SeASONAL AND WITHIN-HERD VARIABILITY OF E. COLI CONCENTRATIONS IN FRESH DAIRY FAECES

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Significance and Impact of the Study: This study provides a comprehensive temporal data set of faecal indicator organism (FIO) counts (both E. coli and other coliforms) in fresh dairy faeces for Scotland. Such faecal audits for the UK are scarce which is surprising given that livestock constitute one of the largest agricultural sources of diffuse microbial pollution of surface waters and contributors to poor bathing water quality. Such FIO concentration data (and evaluation of variability across seasonal, within-herd and year-on-year counts) in fresh faeces is a fundamental precursor to the robust parameterization of models that aim to predict the fate and transfer of both FIOs and pathogens in agricultural catchments.

Keywords
agriculture, cattle, diffuse pollution, Escherichia coli, faecal coliforms, livestock faeces, modelling.

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Abstract
The aim of this study was to determine concentrations of culturable faecal indicator organisms (FIOs) in freshly excreted dairy faeces and assess seasonal, within-herd and year-on-year variability in counts. Such values are essential in order to provide input parameters and associated uncertainty bounds for empirical models designed to determine the burden of FIOs on pasture. A longitudinal faecal analysis survey (n = 80) was conducted at a conventional dairy farm in central Scotland over a 2-year period. The analysis quantified counts of Escherichia coli and other non-E. coli coliforms and compared the concentrations of these FIO groups across contrasting seasons. The overall mean concentration of E. coli was 6.63 and 6.58 log_{10} CFU g^{-1} dry weight in 2012 and 2013, respectively. However, concentrations of E. coli in faecal pats on each seasonal sampling event were highly variable and spanned several orders of magnitude on all occasions. Concentrations of E. coli in faeces excreted in winter were found to be lower than those excreted in all other seasons in 2012, though patterns of seasonal shedding were not consistent in observations the following year highlighting additional sources of uncertainty in FIO loading to land from dairy herds.

Introduction

Escherichia coli are commonly used as a faecal indicator organism (FIO) by environment protection agencies throughout the world. While the presence (or absence) of FIOs does not confirm the presence (or absence) of a pathogen (Wu et al. 2011), their detection in environmental matrices is indicative of pollution originating from a faecal source (Blaustein et al. 2013). These bacteria, which make up the majority of the faecal coliform (FC) group, can be released into the wider environment following livestock defecation and/or manure and slurry applications to land, and via wastewater releases from sewage treatment works or septic tanks (Chadwick et al. 2008; Kay et al. 2008). In catchments dominated by livestock agriculture, the accumulation of FIOs on pasture is a dynamic function of livestock numbers, their faecal excretion and bacterial shedding capacity, and bacterial die-off rates as determined by environmental drivers such as temperature and intensity of UV radiation (Oliver et al. 2010a).

The concentrations of E. coli found in freshly excreted livestock faeces can vary by several orders of magnitude (Cox et al. 2005; Muirhead et al. 2006; Ferguson et al. 2009). The factors that contribute to this variation have been suggested to include diet, animal age and livestock type, among others (Russell et al. 2000; Moriarty et al. 2008; Oliver et al. 2010b). This variability in shedding is
This variation in E. coli shedding poses a significant challenge for the development of modelling approaches to predict the fate and transfer of microbial contaminants through agricultural catchments (Oliver et al. 2012). The growing requirement for the design of ‘programmes of measures’ by Article 11 of the EU Water Framework Directive (WFD), to prevent impairment of ‘protected areas’ (i.e. including bathing and shellfish harvesting waters), is generating an imperative for the development of modelling capacity. This is needed in order to differentiate specific (spatial) effects of land management practices when combined with catchment responses to hydrological drivers at relevant timescales. However, such models need to account for the source strength of faecal reservoirs attributed to different livestock types and while the current evidence-base is growing, it remains far from satisfactory. From a UK perspective, there is an urgent need for an inventory of E. coli concentrations associated with a suite of livestock types for different regions where livestock farming dominates. However, rather than a comprehensive evidence-base that captures variability of regional E. coli counts, there are few studies that provide useful information (e.g. Avery et al. 2004; Hodgson et al. 2009) and arguably not enough for widespread spatial and temporal modelling of FIO accumulation on pasture. This situation is not unique to the United Kingdom. For example, Moriarty et al. (2008) highlighted the dearth of published counts of bacterial indicators in fresh livestock faeces across New Zealand and in response undertook a faecal survey across four farm environments spanning the North and South Islands. With limited national data, those who aim to develop microbial fate and transfer models must either undertake faecal surveys as per Moriarty et al. (2008) or instead draw on microbial counts published in the wider international literature. Of course, these latter values may not be particularly relevant to local conditions.

