 and 7.18 log₁₀ CFU g⁻¹, respectively. We compared the daily differences, up to 134 days, between minimum and maximum log₁₀ CFU g⁻¹ *E. coli* counts in each set of 20 replicates. Overall, the replicates from the ground of the pig pen tended to be less variable (mean difference of 3.69 log₁₀ CFU g⁻¹) than the replicates from cattle and sheep pens (mean differences of 4.37 and 4.36 log₁₀ CFU g⁻¹, respectively), although this was not statistically significant.

The soil temperature at 8 cm depth averaged 6.5°C (range 0.4–15.6°C) during the course of the study, and 5.1°C (range 0.4–8.8°C) during the first 134 days; this was the time when most of the *E. coli* reduction occurred.

DISCUSSION

Escherichia coli levels in freshly deposited faeces from all three species of livestock were similar, around 7 log₁₀ CFU g⁻¹, which is comparable with numbers of coliforms or *E. coli* found in cattle rectal digesta (Grauke *et al.* 2003; C.-A. Reid, S. M. Avery, P. Warriss and S. Buncic, 2002, personal communication).

The average rate of decline for *E. coli* originating from pig faeces was significantly faster than decline rates observed for *E. coli* from either cattle or sheep faeces. Numerous factors influence the speed with which *E. coli* levels on land reduce including the soil type, rainfall, UV radiation, temperature, animal diets, in-soil predation by other microorganisms, the physiological status of the organisms and strain variability. Other relevant factors include the organisms being washed away via precipitation (Bolton *et al.* 1999; Gagliardi and

Karns 2000), as well as their growth (Fukushima *et al.* 1999; Gagliardi and Karns 2000) and death. As none of the soil or external environmental factors varied between the pens used in the current study, the faster rate of *E. coli* decline when originating from pig faeces may have been due to physiological differences in the *E. coli* strains and/or the chemistry of the faeces. A similar observation was reported for declines of pathogenic bacteria inoculated into stored slurries on-farm (Hutchison *et al.* 2002).

The average *D*-values for *E. coli* originating from live-stock faeces ranged from 28 to 38 days. This was longer than *D*-values of 9–19 days reported for *E. coli* O157 in fresh cattle faeces stored at 4°C in the laboratory (Himathongkham *et al.* 1999). This may be due to *E. coli* strain/serotype differences between the current study and that of Himathongkham *et al.* (1999), or to unknown factors that influence the fate of *E. coli* in freshly voided faeces. In addition, bacteria shed in the faeces of naturally colonized animals may survive in soil longer than those cultured and experimentally inoculated under laboratory conditions (Kudva *et al.* 1998).

It is pertinent to examine the overall survival times of bacterial populations in soil from a food safety perspective, as they may contribute to bacterial re-cycling when animals are grazing, and to bacterial loads on crops or in run-off waters. Our study has shown *E. coli* in the faeces of naturally colonized cattle, sheep or pigs had extensive survival times on the ground (on average, 134 days, but ranging up to at least 162 days). When we take into account the fact that some bacteria seen at the end of the study may have been deposited on the ground 14 days before sampling commenced, and the fact that sampling days were at 14-day intervals, the survival times may have ranged up to 190 days. In addition, resuscitation methods were not used and some surviving *E. coli* were likely to have been washed away from the sampling site (Bolton *et al.* 1999). Overall, the survival time for *E. coli* in this agricultural soil could, in fact, be longer than the measured 162 days. On the contrary, *E. coli* has been shown to survive longer at lower (around 5°C) rather than higher (>20°C) temperatures under a range of laboratory conditions (Clavero and Beuchat 1996; Cools *et al.* 2001; Uyttendaele *et al.* 2001; Wait and Sobsey 2001). If this study were repeated during summer, measured survival times could be different (likely shorter) than those determined in this study, and this would be partially due to higher average temperatures that occur during summer.

In other studies, *E. coli* O157 inoculated into fresh livestock faeces and stored in laboratory conditions at 4–5°C survived around 50–70 days (Wang *et al.* 1996; Fukushima *et al.* 1999) or around 100 days (Kudva *et al.* 1998; Bolton *et al.* 1999).

The large variation in *E. coli* numbers observed amongst the replicates examined may have been partially due to the

method used to count the organisms, as a resuscitation step was not included. However, the lack of soil/faeces homogeneity that may naturally occur in ground contaminated with faeces likely contributed some of the observed variation. This may be supported by the fact that the ground in the pig pen appeared more mixed by trampling and digging than the ground in the other pens, and the replicates from the pig pens tended to be less variable than replicates from either the cattle or sheep pens.

The results of this study indicate that the deposition of livestock faeces onto land used for food production could lead to microorganisms, including pathogenic types remaining on land for substantial times. This could provide opportunity for pathogens to enter the food chain. However, further studies are necessary to confirm whether such survival of microorganisms outside animal gastrointestinal tracts does contribute to pathogen re-cycling or to contamination of plants or waters on-farm.

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