DETERMINING HYDROLOGICAL PATHWAYS FOR THE TRANSFER OF POTENTIAL PATHOGENS FROM GRASSLAND SOILS TO SURFACE WATERS

D. M. Oliver *[&]**, C. D. Clegg *, P. M. Haygarth * and A. L. Heathwaite **

** Soil Science and Environmental Quality Team, Institute of Grassland and Environmental Research, North Wyke Research Station, Okehampton, Devon, UK, EX20 2HN*
(E-mail: david.oliver@bbsrc.ac.uk; christopher.clegg@bbsrc.ac.uk; phil.haygarth@bbsrc.ac.uk)
*** Dept. of Geography, University of Sheffield, Winter Street, Sheffield, UK, S10 2TN*
(E-mail: A.L.Heathwaite@sheffield.ac.uk)

ABSTRACT
Within the UK, large amounts of livestock wastes are applied to grassland farming systems both as excreta from grazing animals and as slurries. A consequence of this management practice is the opportunity for potential pathogens to disperse through the soil following the input of hydrological energy derived from rain events. This study reports findings from a field drainage experiment in Devon whereby agricultural drainage waters were monitored for *Escherichia coli* immediately following a five-month cattle grazing period. A comparison was made between the different hydrological export routes in transferring *E. coli* from soil to receiving waters. Storm waters mobilised bacteria at much higher concentrations than occurred during base flows suggesting the existence of energy thresholds for the entrainment and successful transfer of soil, waste and biological colloids from within the soil matrix. The results of the post-grazing phase of this study propose that faecal bacteria, introduced to grasslands via grazing cattle, travel through different transfer pathways when combined with hydrological energy of precipitation events.

Keywords :Agriculture; faecal bacteria; diffuse pollution; Escherichia coli; hydrological pathways; livestock wastes

INTRODUCTION
Within the U.K. each year, an estimated 150 M tonnes of livestock-derived faecal wastes are applied to grasslands (Nicholson *et al*., 2000; Smith and Frost, 2000). A large proportion of this, about 90 M tonnes, is applied to land in the form of manure and slurry (Smith and Frost, 2000). The remainder is deposited directly as excreta by grazing livestock. Contained within faecal material is a broad range of micro-organisms that are common inhabitants of the gastro-intestinal tract of cattle; an unknown proportion of which may be pathogenic to humans. The introduction of livestock wastes to agricultural soils enhances the potential for contamination of soils and surface waters and increases the risk of transmission to a wider community. Many studies have acknowledged agriculture as a vector of food chain contamination through the faecal-oral transmission route (e.g. Bryan, 1977; Bates *et al*., 1994; Wight *et al*., 1997; Natvig *et al*., 2002; Solomon *et al*., 2002). However, it is only recently that attention has shifted to the pathogen transmission risk associated with runoff from pastures in facilitating gastro-intestinal infections within the human population.

The increasing frequency with which agriculture is associated with bacterial contamination of surface waters continues to heighten public awareness of farming as a significant source for the loading of soil with enteric bacteria (e.g. Fenlon *et al*., 2000; Ogden *et al*., 2001; Vinten *et al*., 2002; Aitken, 2003). In response, there exists a need to detail faecally-derived potential pathogen movements at the field scale in order to identify conditions which favour their export via carrying drainage waters and simultaneously to develop management strategies that satisfy the requirements of the EU Water Framework Directive.
Throughout a typical year, grassland management is likely to give rise to three different phases of potential loading and subsequent transport of faecal bacteria: (1) the slurry application period, (2) the grazing season and (3) the post-grazing season. Each period in the agricultural calendar is associated with a different set of environmental conditions that contribute to the potential for transfer. In addition, the survivability of the micro-organisms as a function of both carrier medium and time adds another dimension to the potential for bacterial loading of receiving waters. It has been demonstrated that, within catchments and sub-catchments, grassland farming practices are capable of contributing considerably to the loading of receiving waters with faecally-derived bacteria (Patni *et al*., 1985; Heinonen-Tanski and Uusi-Kamppa, 2001; Nagels *et al*., 2002). However, what remains to be understood is the relative contribution of the suite of hydrological pathways available to these microbes in facilitating their transfer from source to receptor as driven by rainfall events.