Clearly, a national inventory of typical FIO counts would take time to evolve and necessitate significant effort to develop. However, the need for better quality information and a robust empirical evidence-base on FIO concentrations for different geographical areas, livestock types and seasons, is fundamental for underpinning our understanding of diffuse microbial pollution from agriculture (and informing mitigation strategies to reduce its impact). Similar issues have been raised with regard to knowledge of the likely FIO concentrations in raw sewage and treated effluents. Kay et al. (2008) identified that few empirical data had been published in the peer reviewed literature for these effluent types and provided a summary of FIO concentrations determined from 162 sewage discharge sites across the United Kingdom and Jersey, and stressed the importance of this data for prioritizing suitable management approaches to water quality protection.

Without a thorough understanding of how the burden of FIOs on pasture varies through an annual cycle (and its susceptibility to vary year-on-year), our landscape-level models of microbial fate and transfer are immediately disadvantaged in terms of their predictive capability. This study was therefore designed to contribute important information on FIO concentrations in dairy faeces – one of the key sources of diffuse microbial pollution from agricultural landscapes. The aim of the study was to quantify seasonal, within-herd and year-on-year variability of FIO (both E. coli and other coliform) concentrations in freshly excreted dairy faeces from a typical farm enterprise in central Scotland.

**Results and discussion**

This study provides a significant data set relating to the potential for E. coli and coliform loading to agricultural land by dairy cattle in central Scotland. By following the same herd over a 2-year period, the study has documented the temporal profile of this variability and highlighted: (i) seasonal impacts on the magnitude of E. coli excreted in fresh faeces of dairy cows; and (ii) how seasonal shedding patterns can fluctuate over successive years. The importance of FIO concentration data in fresh faeces cannot be understated as it provides information that is crucial for the parameterization of models that aim to predict pathogen and FIO fate and transfer in agricultural catchments (Moriarty et al. 2008; Oladeinde et al. 2014). All microbial counts are presented on a fresh and dry weight basis to enable a wider comparison across the literature. All E. coli counts, and all but the spring 2012 combined coliform counts were confirmed as being log-normally distributed (see Table 1 for normality assessment on the fresh weight counts using the Shapiro–Wilk test).

All method blanks were negative for FIOs, indicating no cross-contamination during sample processing. The mean concentration of E. coli determined in fresh dairy faeces for all samples collected across all seasons was found to be 6·63 and 6·58 log_{10} CFU g^{-1} dry weight for 2012 and 2013, respectively. Interestingly, Martinez et al. (2013) reported that the average E. coli concentration in fresh faecal material (based on combined data from six international studies) equated to 6·5 log_{10} CFU g^{-1}, which is close to the average values recorded in both years of this study. A series of boxplots are presented in Fig. 1 to highlight the contrasting variability in concentrations of E. coli excreted in dairy faeces across different seasons over the
were significantly lower (counts determined for different seasons (ANOVA identified a significant difference between the counts and so the statistical analysis focused on the E. coli counts for brevity. With all data combined, a two-way ANOVA identified a significant difference between the counts determined for different seasons (P < 0.001) but not for the overall mean of E. coli counts in 2012 and 2013, it was revealed that seasonal differences in 2013 did not mirror those observed in 2012. In 2013, autumn and winter faecal deposits (mean of 5.34 log_{10} CFU g^{-1} dry weight) were both found to have significantly lower counts of E. coli (mean of 6.24 and 6.16 log_{10} CFU g^{-1} dry weight) but were not significantly lower than those observed in spring (see Fig. 1).

