

THE SURVIVAL OF *ESCHERICHIA COLI* O157 IN CATTLE MANURE DEPENDING ON HANDLING STRATEGY

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Introduction

Verocytotoxicogenic *Escherichia coli* (VTEC) is a human pathogen and has been implicated in numerous outbreaks of hemorrhagic colitis and the life-threatening haemolytic uremic syndrome (HUS). *E. coli* serotype O157 strains have been considered as the most important VTEC strains in terms of severe human outbreaks (Nataro and Kaper, 1998). VTEC is a zoonotic bacteria and it has been demonstrated that cattle are one of the primary reservoir of the pathogen. The faecal excretion rates of *E. coli* O157 from infected cattle typically range from 10^2 to 10^5 cfu/g faeces (Wang and Doyle, 1996). However, human ingestion of only 50 cells of *E. coli* O157 is required to develop full symptoms (Tilden *et al.*, 1996). Mostly, cattle manure is not specifically treated to reduce their bacterial content. Thus, cattle manure may transmit *E. coli* O157 to pastureland and to associated water systems by the land-based disposal of contaminated cattle manure. However, while soil and vegetation can be expected to directly influence the survival of this pathogen, relatively little is known concerning the transmission routes of *E. coli* O157 in agricultural environment.

The Swedish situation concerning human pathogenic VTEC is around 100 reported cases each year among a population of 9 million people. Around 10% of the Swedish herds are carriers of the pathogen with a geographical concentration to the south and south west of Sweden (23% in Halland and Skåne; Eriksson *et al.*, 2005). In order to control this agent five Swedish governmental institutes have developed a national action plan on how to best handle VTEC within the food chain, including animals in the primary production. In this action plan some knowledge gaps are identified and one deals with biosecurity aspects of re-circulation of manure back to the food chain. This is a subject of interest at the National Veterinary Institute (SVA). Our intention is to identify transmission routes of VTEC by manure to crop, soil and water resources in order to work out recommendations of manure management strategies to the farmers. Our work also includes development of hygienisation methods for infected manure.

The present set of incubation studies were undertaken to; evaluate the survival of *E. coli* O157 in stored manure and in manure amended soil at different temperature regimes (4, 15 and 25°C), evaluate the potential to use urea as disinfections agent to treat infected manure; study the strain-dependent variability in survival of *E. coli* O157.

Materials and methods

The reduction rate of spiked *Escherichia coli* O157 were monitored in stored cattle manure and in manure amended soil at three temperature regimes. Pure culture of a non-verotoxin-producing *E. coli* O157 (CCUG 44857), pre-grown on horse blood agar (Blood agar base no.2, LabM) for 24 h at 37°C and suspended in phosphate buffer, pH 7.2, was used as inoculum. Fresh cattle manure was collected from a dairy barn and spiked with the inoculum. A sandy loam soil was collected from a field (upper 10-30 cm), air dried, sieved at 4 mm and mixed with spiked cattle manure corresponding to a dose of 30 ton/ha. The manure-amended soil was adjusted to 80% of water holding capacity by adding the necessary amount of water. Portions with 300 g composite samples of manure and manure-amended soil were placed in aerated microcosms (0,5 dm³). Three microcosms were placed in each of three incubators at 4°C, 15°C and 25°C, respectively. The recovery and enumeration of *E. coli* O157 was determined at 9 occasions during the incubation period of 70 days.

The strain-dependent variability in survival of *E. coli* O157 in manure-amended soil was monitored. Three vero-cytotoxin-producing strains of *E. coli* O157 (PG 21, PG59 and PG 94), previously isolated from cattle manure, were provided from a strain collection (SVA, Uppsala, Sweden). The non-verotoxin-producing *E. coli* O157 (CCUG 44857) was used as control. The production of each inoculum and the preparation of manure-amended soil were done according to the protocol described above. Three portions with 300 g composite samples of each bacterial strain were placed in aerated microcosms (0,5 dm³) and incubated at 25°C. The recovery and enumeration of each strain were determined at 8 occasions during the incubation period of 64 days. Water losses due to evaporation were corrected for during both incubation studies with sterile distilled water.

