Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

Establishing relative release kinetics of faecal indicator organisms from different faecal matrices

C.J. Hodgson[1], N. Bulmer[1], D.R. Chadwick[1], D.M. Oliver[2], A.L. Heathwaite[2], R.D. Fish[3] and M. Winter[3]

1 North Wyke Research, Okehampton, Devon, UK
2 Centre for Sustainable Water Management, Lancaster Environment Centre, Lancaster University, Lancaster, UK
3 Centre for Rural Policy Research, Department of Politics, Devon, UK

Keywords
Escherichia coli, faecal indicator organisms, intestinal enterococci, manures mobilization, rain water, release.

Correspondence
Chris J. Hodgson, North Wyke Research, Okehampton, Devon, EX20 2SB, UK.
E-mail: chris.hodgson@bbsrc.ac.uk

2008/2132: received 12 December 2008, revised and accepted 17 March 2009

doi:10.1111/j.1472-765X.2009.02630.x

Abstract

Aims: A laboratory assay for comparative characterization of various faecal matrices with respect to faecal indicator organism (FIO) release using, artificial rain water.

Methods and Results: Fresh sheep and beef-cattle faeces, dairy cattle slurry and beef cattle farm yard manure (FYM) were collected from commercial units in south-west England and applied to 20 randomized 1 m^2 plots established on permanent grassland. Representative samples from each faecal matrix ($n = 5$) were collected on four occasions over 16 days. One gram of each sample was transferred to a sterile vial to which 9 ml of standard local rain was carefully pipetted. The vial was then rotated through 360°, 20 times in 60 s to 'simulate' a standardized interaction of the faecal material with rainfall, providing an assay of comparative release potential. Appropriate decimal dilutions were prepared from the eluent. Following agitation, with a sterile spatula, the remaining faecal material and eluent in the vials were vortex mixed for 60 s before decimal dilutions were prepared from the resulting mixture, providing a quantitative assessment of the total FIO in the sample from which percentage release could be determined. Bacterial concentrations were enumerated in duplicate by membrane filtration following standard methods for FIO. Significant differences in release kinetics of *Escherichia coli* and enterococci from each of the faecal matrices were determined.

Conclusions: Differences in release from each faecal substrate and between FIO type (*E. coli* and intestinal enterococci) were observed in this laboratory study. The order of release of *E. coli* from the faecal matrices (greatest to least, expressed as a percentage of the total present) was dairy cattle slurry > beef cattle FYM > beef-cattle faeces > sheep faeces. For intestinal enterococci the order of percentage release was dairy cattle slurry > beef-cattle faeces > beef cattle FYM > sheep faeces.

Significance and Impact of the Study: This laboratory-based method provides the first data on the relative release kinetics of FIO from different faecal matrices in rain water. This is fundamental information needed to parameterize laboratory-based microbial models and inform approaches to field and catchment risk assessment.

Introduction

Faecal indicator organisms (FIO) are currently defined as *Escherichia coli* and intestinal enterococci (Anon, 2006) whose presence in large numbers in the faeces of mammals make them good bacterial indicators of faecal pollution. FIO are key parameters used to index pollution of public health significance in the new catchment scale

water quality management approach advocated by the Water Framework Directive (WFD) (EU) and the Clean Water Act (USA) (Kay *et al.* 2008). Microbial pollution as determined by FIO concentrations has been cited as the most significant reason for water quality impairment in recreational and shell-fish harvesting waters over the last decade in the United States (Hyer and Moyer 2004; Kay *et al.* 2008).

There is little information on the relative differences in cell dispersal from differing faecal matrices found in agricultural environments (Guber *et al.* 2006). Various factors will impact on release kinetics of faecal bacteria in the field (e.g. rainfall intensity, solution salinity and age of faeces) but as a first approximation there is a fundamental need to understand which livestock manures are more or less likely to release faecal bacteria under rainfall. These release rates are crucial to underpin an evaluation of combined transport and fate of cells in agricultural environments and their subsequent delivery to surface waters, yet few studies have investigated the release behaviour of indicator bacteria. Furthermore, this information is complementary to studies of the differential die-off patterns of FIO in faecal matrices (e.g. Oliver *et al.* 2006). Initial studies have investigated other microbial contaminants such as, for example *Cryptosporidium* dispersal from faecal material in agricultural systems, but have not considered the relative difference between different types of faecal material with regard to their potential to disperse faecal microbes (e.g. Schijven *et al.* 2004). With an improved understanding of the likelihood of cell release from different faecal substrates we can begin to target simple mitigation strategies at the most risky faecal sources found on pasture.