A number of studies have been published that report, to varying extents, on concentrations of FIOs in fresh cattle faeces in New Zealand (Moriarty et al. 2008; Sinton et al. 2008; Sinton et al. 2008).
Donnison et al. 2008; Muirhead and Littlejohn 2009), the US (Weaver et al. 2005; Van Kessel et al. 2007; Soupir et al. 2008), Canada (Meays et al. 2005), Australia (Cox et al. 2005) and the United Kingdom (Avery et al. 2004; Hodgson et al. 2009). All of these studies report variability in concentrations of FIOs in fresh faeces, often in excess of at least one order of magnitude, and this result is consistent with the data reported in this current study. There are contrasting observations evident in the international literature with studies reporting peak concentrations of FIOs associated with different seasons (e.g. Sinton et al. 2007; Moriarty et al. 2008; Muirhead and Littlejohn 2009). Differences in observations at a national level may reflect variations in dietary supplements available to livestock during housing periods (Russell et al. 2000) or anxiety levels of livestock associated with management regimes (Bach et al. 2004). Studies also vary in their use of ‘naturally’ deposited cowpats versus artificially homogenized fresh faecal material crafted into replicate cowpats, and this may also play a role in the observed variability. For example, recent research by Martinez et al. (2013) analyzed data on FIOs in fresh cowpats obtained from a number of studies at different locations across the world and identified that repackaged cowpats had a significantly higher E. coli content than naturally intact cowpats. The same authors also reported that using this combined international data set, artificial repackaged cowpats exhibited relatively small differences in initial concentrations of E. coli in cowpats across different seasons compared with seasonal differences observed in their naturally intact counterparts.

The results of the current study confirm that in 2012, autumn > spring > summer > winter with regard to the concentrations of E. coli detected in fresh dairy faeces on the monitored farm in Scotland. For 2013, this ranking shifted to summer > spring > autumn > winter. Two observations are clear from an inspection of these seasonal rankings: (i) patterns and seasonal peaks of E. coli shedding by dairy cattle are not consistent year-on-year; but (ii) winter does appear to be somewhat consistent in generating dairy faeces with substantially lower E. coli counts relative to other seasons (for a 2 year cycle at least). The apparent shifts in ranking of seasonal E. coli shedding for this study in Scotland may reflect local conditions linked to diet and management that were indirectly impacted by weather conditions. While climatic variables (e.g. temperature and rainfall) cannot be held directly accountable for fresh E. coli concentrations in faeces, because the cells will be held within the animal gut and gastrointestinal tract at 37°C prior to excretion, such environmental factors might influence on-farm management decisions (e.g. changes in grazing management that necessitate a shift in livestock diet) that may then have consequential impacts on E. coli shedding by cattle.

For example in this study, during 2012, dairy cattle were put out to pasture for grazing at the end of April (i.e. mid-spring) but were re-housed relatively early (i.e. July; mid-summer) because of exceptionally wet conditions that rendered grazing activity detrimental to soil and pasture quality. Indeed, summer 2012 ranked as the second wettest in the United Kingdom since records began in 1910, and 121% of the 1961–1990 UK average rainfall was recorded during 2012 (MET Office 2012). The cattle were reintroduced to pasture later in the summer of 2012 and grazed until early September before being rehoused again for autumn and winter. In contrast, the 2013 grazing regime was more straightforward with cattle grazing from the end of April through to the beginning of October. The diet of the cows was necessarily different during the contrasting grazing and housed periods. During grazing, the dietary intake of cattle was predominantly perennial ryegrass Lolium perenne, and this was supplemented with dairy cake (an 18% protein mix containing wheat and distiller’s grains) during milking. During the housed period, their diet consisted mainly of silage combined with distiller’s grains, brewer’s barley and molasses, and again this was supplemented with dairy cake (at an increased 20% protein mix) during milking. Given that the winter period in both years resulted in the lowest counts of E. coli in fresh dairy faeces, it is possible that the housed diet of predominantly silage helped to reduce generic E. coli levels excreted, or at least rendered a proportion as viable but nonculturable. In a comparison of faeces excreted from silage- and pasture-fed cows the concentrations of E. coli have been shown to be lower (by c. 1 order of magnitude) and more variable for those given a silage diet (Donnison et al. 2008). The fermentation process typical of silage production results in the generation of acids, such as lactic acid, that preserves the silage and the resulting reduction in rumen pH once consumed can reduce naturally occurring E. coli that do not grow well at low pH values (Russell et al. 2000). In addition, Donnison et al. (2008) hypothesize that the higher counts associated with pasture-fed diet may reflect a continuous ingestion of FIOs from faecally contaminated pasture. Interestingly, the 2012 summer FIO concentrations ranked lower relative to their 2013 ranking, and this might reflect the removal of the cows from a pasture-fed diet to one of silage during their temporary summer housing because of the exceptionally wet weather in 2012 which was not repeated in 2013.

