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ORIGINAL ARTICLE

Numbers and transported state of *Escherichia coli* in runoff direct from fresh cowpats under simulated rainfall*

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Abstract

Aims: To investigate the number of *Escherichia coli* in runoff derived directly from fresh cowpats and to determine if the *E. coli* are attached to dense particles, in flocs or as individual cells.

Methods and Results: Three cowpats were collected monthly from the same farm for 13 months and the number of *E. coli* in them estimated. A rainfall simulator was used to generate runoff from the individual cowpats, which was fractioned to determine the transported state of any *E. coli* present. The number of *E. coli* in the cowpat runoff was highly variable and was strongly correlated with the number of *E. coli* in the cowpat. Only a small percentage (approx. 8%) of the *E. coli* in runoff were attached to dense (>1·3 g ml^{-1}) particles and there was no evidence of flocculation of the cells.

Conclusions: *Escherichia coli* in runoff from cowpats are transported predominantly as individual cells.

Significance and Impact of the Study: Mitigation strategies to reduce the number of faecal bacteria in overland flow from agricultural land need to be designed to trap single bacterial cells.

Introduction

The erosion of bacteria from cowpats into overland flow is a controlling process in the faecal contamination of runoff upon agricultural land. Faecal coliform numbers in runoff from fresh cowpats have previously been reported as $\approx 10^7$ CFU 100 ml^{-1} decreasing to 10^5 and 10^4 CFU 100 ml^{-1} as the cowpats aged over 30 and 100 days, respectively (Thelin and Gifford 1983; Kress and Gifford 1984). However, whilst Muirhead *et al.* (2005) reported runoff *Escherichia coli* numbers eroded from fresh cowpats of approximately 10^7 MPN 100 ml^{-1}, no significant decrease in concentration was observed in runoff from cowpats aged up to 30 days. They also observed a very strong correlation between the number of *E. coli* in the cowpat, and the numbers measured in the runoff (Muirhead *et al.* 2005). As the numbers of *E. coli* in faeces has been reported to vary by six orders of magnitude (Davidson and Taylor 1978), such variability may have a dramatic effect on the number of *E. coli* present in overland flow from agricultural land.

An important factor influencing the transport of bacteria in overland flow is the state in which the bacteria are present, with bacteria attached to soil particles, in flocs or as individuals, postulated to have different transport mechanisms (Fiener and Auerswald 2003; Tyrrel and Quinton 2003; Jamieson *et al.* 2004). The greater the degree of attachment to dense particles, the more readily bacteria are likely to deposit or settle out from overland flow, rendering them more susceptible to entrapment in buffer strips and wetlands. Those studies examining bacterial attachment to date have, however, provided contrasting results. Mahler *et al.* (2000) observed that up to 100% of the faecal coliforms recovered from ground water were associated with particles, whilst in an earlier study by the authors of this paper (Muirhead *et al.* 2005) found no evidence of flocculation of *E. coli* and showed that only approximately 8% of the *E. coli* were attached to particles in either fresh or aged (up to 30 days old) cowpats.

In the earlier study of Muirhead *et al.* (2005), the artificial cowpats were created by compositing the faeces from a number of animals together, to allow a more accurate monitoring of changes in the number of *E. coli* in the cowpats over time, and this may have masked some of the potential differences between the faeces of individual animals. Also, only a limited number of samples were analyzed for the presence of flocs (Muirhead *et al.* 2005). In the current study described in this paper we collected faeces from individual animals to confirm our previous observations of the transported state of the *E. coli* including a greater number of samples analyzed for flocs. We also investigated the relationship between the *E. coli* numbers in the cowpat and the direct runoff from the individual cowpats, which we predicted would provide a wider concentration range of *E. coli* than was measured previously in the composite samples.