The evolving mandates of policy in the area of water quality, such as the EU WFD 2000/60/EC (Anon, 2000) and revised Bathing Water Directive 2006/7/EC (Anon, 2006) reflects growing acknowledgement of diffuse pollution derived from agriculture as the single biggest threat to recreational water quality in England and Wales (DEFRA, 2007). The implications of the WFD are such that farmers and land owners will have legal obligations to safeguard water bodies and protect the environment. Risk assessment frameworks for some agricultural contaminants, such as the Phosphorous Index and nutrient-management planning are well established, allowing land owners to make informed decisions at the field scale (Lemunyon and Gilbert 1993; Coale *et al.* 2002; Buczko and Kuchenbuch 2007). However, there are few risk indexing tools that address the impacts of diffuse microbial pollution from agriculture (e.g. Oliver *et al.*2009), which is perhaps surprising considering that FIO have been established for a considerable time as a surrogate measure of infection risk to humans (Kay *et al.* 2007). Furthermore the 'evidence-base' necessary to inform good regulatory practice in the context of microbial pollution from agriculture (Kay *et al.* 2008) is partial. Empirical science takes time to accumulate and does not map neatly on to the timescales and exigencies of policy frameworks, most notably the need for EU member states to meet their obligations under the WFD by 2015. Consequently, laboratory-based experiments that can provide an indication of relative release kinetics for FIO from various faecal matrices in rain water are essential to provide the data to drive early iterations of risk tools that focus on identifying and mitigating microbial diffuse pollution at the farm scale. The objective of this study was to use a laboratory scale experiment to determine the relative release kinetics of FIO in artificial rain water over 16 days, from four distinct and common faecal matrices: beef-cattle faeces; beef cattle (farm yard manure, FYM); dairy cattle slurry; and sheep faeces.

Materials and methods

Collection of faecal material

Beef-cattle faeces, FYM and sheep faeces were collected from a research farm at North Wyke Research, Devon, UK (Grid ref: 985659). The cattle slurry was collected from a nearby dairy unit. All faecal material was stored immediately after collection, in the dark and at 4°C and applied to land within 2–6 h. Beef-cattle faeces and FYM were collected from cattle sheds housing 20 beef cattle (Hereford × Friesian). Prior to collection of the faeces the concrete feeding area was scraped and thoroughly cleaned with a commercial farm disinfectant followed by washing with a pressure hose as per standard farming practice. This removed old remnants of different faecal types. The cattle were then reintroduced onto the clean concrete area and fresh faecal deposits were collected immediately after defecation. Five deposits, from separate animals, were collected in total using sterile plastic bags. Approximately 25 kg of beef cattle FYM was collected by scraping away the top 10 cm of bedding material and digging out the bedding to the concrete floor. The FYM was mixed thoroughly on a clean concrete surface. For the sheep faeces, 15 faeces (each faeces is comprised of a group of faecal pellets) were collected from a flock (*c.* 60 head) of Suffolk × ewes, which had been out wintering on a grassland paddock. A concrete collection yard was scraped and cleaned thoroughly; the flock was corralled on the collection yard and left for 90 min. The flock was then released and intact faecal deposits were collected in sterile plastic bags. Approximately 27 l of dairy cattle slurry was collected from a reception pit. The slurry had been mixed by an automated stirrer for 24 h prior to collection to ensure that it was as homogeneous as possible.