Statistical analysis using a paired t-test on duplicate samples taken from 40 cowpats across all seasons recorded no significant difference (P = 0.58) in E. coli counts. This suggests that faecal excretion by dairy cattle is effective in homogenizing E. coli populations in the faecal matrix and supports the hypothesis that FIOs are
thoroughly mixed following faecal passage through the ruminant digestive system and gut. This contrasts with observations for specific pathogens such as *E. coli* O157 (Robinson et al. 2005) where cells remain heterogeneously distributed within the faeces. The mean % DM of fresh dairy faeces for all samples collected across all seasons was 13.83 and 13.22% for 2012 and 2013, respectively. The underlying dry matter content of all faecal deposits is presented in Table 2 (mean, median and range), and the variability in % dry matter is shown in Fig. 2 for all seasons across both years. For all data combined, two-way ANOVA identified a significant difference between the % dry matter determined in different seasons (*P* < 0.05) despite accommodating the largest range of % DM recorded across both years of the study. No correlation between % DM content and FIO concentrations in fresh dairy faeces was observed. Moriarty et al. (2008) observed a consistent increase in total solid content of fresh dairy faeces from spring to winter and found the winter total solids content to be approximately double that observed in faeces excreted in spring. In our study, this pattern was not observed, and for both 2012 and 2013, the faeces excreted in summer contained the lowest dry matter content. The lower DM in summer is probably a consequence of diet with pasture forming the predominant source of feed. The higher DM in winter through spring is likely to reflect the diet shift from pasture to silage.

The empirical data reported in this study has highlighted considerable variability in *E. coli* and coliform concentrations and their susceptibility to change seasonally, both between and within annual cycles. This has important implications for modelling approaches that choose to use a single parameter for an *E. coli* concentration typical of dairy faeces (and most probably other faeces associated with other livestock types too) without considering (i) within-herd variation in shedding and (ii) how this seasonal shift in variability might impact on predictions of FIO risk dynamics over time for a given area. Studies such as the one presented here need to be repeated across different regions of the United Kingdom

### Table 2: Dry matter (DM) content of dairy faeces collected throughout the 2-year study. All counts derived from 10 cowpats per sampling event.

| Sampling date | Mean % DM | Median % DM | Range of % DM (magnitude) |
|---------------|-----------|-------------|---------------------------|
| Spring 2012   | 15.19     | 15.16       | 13.96–16.99 (3.04)        |
| Summer 2012   | 11.95     | 11.82       | 9.72–14.52 (4.80)         |
| Autumn 2012   | 14.07     | 13.93       | 11.97–18.31 (6.33)        |
| Winter 2012   | 14.10     | 13.90       | 13.03–15.26 (2.23)        |
| Spring 2013   | 13.92     | 13.86       | 12.34–15.55 (3.21)        |
| Summer 2013   | 11.85     | 11.54       | 9.25–17.44 (8.19)         |
| Autumn 2013   | 12.35     | 12.21       | 9.89–14.25 (4.36)         |
| Winter 2013   | 14.76     | 14.35       | 13.28–17.42 (4.14)        |

Figure 2: Seasonal, within-herd and year-on-year variability of % dry matter content in fresh dairy faeces. Boxplots with different letter codes differ significantly from one another (2012 data: one-way ANOVA, *P* < 0.001; Tukey multiple comparison test, *P* < 0.05 & 2013 data: one-way ANOVA, *P* < 0.001; Tukey multiple comparison test, *P* < 0.05). Centre horizontal dash, box and whiskers represent median, interquartile range and upper and lower limits, respectively. *signifies an extreme value. Values are the mean of 10 replicates.
to build up a better profile of how FIO concentrations vary spatially and in time. Developing an inventory of microbial magnitudes in fresh faeces and improving our understanding of their scope to vary is an important factor to build into modelling approaches and to communicate to catchment stakeholders interested in microbial risks associated with land and water. A concerted effort is essential in order to consolidate this important evidence-base so that uncertainties surrounding FIO concentrations can not only be acknowledged but also used to improve the quality of models of microbial fate and transfer in catchments.