This study evaluates the transfer of *E. coli* from 1 ha experimental plots during the post-grazing phase to assess the differences in the number of cells exported via defined hydrological pathways. This bacterium was used as an indicator for the routes of faecally-derived cells in connecting pathways. Comparisons were made between the routes of transfer associated with both undrained grasslands and those with artificial drainage.

MATERIALS AND METHODS
The experimental field site was established in 1982 as the Rowden Drainage Experiment (NGR SX 650 995) at North Wyke, Devon. This facility provided the current study with a total of 8 hillslope lysimeters of 1 ha area, half of which were installed with mole and pipe drains (40 and 85 cm, respectively) to re-route water loss from the field. The hydrological

isolation of each plot allowed for a comparison of transfer routes from drained and undrained grassland lying on a slope of between 5 and 10 degrees. The soil comprised an Ap horizon (0-30 cm) of 37% clay, 5% organic matter and a well-developed fine subangular block structure overlying a well developed coarse prismatic-structured subsoil of 40% clay (Findlay *et al*. 1984). This clay loam of the Hallsworth series is comparable with many other soils where grassland production predominates (Armstrong and Garwood, 1991) and represents the single most common hydrological soil type in the UK (Haygarth *et al*., 1998). The impermeable clay layer in the B horizon prevents any natural vertical drainage below 30 cm making the soil conducive to lysimetry.

The undrained plots have a single hydrological pathway that incorporates all overland flow and interflow to a depth of 30 cm. This composite flow is collected in gravel filled ditches installed at 30 cm depth at plot boundaries. Mole and pipe drains provide drained plots with a secondary export route in addition to the composite pathway that is found in undrained plots. Samples were collected with a 2-tiered approach using a manual weekly baseline sampling programme complemented with a manual plus automated intensive storm event sampling strategy. Sampling commenced with the emergence of drainage water and spanned the complete drainage period. Each of the 4 replicate drained and undrained plots had been grazed for 5 months with 4 steers. Soil cores to a depth of 7 cm were also sampled to relate numbers emerging in drainage waters to those determined in the upper soil matrix. A weather station already located at the site accommodated a rain gauge and soil temperature probes. Detailed instrumentation was installed for one replicate of each plot and included a flow level recording device as described by Talman (1983), TDR probes (Campbell Scientific CS616) and automated water sampling units (Buhler Montec 1011 Epic). Operation of all devices was controlled using a Campbell Scientific CR10 datalogger and wiring panel.

When flow levels exceeded a pre-determined threshold level the auto-sampler was triggered through an inline solid state switch (Campbell Scientific PSW12) and via an auxiliary signal lead. This control output from the datalogger was simply a prompt for the autosampler to run its programme to completion for a pre-configured period regardless of the water flow in relation to the threshold once the initial contact closure had been enabled. Sampling was initiated on a time-wise basis (see also Roser *et al*., 2002) and allowed the characterisation of flow hydrograph signatures during storm events.

For the microbiological analysis of soil and water samples, the following procedures were undertaken: Fresh soil samples were crumbled and 10 g was added to 90 ml of sterile water prior to mixing for 40 minutes on a rotary agitator. The resulting soil suspensions, and water samples, were serially diluted then spread-plated onto MacConkey agar and incubated at 37 ^{o}C for 24 hours before enumeration of colony forming units (CFU). Colonies of E. coli were enumerated using particular colony characteristics on MacConkey agar, after confirmation of their identity by partial 16S rRNA gene sequences. Briefly, a region of the 16S rRNA gene was amplified by the polymerase chain reaction (PCR) using primers 968f and 1401r (Heuer and Smalla, 1997). All PCR amplifications were carried out in a 40 µl reaction volume containing 20 pmol each of primers 968f and 1401r, 250 µM dNTPs, 3 U Expand High Fidelity polymerase (Roche Diagnostics, U.K.) and 4 µl 10x reaction buffer. To each reaction mix were added a few cells taken from individual bacterial colonies representative of seven different colony types on MacConkey agar. PCR was performed using the following cycle, 95 ^{o}C for 5 min followed by 30 cycles of 95 ^{o}C for 1 min, 55 ^{o}C for 30 s, 72 ^{o}C for 1 min. A final extension time of 5 min at 72 ^{o}C was included. All PCR samples were amplified using a Primus Thermocycler (MWG-Biotech, U.K.), and PCR products were checked on a 1.2% low melting temperature agarose gel stained with ethidium bromide. Bands of the expected product size were excised from the gel which was then melted at 65 ^{o}C before digestion of the agarose with agarase (Roche Diagnostics, U.K.) and recovery of the PCR products according to instructions. For sequencing of PCR products, forward and reverse reactions were undertaken using primers 968f and 1401r using the BigDye(TM) Terminater v3.0 Cycle Sequencing kit as described by the manufacturers, and the sequencing was performed by capillary electrophoresis on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Warrington, UK).