Sampling of faecal material

The faecal material was exposed to field conditions and sampled on a series of dates in March and April 2008 for use within a controlled laboratory experiment. Faecal material was placed onto 20 randomized 2 m^2 plots established on permanent grassland that had not been grazed or received livestock manure for 20 years. The treatments comprised five plots of one beef-cattle faeces, five plots of sheep faeces comprising three faecal deposits, five plots of beef cattle FYM and five plots of dairy slurry. The FYM and slurry were broadcast (surface) applied at the equivalent rate of 45 t and 45 m^3 ha^{-1}, respectively, within the confines of a 1 m^2 quadrat. This application rate was chosen because it represents a realistic upper level used in the UK and is within the limit outlined in codes of good agricultural practice (MAFF, 1998). To facilitate broadcast application at the plot scale a miniature 'splash-plate' device was used. The faecal material was exposed to natural weather conditions over the course of the study; meteorological data were collected in the field using a Skye Minimet 4 Meteorological Station (Skye Instruments Ltd, Powys, UK). Representative samples, *c.* 2 g, from the four faecal matrices were taken, using a sterile spatula, from each of the five replicates on four separate occasions, for the beef cattle and sheep faeces and three separate occasions for the Beef FYM and dairy cattle slurry, over 16 days (day 1, 3, 9 and 16) to investigate release from freshly deposited/applied faecal matrices and also ageing faecal matrices. The rationale for not taking a sample on day 1 for the FYM and slurry was related to current best practice, which recommends that farmers do not apply animal manures when rain is imminent. The spatula was sterilized by immersion in Virkon (Anachem Ltd, Luton, UK) and rinsing with sterile-deionized water between each plot. Faecal samples were placed into sterile vials and immediately returned to the laboratory for analysis.

Generation of standardized, sterile rainwater

Standard rain water characteristic of the local area was generated by dissolving salts in deionized water. The resulting composition was typical of rainwater collected at North Wyke Research, pH 5·64, (composition (g l^{-1}): CaCl, 2·465; MgCl, 1·919; FeCl, 0·0445; NH_4NO_3, 0·430; K_2SO_4, 0·617; NaCl, 3·317. The artificial rainwater was sterilized using an autoclave (15 min at 121°C).

Determination of FIO concentrations

A laboratory experiment following a protocol used to measure phosphorus mobilization from soil was used (The DESPRAL test) but adapted to measure FIO release from faecal matrices Withers *et al.* 2007). Briefly, one gram of each of the faecally derived substrates was added to a sterile vial in replicate (n = 5) to which 9 ml of sterile-standardized rain water was pipetted slowly down the side of the vial so as to avoid agitating the organic matter. The vial was then rotated through 360°, 20 times in 60 s to simulate a standardized interaction of the faecal material with rainfall, providing an assay of comparative release potential under controlled laboratory conditions. One millilitre of the eluent was aseptically transferred to 9 ml of sterile Ringers (Oxoid, Basingstoke, UK) and appropriate serial 10-fold dilutions were made, standard methods of membrane filtration were used to determine bacterial concentrations (Anon, 2002). Samples were washed through the filtration unit with 20 ml of sterile Ringer's solution. Membrane filters of 0·45 μm pore size (Pall Gellman Sciences, East Hills, NY) were aseptically transferred to Membrane Lactose Glucuronide Agar (Oxoid) and incubated inverted, at 44·5°C (±0·2°C) for 18–24 h for *E. coli* and Slanetz and Bartley (Oxoid) incubated at 37·0°C (±0·2°C) for 44–48 h, for intestinal enterococci. The remaining rain and faecal material was homogenized by vortex mixing for 60 s and agitating with a sterile spatula. Appropriate decimal dilutions were prepared in 9 ml of Ringers and duplicate FIO concentrations were determined as described above providing a quantitative assessment of the total FIO in the sample from which release percentage could be determined. FIO concentrations were analysed in the laboratory within 2 h of sample collection. The remaining faecal material was used to determine the gravimetric water content by drying at 105°C for 24 h.

Statistical analyses

Raw FIO counts were normalized by transforming to Log_{10} colony forming units (CFU) g^{-1} (dry wt., dry weight). Means and associated standard deviations and percentage of FIO released in the artificial rain water were calculated using Excel (Microsoft Excel 2003; Microsoft Corporation, 2003). A general linear model with repeated measures was performed on each faecal matrix at each sampling time to test for differences in cells released from each treatment (GenStat 10th edition; VSN International Ltd, Hemel Hempstead, UK).