Materials and methods

Sample collection

Ten fresh dairy cowpats were collected on eight sampling occasions over a 2-year period. Samples were collected in March, June, September and December of 2012 and 2013 and represented faeces excreted at the start of each season (spring, summer, autumn and winter, in the northern hemisphere). The ten cowpats served as replicate samples and were collected from ten different cows on each sampling occasion. Thus, a total of 80 cowpats were collected throughout the study period. The cowpats were collected from a single conventional 165 ha dairy farm in Stirlingshire, Scotland. The dairy herd totalled 80 head of cattle, was normally housed from October through to the end of March, and produced an average of 8000 l of milk per year per cow. All cowpats were collected within 30 min of excretion. Fresh samples were collected from a covered holding-barn that was used during the transfer of dairy cows to the parlour for morning milking. This barn was scraped clean twice daily, and so all cowpats collected were assumed to be fresh deposits.

All cowpats were collected from Holstein Friesians used for milk production and were sampled and analyzed for Escherichia coli, coliforms and dry matter (DM) content. Microbial analysis was initiated within 1 h of samples being collected. Approximately 15 g of faeces was randomly sampled from each cowpat using a sterile spatula (70% IMS, rinsed with sterile water) and placed into sterile 50-ml centrifuge tubes. Samples were assumed to be well mixed and homogeneous following faecal passage through the ruminant digestive system and gut. However, for 50% of the cowpats, a duplicate random sample was taken from the faeces to investigate whether the sampling approach could potentially impact on recorded FIO counts because of uneven distribution of cells within the faecal matrix (i.e. spatial bias in counts). Only the original sample was used in the wider analysis reported in this study but the duplicate sample served an important purpose as a subcomponent of this faecal survey, as described.

Sample analysis

One gram of fresh faeces was used for microbial analysis, and the remainder was used to determine the gravimetric water content by drying at 105°C for 24 h (until constant mass) and weighing the residual. For microbial analysis, one gram of faeces was transferred to 9 ml of sterile phosphate buffered saline (PBS) and then thoroughly mixed using an orbital shaker (160 rpm for 60 min at ambient temperature) to disperse cells from the faecal matrix. Further serial 1:10 dilutions were then made as appropriate to ensure capture of between 20 to 200 colony-forming units (CFU) once the sample had been transferred to an agar growth medium. To get to this stage, 1 ml of each serially diluted sample was washed through a filtration unit (Sartorius, Göttingen, Germany) with c. 20 ml of sterile PBS. Membrane filters of 0.45 micron pore size (Sartorius) were aseptically transferred to membrane lactose sucrose agar (MLGA) (Oxoid, Basingstoke, United Kingdom) and incubated inverted at 37°C (±0.2°C) for 18–24 h for the determination of presumptive E. coli and other coliform colonies. Equipment was flame sterilized between samples, and method blanks (i.e. sterile PBS) used to confirm the sterilization procedure. The limit of detection was 100 CFU per g fresh weight faeces.

Statistical analysis

All counts were transformed to log_{10} CFU, and distributions of E. coli were log-normally distributed as determined using the Shapiro-Wilk goodness-of-fit test. Treatment (season, year) differences in E. coli and % DM were compared by two-way analysis of variance (ANOVA) for all data combined. One-way ANOVA was used to test for differences across individual years and Tukey multiple comparison tests applied (Minitab 12.0 software, Minitab Inc., State College, PA). A paired t-test was used to determine whether there was any significant difference between repeated sampling of different subcomponents of the same cowpat.

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Conflicts of Interest

No conflict of interest declared.

References

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

Bach, S.J., McAllister, T.A., Mears, G.J. and Schwartzkopf-Genswein, K.S. (2004) Long-haul transport and lack of preconditioning increases faecal shedding of Escherichia coli and Escherichia coli O157 by calves. J Food Prot 67, 672–678.

Blaustein, R.A., Pachepsky, Y., Hill, R.L., Shelton, D.R. and Whelan, G. (2013) Escherichia coli survival in waters: temperature dependence. Water Res 47, 569–578.

Chadwick, D., Fish, R., Oliver, D.M., Heathwaite, L., Hodgson, C. and Winter, D.M. (2008) Management of livestock and their manure to reduce the risk of microbial transfers to water: the case for an interdisciplinary approach. Trends Food Sci Technol 19, 240–247.