Materials and methods

Sampling of cowpats

Each month, for 13 months starting in October 2003, three individual cowpats were collected for analysis and for use in simulated rainfall experiments from the same herd of mixed age dairy cows (predominantly Friesian) from a farm on the Taieri Plans, Otago, New Zealand. The cows grazed a predominantly ryegrass pasture with only small amounts of supplements required to fill winter and summer feed deficits. To ensure that all cowpats were fresh, the herd was observed until a cow defecated and then the cowpat was immediately collected from the ground with a shovel and placed in a metal tray for transport to the laboratory. All cowpats were exposed to simulated rainfall within 2 h of collection and all sub-samples analyzed for *E. coli* within 6 h of collection.

Analysis of cowpats

A small sub-sample (approximately 50 g wet weight) of each cowpat was collected (before the simulated rainfall experiments) and the number of *E. coli* present and the total solids (% TS) content estimated. *Escherichia coli* were quantified using the Colilert-Quanti-Tray system (IDEXX Laboratories, Westbrook, ME, USA) as described by Muirhead *et al.* (2004). Total solids content was measured by drying to a constant weight (Anon 1992).

Simulated rainfall experiments

The rainfall simulator used one TeeJet 1/4HH-SS30WSQ nozzle (Spraying Systems Co., Wheaton, IL, USA) sited approximately 250 cm above the cowpat to gain terminal velocity (McDowell and Sharpley 2002). Simulated rainfall had drop-size, velocity and impact energies approximating those of natural rainfall (Shelton *et al.* 1985). The cowpats were placed in a metal tray (250 mm long, 200 mm wide with sides 50 mm high) and the tray tilted on a 10% (approx.) slope, which allowed runoff to be collected from one of its corners. The rainfall simulator was run for 20 min at a rate of 25 mm h^{-1} and all runoff from this time period (approximately 400 ml) collected for analysis. Each individual cowpat was rained on once only.

Analysis of runoff

A sub-sample of the runoff was diluted 1 : 10 with sterile water before it was fractioned to determine the number of *E. coli* attached to particles using the buoyant density/centrifugation technique described by Muirhead *et al.* (2005). The un-attached fraction was further analyzed to determine if the *E. coli* were in flocs, by splitting the un-attached fraction in to two samples and vortexing one of the samples with microbeads, before both samples were analyzed for *E. coli* (Muirhead *et al.* 2005). The total *E. coli* concentration in the runoff was calculated from the numbers present in the respective fractions.

Statistical analysis

The data were analyzed using GENSTAT VERSION 7. The *E. coli* numbers in the cowpat and in the runoff were $\log_{10}$ transformed. The data set contained three censored values for the *E. coli* concentration in runoff as follows. Two occurred when the concentration of *E. coli* attached to particles in the runoff was below the detection limit (5 MPN 100 ml^{-1}) and the other, when the total runoff concentration was below the detection limit (5 MPN 100 ml^{-1}). The log of each censored observation was estimated using the CENSOR procedure (Taylor 1973). The censored units in the data were then replaced by their estimated values and these were used in all subsequent analyses. One-way ANOVAS were used to test for differences between seasons for (i) percentage TS and log *E. coli* concentrations in the cowpat (ii) percentage attachment to particles and (iii) log total *E. coli* concentration in the runoff. The relationship between *E. coli* in the cowpat and runoff was examined using log-transformed concentrations. The degree of flocculation of *E. coli* in runoff was determined by plotting the vortexed sample against the un-vortexed sample with the intercept constrained to 0. If the *E. coli* in the un-attached fraction does occur in flocs, then the slope of this relationship would be expected to be greater than one (i.e. if there were an average of five *E. coli* cells in each floc then a slope of five would be expected).

Results

The percentage TS from the individual cowpats ranged from 8 to 23% with an overall average of 12%. The changes in mean percentage TS between seasons were not statistically significant ($P > 0{\cdot}05$) due to the high variability between individual replicate samples (Table 1). There were no significant relationships identified between the percentage TS and the percent of *E. coli* attached to particles in the runoff, or numbers of *E. coli* in the faeces or the runoff. The *E. coli* numbers in the individual cowpats ranged from $9{\cdot}7 \times 10^1$ to $1{\cdot}9 \times 10^7$ MPN g^{-1} dry weight with a geometric mean of $2{\cdot}1 \times 10^5$ MPN g^{-1} dry weight. The number of *E. coli* in the fresh faeces were similar during spring, summer and autumn but were significantly ($P < 0{\cdot}01$) lower in winter (Table 1).