Results

Meteorological conditions

Meteorological conditions during the experiment were relatively dry. A total of 12 mm of rain fell during the

Table 1 Mean *Escherichia coli* concentrations and dry matter in each of the four faecal matrices (n = 5 for each faecal matrix) and concentrations and percentage of cells lost following dispersion experiment, standard deviations are shown in parentheses

Faecal matrix	Day	Total cell concentration detected in faecal material Log_{10} CFU g^{-1} dry wt.	Cell concentration detected in rain water Log_{10} CFU g^{-1} dry wt.	*Escherichia coli* released in rain water (%)	Dry matter of faecal material (%)
Beef-cattle faeces	1	5·74 (0·79)	5·15 (1·02)	30·7 (17·6)	10·8 (1·7)
	3	4·61 (0·55)	2·94 (1·74)	20·9 (27·4)	13·5 (1·6)
	9	4·47 (0·75)	3·94 (0·66)	32·9 (17·1)	15·4 (1·5)
	16	2·85 (1·87)	1·84 (1·82)	15·1 (25·4)	41·1 (28·6)
Sheep faeces	1	6·91 (0·61)	6·15 (0·367)	22·5 (21·3)	20·2 (1·9)
	3	5·01 (2·88)	3·27 (1·95)	0·8 (0·9)	30·6 (15·0)
	9	8·03 (0·17)	4·88 (2·73)	1·0 (0·8)	27·1 (4·7)
	16	6·76 (0·84)	4·56 (0·64)	0·8 (0·5)	60·8 (17·7)
Beef farm yard manure	1	ND			
	3	5·66 (0·50)	4·91 (1·02)	34·6 (35·9)	24·7 (3·7)
	9	5·28 (1·32)	4·37 (1·19)	21·0 (24·7)	21·7 (3·0)
	16	4·21 (1·20)	1·34 (2·10)	1·8 (3·9)	65·1 (21·7)
Dairy cattle slurry	1	ND			
	3	6·53 (0·02)	6·23 (0·02)	50·2 (0·7)	17·0 (0·9)
	9	5·71 (0·15)	5·40 (0·17)	49·5 (2·1)	20·2 (1·6)
	16	3·52 (0·37)	1·95 (1·16)	6·5 (6·9)	55·6 (9·8)

ND, no data (refer to Materials and methods for rationale).

16 days, with no single day recording more than 5 mm, air temperature, recorded at 10 cm above the ground, ranged from −1·2 to 11·1°C. There was very little visible deterioration of the faecal material as a result of weather conditions from day 1 to 9. However, by day 16, it was apparent that all four matrices were drying as evidenced by the reported percentage dry matters (see Tables 1 and 2). By day 16, there was little physical evidence of the faecal material being incorporated into the soil and each faecal matrix was still occupying its original area within its respective grassland plot.

FIO concentrations in faecal material and FIO release

Fresh beef and sheep faeces collected on day 1 had mean (n = 5) *E. coli* concentrations of 5·74 and 6·91 Log_{10}

Table 2 Mean intestinal enterococci (IE) concentrations and dry matter in each of the four faecal matrices (n = 5 for each faecal matrix) and concentrations and percentage of cells lost following dispersion experiment, standard deviations are shown in parentheses

Faecal matrix	Day	Total cell concentration detected in faecal material Log_{10} CFU g^{-1} dry wt.	Cell concentration detected in rain water Log_{10} CFU g^{-1} dry wt.	IE released in rain water (%)	Dry matter of faecal material (%)
Beef-cattle faeces	1	6·92 (0·28)	6·30 (0·31)	26·8 (13·1)	10·8 (1·7)
	3	6·14 (0·60)	5·34 (0·94)	22·4 (17·3)	13·5 (1·6)
	9	5·12 (0·79)	4·78 (0·82)	44·0 (7·4)	15·4 (1·5)
	16	4·37 (0·29)	3·59 (0·76)	27·4 (23·4)	41·1 (28·6)
Sheep faeces	1	6·16 (0·39)	4·61 (0·16)	5·2 (7·6)	20·2 (1·9)
	3	6·37 (0·44)	4·30 (0·58)	1·3 (1·3)	30·6 (15·0)
	9	6·06 (0·68)	4·53 (1·04)	4·7 (4·7)	27·1 (4·7)
	16	5·47 (0·81)	1·74 (1·69)	0·2 (0·4)	60·8 (17·7)
Beef farm yard manure	1	ND			
	3	5·77 (0·65)	4·72 (0·65)	26·5 (33·9)	24·7 (3·7)
	9	5·69 (0·18)	4·82 (0·58)	17·2 (9·1)	21·7 (3·0)
	16	4·56 (0·66)	2·43 (1·54)	2·5 (2·8)	65·1 (21·7)
Dairy cattle slurry	1	ND			
	3	8·41 (0·05)	8·11 (0·06)	49·5 (1·3)	17·0 (0·9)
	9	6·39 (0·16)	6·07 (0·17)	47·9 (1·4)	20·2 (1·6)
	16	4·31 (0·09)	2·71 (1·66)	17·4 (16·5)	55·6 (9·8)

ND, no data (as Table 1, see rationale in Materials and methods).