Cox, P., Griffith, M., Angles, M., Deere, D. and Ferguson, C. (2005) Concentrations of pathogens and indicators in animal feces in the Sydney watershed. Appl Environ Microbiol 71, 5929–5934.

Donnison, A., Ross, C. and Clark, D. (2008) Escherichia coli shedding by dairy cows. NZ J Agric Res 51, 273–278.

Ferguson, C.M., Charles, K. and Deere, D.A. (2009) Quantification of microbial sources in drinking-water catchments. Crit Rev Environ Sci Technol 39, 1–40.

Hodgson, C.I., Bulmer, N., Chadwick, D.R., Oliver, D.M., Heathwaite, A.L., Fish, R.D. and Winter, M. (2009) Establishing relative release kinetics of faecal indicator organisms from different faecal matrices. Lett Appl Microbiol 49, 124–130.

Kay, D., Crowther, J., Stapleton, C.M., Wyer, M.D., Fewtrell, L., Edwards, A., Francis, C.A., McDonald, A.T. et al. (2008) Faecal indicator organism concentrations in sewage and treated effluent. Water Res 42, 442–454.

Martinez, G., Pachepsky, Y.A., Shelton, D.R., Whelan, G., Zepp, R., Molina, M. and Panhorst, K. (2013) Using the Q10 model to simulate E. coli survival in cowpats on grazing lands. Environ Int 54, 1–10.

Meays, C.L., Broersma, K., Nordin, R. and Mazumder, A. (2005) Survival of Escherichia coli in beef cattle faecal pits under different levels of solar exposure. Rangeland Ecol Manage 58, 279–283.

MET Office. (2012). Regional annual summaries of UK rainfall 2012. http://www.metoffice.gov.uk/climate/uk/summaries/2012/annual/regional-values. Accessed 29th January 2014.

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.

Muirhead, R.W. and Littlejohn, R.P. (2009) Die-off of Escherichia coli in intact and disrupted cowpats. Soil Use Manage 25, 389–394.

Oladeinde, A., Bohrmann, T., Wong, K., Purucker, S.T., Bradshaw, K., Brown, R., Snyder, B. and Molina, M. (2014) Decay of fecal indicator bacterial populations and bovine-associated source-tracking markers in freshly deposited cow pats. Appl Environ Microbiol 80, 110–118.

Oliver, D.M., Page, T., Heathwaite, A.L. and Haygarth, P.M. (2010a) Re-shaping models of E. coli population dynamics in livestock faeces: increased bacterial risk to humans? Environ Int 36, 1–7.

Oliver, D.M., Page, T., Hodgson, C.I., Heathwaite, A.L., Chadwick, D.R., Fish, R.D. and Winter, M. (2010b) Development and testing of a risk indexing framework to determine field scale critical source areas of faecal bacteria on grassland. Environ Model Softw 25, 503–512.

Oliver, D.M., Page, T., Zhang, T., Heathwaite, A.L., Beven, K., Carter, H., McShane, G., Keenan, P.O. et al. (2012) Determining E. coli burden on pasture in a headwater catchment: combined field and modelling approach. Environ Int 43, 6–12.

Robinson, S.E., Brown, P.E., Wright, E.J., Bennett, M., Hart, C.A. and French, N.P. (2005) Heterogeneous distributions of Escherichia coli O157 within naturally infected bovine faecal pats. FEMS Microbiol Lett 244, 291–296.

Russell, J.B., Diez-Gonzalez, F. and Jarvis, G.N. (2000) Effects of diet shifts on Escherichia coli in cattle. J Dairy Sci 83, 863–873.

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

Songir, A.L., Mostaghimi, S. and Lou, J. (2008) Die-off of E. coli and enterococci in dairy cowpats. Trans ASABE 51, 1987–1996.

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

Weaver, R.W., Entry, J.A. and Graves, A. (2005) Numbers of fecal streptococci and Escherichia coli in fresh and dry cattle, horse, and sheep manure. Can J Microbiol 51, 847–851.

Wu, J., Long, S.C., Das, D. and Dorner, S.M. (2011) Are microbial indicators and pathogens correlated? A statistical analysis of 40 years of research. J Water Health 9, 265–278.