The number of *E. coli* in runoff was strongly correlated with the number in the cowpats (Fig. 1), deriving the following relationship:

$$\text{runoff}(\log \text{MPN } 100\ \text{ml}^{-1}) = [1{\cdot}01 \times \text{cowpat } (\log \text{MPN } \text{g}^{-1}\ \text{dw})] - 0{\cdot}49,$$

which was statistically significant ($P < 0{\cdot}001$ with an r^2 of 90%).

The percentage of *E. coli* attached to particles within individual samples ranged from 'not detected' to 27% with an overall average of 9%. The percentage of *E. coli* attached to particles appeared to decrease slightly in summer compared to the other seasons though this decrease was not significant ($P = 0{\cdot}91$) (Table 1). The total number of *E. coli* in runoff ranged from 'not detected' (<5 MPN 100 ml^{-1}) to $1{\cdot}4 \times 10^7$ MPN 100 ml^{-1} with a geometric mean of $7{\cdot}3 \times 10^4$ MPN 100 ml^{-1}. The number of *E. coli* in the runoff were similar during spring, summer and autumn but were significantly ($P < 0{\cdot}01$) lower in winter (Table 1).

Regression analysis of the results from the vortexed and un-vortexed samples of the runoff calculated a slope of 1·01 ($P < 0{\cdot}001$), hence there was no evidence of flocculation of the *E. coli* in the runoff samples (data not shown).

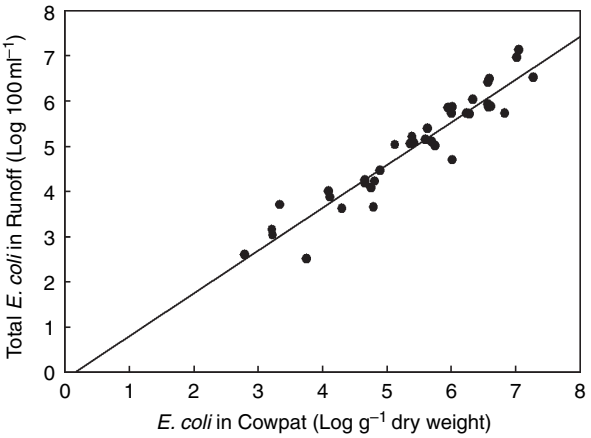

Figure 1 The relationship between the *Escherichia coli* numbers in the individual cowpats and in the runoff generated by simulated rainfall. The regression line, which was statistically significant ($P < 0{\cdot}001$ and $r^2 = 90\%$), is also shown.

Discussion

Despite the importance of understanding the transported state of bacteria in overland flow few studies have been published on this issue. In our previous study on the state of *E. coli* eroded from cowpats of dairy cows, we reported that the *E. coli* were not in flocs and that only 8% were attached to dense particles (Muirhead *et al.* 2005). The method used to determine flocculation in these studies determined the average number of *E. coli* cells per floc and it is possible that the *E. coli* could still floc with other species of bacteria. However, the high concentrations of *E. coli* in faeces and our current understanding of the interactions of bacteria (Rickard *et al.* 2003) indicate that it is unlikely that there would be multi-species flocs with only one *E. coli* cell per floc.