CFU g^{-1} dry wt. respectively (Table 1). Mean ($n = 5$) concentrations of intestinal enterococci were 6·92 (beef) and 6·16 (sheep), Log_{10} CFU g^{-1} dry wt. (Table 2). On day 1, the percentage loss (release) of *E. coli* in rain water for the beef cattle and sheep faeces was similar at 30·6% and 22·5% respectively (Fig. 1). In contrast there was a statistically significant difference ($P = 0·013$) in the percentage loss of intestinal enterococci in rain water between beef cattle and sheep faeces, 26·8% and 5·2% respectively (Fig. 1).

There was a general decline observed in total viable bacterial cells, both *E. coli* and intestinal enterococci, for the three bovine faecal matrices over the 16 days. In contrast the total viable *E. coli* concentration in sheep faeces showed no general decline over the 16 days, with an actual increase observed on day 9 (a mean concentration of 8·03 Log_{10} CFU g^{-1} dry wt.). At day 16, the total viable concentration of *E. coli* and intestinal enterococci was significantly greater in the sheep faeces compared with all three bovine faecal matrices ($P < 0·001$) and ($P < 0·010$), respectively.

When the mean Log_{10} CFU g^{-1} recovered in the rain water were compared over time it was found that between day 3 to day 16 there were significant differences between both *E. coli* and intestinal enterococci across all four faecal matrices ($P < 0·001$). The order of release of *E. coli* from the faecal matrices (greatest to least, expressed as a percentage of the total present) was dairy cattle slurry > beef cattle FYM > beef-cattle faeces > sheep faeces. For intestinal enterococci the order of percentage release was dairy cattle slurry > beef-cattle faeces > beef cattle FYM > sheep faeces.

Discussion

The initial concentrations of faecal indicators were broadly similar across all manure types, which is consistent with concentrations reported in the literature for cattle faeces (Sinton *et al.* 2007; Van Kessel *et al.* 2007; Moriarty *et al.* 2008) and sheep faeces (Avery *et al.* 2004). There are very few studies that have attempted to quantify and compare the release of FIO by rain water from a suite of faecal matrices. This laboratory scale investigation is unique in its attempt to 'order' the relative release of FIO from four typical faecal matrices, found predominantly in grassland farming systems in the UK. The research conducted has concentrated on the release of specific pathogens. For example Davies *et al.* (2004) have examined the dispersion of *Cryptosporidium* oocysts from faecal pats under simulated rainfall events while Bradford and Schijven (2002) evaluated the impact of solution salinity on the release of *Giardia* and *Cryptosporidium* from dairy calf manure. Ferguson *et al.* (2007) investigated the microbial (*Cryptosporidium*, *E. coli* and a bacteriophage, PRD 1) transport from cattle faeces under simulated rainfall events at the field scale, but did not