The potential to use urea as disinfections agent to treat infected manure was evaluated. Cattle manure was spiked with *E. coli* O157 (CCUG 44857) as described above. Urea (Harnstoff, MERcuenrolab) was incorporated to a concentration of 2%. Four composite samples of 300 g were placed in aerated microcosms (0,5 dm³), incubated at 4°C, and sampled on day 1, 2 and 5.

The recovery and enumeration of *E. coli* O157 was quantified by direct viable counting on selective media. Thus, 3 g sample was stomached with 27 ml phosphate buffer (pH 7.2) followed by 10-fold serial dilutions in sodium chloride, 0.86-0.90%. Three dilutions of each sample were plated on Cefixime-Tellurite Sorbitol MacConkey (CT-SMAC; Oxoid) and incubated at 37 °C for 24. The colonies were counted by colony appearance and observation of cell morphology. Confirmation was done by agglutination with diagnostic reagents for *E. coli* O157 latex test (Oxoid, England).

The exponential death of *E. coli* O157 was expressed in the form $N = N_0e^{-kt}$ where N is the concentration of microorganisms at time t , N_0 the initial concentration of *E. coli* O157 and k the specific reduction rate. Students t-test was used to find significant differences ($p < 0.05$) between treatments.

Results and discussion

We found a clear relationship between temperature and reduction rate, k , of *E. coli* O157 during storage of infected cattle manure. The k at each temperature showed that 25°C (0.037 h⁻¹), 15°C (0.0046 h⁻¹) and 4°C (0.0015 h⁻¹) all differed from each other (fig. 1). Similar incubation studies have shown the *E. coli* O157 to survive longer at low temperatures (Wang and Doyle, 1996). In a out-door study Kudva *et al.* (1998) found *E. coli* O157 to remain viable in non-aerated cattle manure for longer than 12 months. Our study further confirms that *E. coli* O157 can survive well in faces, and that the survival to some extent is depending on the temperature during manure storage.

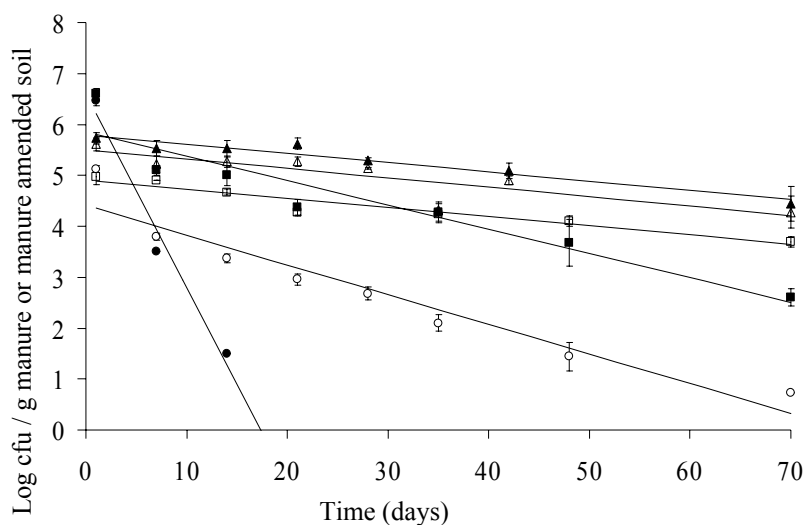


Figure 1. Reduction of *Escherichia coli* O157 in cattle manure and manure amended soil during incubation at three temperature regimes. (▲, ■, ●) storage at 4, 15 and 25°C respectively; (△, □, ○) manure amended soil at 4, 15 and 25°C respectively.