The method used to determine the percentage of *E. coli* attached to particles is based on buoyant density separation and is proposed to identify bacteria attached to dense particles (such as soil) that are more likely to be removed by sedimentation mechanisms in overland flow (Fiener and Auerswald 2003). The average percentage of *E. coli* attached to dense particles in the runoff was 9%. This low

Table 1 Seasonal variation in the measurements from the fresh faeces and in the runoff from the faeces generated by simulated rainfall including the pooled standard errors

	Autumn	Winter	Spring	Summer	SED
Faeces					
Percentage of total solids	13·0%	12·7%	10·3%	11·5%	1·3%
E. coli ($\log_{10}$ MPN g^{-1} dry weight)	5·97*	3·97†	5·49*	5·77*	0·51
Runoff					
Percentage of *E. coli* attached to particles	9·9%	9·2%	9·2%	7·4%	3·4%
E. coli ($\log_{10}$ MPN 100 ml^{-1})	5·83*	3·46†	4·90*	5·25*	0·52

Means in a row without a common superscript were significantly different ($P < 0{\cdot}01$).

value was not surprising assuming that there are only low quantities of dense particles in the fresh faeces. There was no relationship between the percentage of *E. coli* attached to dense particles and the total solids content of the faeces and again this is not surprising as most of the total solids measured are organic material with neutral buoyancy. The current study based on fresh faeces collected from individual cows therefore, confirms our earlier observations (based on composite faeces samples) that *E. coli* are predominantly transported from cowpats as individual cells.

The number of *E. coli* in the runoff from the cowpats was highly variable, ranging over seven orders of magnitude, which is greater than the ranges previously published (Thelin and Gifford 1983; Kress and Gifford 1984; Muirhead *et al.* 2005). The number of *E. coli* in runoff was strongly correlated with the number of *E. coli* in fresh faeces. The observed variability in numbers of *E. coli* in faeces is similar to that reported in the faeces of beef cattle presented for slaughter (Davidson and Taylor 1978). The relationship between the number of *E. coli* in the runoff and faeces exhibited a best-fit regression line with a slope of 1·01, suggesting that the faecal material was uniformly eroded into the runoff, across the simulated events. Muirhead *et al.* (2005) also observed this relationship but reported a less steep regression (of 0·67), which may have been influenced by the analysis of aged cowpats that exhibited crusted surfaces that are postulated to slow down the release of microbes under rainfall (Thelin and Gifford 1983; Kress and Gifford 1984). The mean total solids content was 12% and our observation that there is no relationship between percentage TS and the number of *E. coli* in faeces is consistent with findings from research on the preslaughter management of beef cattle (Gregory *et al.* 2000; Jacobson *et al.* 2002).

There was a significant decrease in the number of *E. coli* in faeces and runoff during winter. The cause(s) of this decrease was not identified but could be related to the cessation of milking and the corresponding decrease in feed intake of the cows, or other ambient conditions. The environmental impact of this decrease during winter is difficult to predict since it will, potentially, be affected by a range of seasonal effects, such as the rate of die-off in the cowpats (Crane and Moore 1986; Miller *et al.* 2003) and the likelihood of overland flow actually occurring (Collins and Rutherford 2004). For example, the low *E. coli* numbers observed during winter may still pose a significant risk to surface waters as there is a higher probability of overland flow occurring on wet soils. Considering the importance of the number of faecal bacteria excreted by cattle as an input to catchment-scale bacterial water quality models (Collins and Rutherford 2004; Tian *et al.* 2002), this variability between individual animals and the potential for seasonal variation warrants further investigation.

The observation that *E. coli* are eroded and transported from cowpats as individual bacterial cells indicates that they will be highly mobile in overland flow (Fiener and Auerswald 2003; Jamieson *et al.* 2004). This apparent lack of a propensity for bacteria to settle or deposit, is also likely to be a cause of the high concentrations of bacteria frequently observed in overland flow from agricultural land (Doran and Linn 1979; Edwards *et al.* 2000; Vinten *et al.* 2004; Collins *et al.* 2005) and the sometimes limited attenuation of bacteria through buffer zones (Collins 2004; Collins *et al.* 2004). The key implication of this finding is that mitigation methods to reduce the number of bacteria in runoff from agricultural land will need to be designed to entrap single bacterial cells.

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