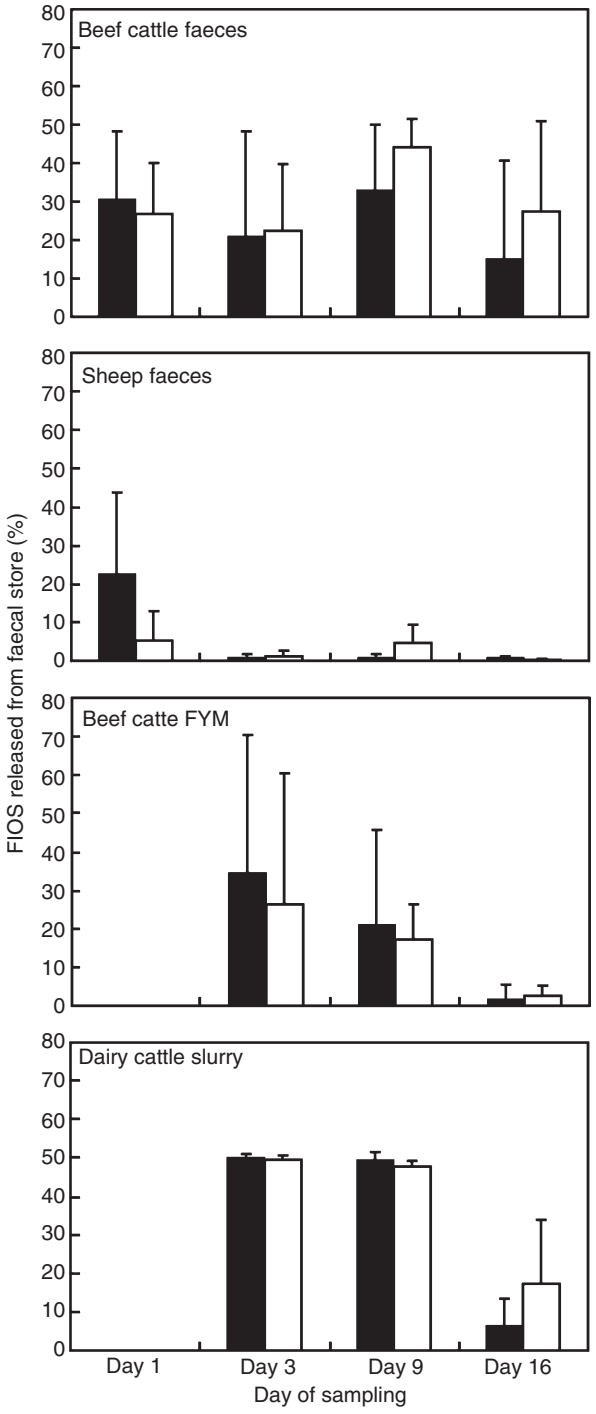

Figure 1 Percentage of faecal indicator organisms released from each faecal matrix with increasing time. Black bars show *Escherichia coli*, white bars show intestinal enterococci, error bars show standard deviation.

assess the difference in release from the diversity of faecal types found in agricultural environments. Others have assessed the release kinetics of a range of manure-borne contaminants, including FIO, alongside nutrient release from dairy manure applied to runoff plots, but again did not compare release kinetics attributed to different faecal matrices (Stout *et al.* 2005; Guber *et al.* 2006; Dao *et al.* 2008). Of course, while the study we report here has made an assessment of rainfall-induced release of FIO from faecal material others have commented that insect and worm communities can also facilitate transfer of FIO from faecal sources into the environment (Texier *et al.* 2008).

As the faecal material aged its percentage dry matter content increased and for sheep faeces, beef FYM and dairy cattle slurry this increase was coincident with a reduction in the percentage of FIO recovered in the rain water, which was far more pronounced by day 16 (i.e. fewer cells were released and the risk of mobilization had decreased). This would not appear to be an artefact of cell death as the total bacterial concentrations in the faecal material were still relatively high. It is more likely that the bacteria became encapsulated within the faecal matrix (thus less mobile) and were not released in the rain water during the dispersion experiment. While there was a corresponding increase in the percentage dry matter content in the beef-cattle faeces over time, 41% by day 16, the percentage loss of FIO in the rain water was far greater than that observed in the other faecal matrices on day 16. It is arguable that had the faecal material dried to the same dry matter content the percentage release of FIO may have been the same across all faecal matrices.

Notably, the loss of intestinal enterococci from beef-cattle faeces, as a percentage, was actually greater at day 16, when the dry mater content was around 41%, than day 3, when the dry matter content was 13·5%. This would suggest that, for beef-cattle faeces, intestinal enterococci are associated with the more solid fraction of the faecal material. This observation concurs with Guber *et al.* (2007) who found substantial numbers of enterococci were apparently present in the less readily suspended, possibly solid, parts of cattle manure. The relatively high percentage of *E. coli* released from all four faecal materials at the first analysis (day 1 for the beef cattle and sheep faeces and day 3 for the FYM and slurry) suggests they are probably associated with the more liquid fraction of animal manures (Guber *et al.* 2007). The observed significant difference in enterococci release for beef cattle *vs* sheep faeces is potentially related to differences in physical composition of the two faecal materials when the rain water was added. Generally, the sheep-faecal material remained relatively intact during the release phase (rotation through 360°C, 20 times in 60 s) of the laboratory experiment, whereas the beef-cattle faeces tended to disaggregate more readily during the release phase.