The reduction rate of *E. coli* O157 in manure amended soil at each of three temperatures showed that 25°C (0.055 h⁻¹) was different from 15°C (0.0018 h⁻¹) and 4°C (0.0017 h⁻¹), but that there was no significant difference between 15 and 4°C (fig. 1). Cools *et al.* (2001) showed that a low incubation temperature (5°C)

favoured the *E. coli* O157 survival in soil. Further, Gagliardi and Karns (2002) found a prolonged persistence of *E. coli* O157 when the soil was frozen. However, repeated freeze-thaw cycles seem to be detrimental to the survival of *E. coli* O157 on arable land (Natvig *et al.*, 2002). In addition to the climate conditions other factors such as soil type, tillage practice and indigenous microorganisms of the soil appears to be contributory factors to the pathogen's survival when introduced to the soil (Gagliardi and Karns, 2000; Jing *et al.*, 2002). Moreover, some field studies have been made and it appears that vegetation may provide a protective environment for *E. coli* O157, which enhances survival of this pathogen (Sjogren, 1995; Ogden *et al.*, 2002).

We found the k of *E. coli* O157 strain PG 59 (0.0051 h^{-1}) and strain PG 94 (0.0051 h^{-1}) to be different from the non-toxigenic control CCUG 44857 (0.0060 h^{-1}) but no difference was found between PG 21 (0.0056 h^{-1}) and the control (fig. 2). We also found a strain-dependent variability in reduction rate of *E. coli* O157 between strain PG 21 and PG 59

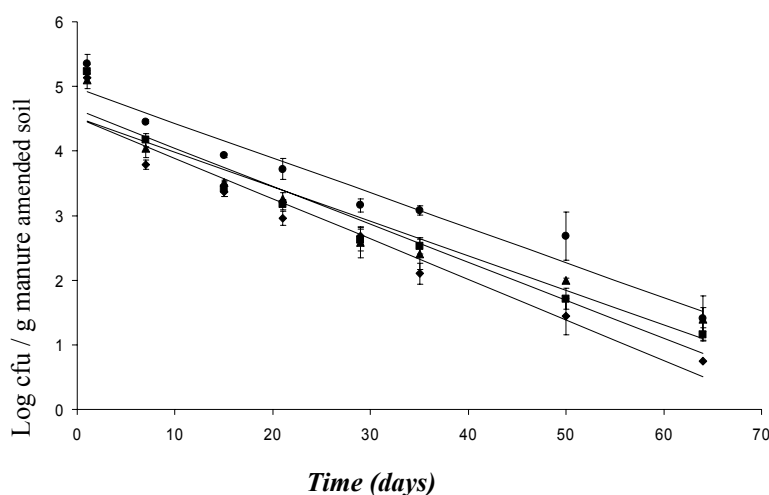


Figure 2. Reduction of four strains of *E. coli* O157 in manure amended soil during incubation at 25°C. (◆) strain CCUG 44857; (■) strain PG 21; (▲) strain PG 59; (●) strain PG 94.

(fig. 2). In similar Fukushima *et al.* (1999) examined five strains of *E. coli* O157 and showed a variation in the survival rate between the strains. The variation in environmental survival between different strains of *E. coli* O157 and the mechanisms behinds have been poorly studied. Since not all strains of *E.*

coli O157 are pathogenic to humans this has to be further examined in order to do appropriate risk assessment analysis.

Storage is a passive hygienisation method and our results shows that the reduction rate is very slow especially during cold climate conditions. An active treatment of the manure will reduce the pathogen to low levels. We found that treatment with 2% urea at 4°C increased the reduction rate of *E. coli* O157 to 0.073 h^{-1} and the pathogen could not be re-isolated after five days of storage. Thus, treatment with urea may be one method to use on contaminated cattle manure before disposed on arable land used for food production or

grazing. Other active treatment methods include composting, digestion, drying and addition of lime (Himathongkham and Riemann, 1999; Bujoczek *et al.*, 2001; Lung *et al.*, 2001).

Conclusions

The present study has been shown that *E. coli* O157 is able to survive for extended periods, i.e. several months, in cattle manure during storage as well as in manure-amended soil. The temperature regime seems to be one important factor for the reduction rate of *E. coli* O157, especially during manure storage. A strain dependent variability in the environmental survival was shown to occur between the different strains of *E. coli* O157 tested. Further we address the importance of an adequate treatment of contaminated cattle manure, e.g. urea treatment, in order to minimize the risk of horizontal transfer of *E. coli* O157 from animal to animal, and to the human food chain.

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