An interesting observation was that the proportion of FIO released from the sheep faeces was relatively small throughout the duration of the experiment. However, it should be noted that although the percentage loss was small the actual concentration of viable FIO in the rain water was still relatively large at 4·56 Log_{10} CFU g^{-1} dry wt. Furthermore, there was no depreciable decline in FIO concentrations recorded in the sheep-faecal material over the 16 days [i.e. they did not undergo first-order die-off, but instead persisted – a trait observed by others for bovine faeces (Sinton *et al.* 2007)]. This is potentially significant in that it shows that viable FIO can remain within sheep faeces at elevated concentrations for at least 16 days. When this is considered in the context of sheep and lamb numbers of which there are 34 million in the UK (Anon, 2008), it highlights that faeces from grazing sheep may: (i) act as a significant reservoir of FIO in the environment and (ii) pose a considerable risk to microbial water quality.

Data from this laboratory experiment provides a step-change improvement in elucidating the relative order and magnitude of rainfall-induced release of FIO from a suite of faecal matrices commonly found on UK grassland farms. Such data are needed to inform and parameterize farm scale risk assessment tools for FIO (e.g. Oliver *et al.* 2009) to target management and mitigation strategies to effectively protect watercourses from microbial contamination.

Acknowledgements

The authors would like to acknowledge the valuable and constructive comments made by the two anonymous referees. This work was funded as part of project RES-224-25-0086 by the Rural Economy and Land Use (RELU) programme who receive financial support from the Biotechnology and Biological Sciences Research Council (BBSRC), the Natural Environment Research Council (NERC), the Economic and Social Research Council (ESRC), the Department for Environment, Food and Rural Affairs (DEFRA) and the Scottish Office.

References

Anon (2000) Directive 2000/60/EC of the European parliament and of the council establishing a framework for community action in the field of water policy. *Off J Eur Union* **L327**, 1–72.

Anon (2002) *The microbiology of drinking water—methods for the examination of waters and associated materials*. London, UK: UK Environment Agency Report, Parts 4 and 5.

Anon (2006) Council Directive 2006/7/EC of the European Parliament and of the council of 15th February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. *Off J Eur Union* **L64**, 37–51.

Anon 2008. *Survey of Agriculture and Horticulture: 1 June 2007 UK – Final Results*. London: DEFRA.

Avery, S.M., Moore, A. and Hutchinson, M.L. (2004) Fate of *Escherichia coli* originating from livestock faeces deposited directly onto pasture. *Lett Appl Microbiol* **38**, 355–359.

Bradford, S.A. and Schijven, J. (2002) Release of *Cryptosporidium* and *Giardia* from dairy calf manure: impact of solution salinity. *Environ Sci Technol* **36**, 3916–3923.

Buczko, U. and Kuchenbuch, R.O. (2007) Phosphorus indices as risk assessment tools in the USA and Europe – a review. *J Plant Nutr Soil Sci* **170**, 445–460.

Coale, F.J., Sims, T. and Leytem, A.B. (2002) Accelerated deployment of an agricultural nutrient management tool: the Maryland phosphorus site index. *J Environ Qual* **31**, 1471–1476.

Dao, T.H., Guber, K.H., Sadeghi, A.M., Karns, J.S., van Kessel, J.S., Shelton, D.R., Pachepsky, Y.A. and McCarty, G. (2008) Loss of bioreactive phosphorus and enteric bacteria in runoff from dairy manure applied to sod. *Soil Sci* **173**, 511–521.

Davies, C.M., Ferguson, C.M., Kaucner, C., Altavilla, N., Deere, D.A. and Ashbolt, N.J. (2004) Dispersion and transport of *Cryptosporidium* oocysts from faecal pats under simulated rainfall events. *Appl Environ Microbiol* **70**, 1152–1159.

DEFRA 2007. Available at: http://www.defra.gov.uk/farm/environment/water/csf/index.htm last accessed on 12 May 2008.

Ferguson, C.M., Davies, C.M., Kaucner, C., Krogh, M., Rodehutskors, J., Deere, D.A. and Ashbolt, N.J. (2007) Field scale quantification of microbial transport from bovine faeces under simulated rainfall events. *J Water Health* **5**, 83–95.

Guber, A.K., Shelton, D.R., Pachepsky, Y.A., Sadeghi, A.M. and Sikora, L.J. (2006) Rainfall-induced release of fecal coliforms and other manure constituents: comparison and modelling. *Appl Environ Microbiol* **72**, 7531–7539.

Guber, A.K., Karns, J.S., Pachepsky, Y.A., Van Kessel, J.S. and Dao, T.H. (2007) Comparison of release and transport of manure-borne *Escherichia coli* and enterococci under grass buffer conditions. *Lett Appl Microbiol* **44**, 161–167.

Hyer, K.E. and Moyer, D.L. (2004) Enhancing fecal coliform total and maximum daily load models through bacterial source tracking. *J Am Water Resour Assoc* **40**, 1511–1526.

Kay, D., Edwards, A.C., Ferrier, R.C., Francis, C., Kay, C., Rushby, L., Watkins, J., McDonald, A.T. *et al.* (2007) Catchment microbial dynamics: the emergence of a research agenda. *Prog Phys Geogr* **31**, 59–76.

Kay, D., Crowther, J., Fewtrell, L., Francis, C.A., Hopkins, M., Kay, C., McDonald, A.T., Stapleton, C.M. *et al.* (2008) Quantification and control of microbial pollution from agriculture: a new policy challenge? *Environ Sci Policy* **11**, 171–184.

Lemunyon, J.L. and Gilbert, R.G.. (1993) The concept and need for a phosphorus assessment tool. *J Prod Agri* **6**, 483–486.

MAFF (1998) *Code of Good Agricultural Practice for the Protection of Water (The Water Code)*. London: Ministry of Agriculture, Fisheries and Food.

Moriarty, E.M., Sinton, L.W., Mackenzie, M.L., Karki, N. and Wood, D.R. (2008) A survey of enteric bacteria and protozoans in fresh bovine faeces on New Zealand dairy farms. *J Appl Microbiol* **105**, 2015–2025.

Oliver, D.M., Haygarth, P.M., Clegg, C.D. and Heathwaite, A.L. (2006) Differential *E. coli* die-off patterns associated with agricultural matrices. *Environ Sci Technol* **40**, 5710–5716.

Oliver, D.M., Fish, R.D., Hodgson, C.J., Heathwaite, A.L., Chadwick, D.R. and Winter, M. (2009) A cross-disciplinary toolkit to assess the risk of faecal indicator loss from grassland farm systems to surface waters. *Agric Ecosyst Environ* **129**, 401–412.

Schijven, J.F., Bradford, S.A. and Yang, S. (2004) Release of *Cryptosporidium* and *Giardia* from dairy cattle manure: physical factors. *J Environ Qual* **33**, 1499–1508.

Sinton, L.W., Braithwaite, R.R., Hall, C.H. and Mackenzie, M.L. (2007) Survival of indicator and pathogenic bacteria in bovine feces on pasture. *Appl Environ Microbiol* **73**, 7917–7925.

Stout, W.L., Pachepsky, Y.A., Shelton, D.R., Sadeghi, A.M., Saporito, L.S. and Sharpley, A.N. (2005) Runoff transport of faecal coliforms and phosphorus released from manure in grass buffer conditions. *Lett Appl Microbiol* **31**, 230–234.

Texier, S., Prigent-Combaret, C., Gourdon, M.H., Poirier, M.A., Faivre, P., Dorioz, J.M., Poulenard, J., Jocteur-Monrozier, L. *et al.* (2008) Persistence of culturable *Escherichia coli* fecal contaminants in dairy alpine grassland soils. *J Environ Qual* **37**, 2299–2310.

Van Kessel, J.S., Pachepsky, Y.A., Shelton, D.R. and Karns, J.S. (2007) Survival of *Escherichia coli* in cowpats in pasture and in laboratory conditions. *J Appl Microbiol* **103**, 1122–1127.

Withers, P.J.A., Hodkinson, R.A., Berberis, E., Presta, M., Hartikainen, H., Quinton, J., Miller, N., Sisák, I. *et al.* (2007) An environmental soil test to estimate the intrinsic risk of sediment and phosphorus mobilization from European soils. *Soil Use Manage* **23**(Suppl. 1), 57–70.