RESULTS AND DISCUSSION

MacConkey agar is a differential medium often used in the isolation of coliforms. The identity of *E. coli* colonies in this study was confirmed by partially sequencing 16S rRNA genes of isolates representative of seven different colony types. The data presented provide an account of *E. coli* contamination of agricultural drainage waters following a typical grazing season. Cattle were removed from the plots in October 2002 (t = 0 d; see Table 1) and the emergence of *E. coli* cells in drainage waters was observed in the weeks that followed. Background soil counts of *E. coli* from an ungrazed plot were negligible in comparison with those from grazed plots.

Differences through time

Observations revealed a marked decline of *E. coli* detected in the upper 7 cm of soil and an increased number of cells exported from within the soil matrix. Table 1 details average *E. coli* concentrations and loads exported in the 3 hydrological pathways in relation to daily rainfall and the average flow rate in each transfer route for selected sampling dates. Higher concentrations of *E. coli* in drainage waters from both drained and undrained plots coincided with periods of high rainfall and with times of increased outflow through the draining hydrological pathways. This was possibly due to the entrainment and subsequent flush of cells from land to water that corresponded with the increased hydrological energy transferred to the soil habitat. Such an observation parallels work associated with faecal bacteria concentrations in rivers draining agricultural land, where positive correlations of cells with flow have been recorded (Nagels *et al*., 2002). Highest

concentrations were observed in both of the continuously flowing pathways in the first month and a half following the removal of cattle, after this period there was a noticeable decline in the concentration of the indicator organism in the drainage waters. The extent to which this is a function of organism die-off or cell wash-out now needs to be determined.

Table 1: Average flow, E. coli concentration and E. coli load exported from each hydrological pathway

Days since cattle removed (d)	Daily rainfall (mm)	Average Sampled Flow (ls^{-1})			Concentration (CFU ml^{-1})			Load per hour (Flow x Conc)		
		S	M	C	S	M	C	S	M	C
16	0.4	X	0.117	0.090	X	8	30	X	3.79E+06	9.72E+06
23	4.8	0.062	0.472	0.419	X	50	99	X	8.50E+07	1.51E+08
24	24.4	2.177	2.728	4.267	382	389	286	2.92E+09	4.00E+09	4.47E+09
30	7.2	0.027	0.398	0.342	X	69	88	X	9.89E+07	1.08E+08
37	2.8	X	0.062	0.065	X	7	15	X	1.56E+06	3.51E+06
38	18.8	1.579	2.810	4.211	320	358	219	1.82E+09	3.62E+09	3.32E+09
58	0.0	X	0.132	0.084	X	11	23	X	5.70E+06	7.26E+06
131	8.4	0.554	1.194	1.990	5	9	6	2.59E+07	4.73E+07	1.43E+08

S = Surface pathway, drained plot; M = Mole and pipe drain pathway, drained plot; C = Composite pathway, undrained plot
X = No flow

During the rain event 24 days after the removal of cattle the artificial drain pathway accommodated *E. coli* at export in the range (mean ± standard error) of 380 ± 59 CFU ml^{-1} in comparison with 8 ± 3 CFU ml^{-1} only 16 days following removal. This demonstrates the ability of cells to persist for extended periods in unfavourable environmental conditions until exported when there is sufficient hydrological energy derived from a rainfall event. A similar situation in the composite undrained pathway was observed with increased hydrological energy providing the vehicle for the export of increased *E. coli* concentrations.

Data in table 1 suggest that, although introduced *E. coli* is able to survive in the soil, there is a decline in cell numbers over time within all 3 hydrological pathways. This is noted in the average concentration and loads of cells exported when comparing data from the storm events of day 24 and 38. The observed decline is emphasised to a greater extent 131 days after the removal of livestock. Then daily rainfall levels similar to those noted 30 days post grazing failed to export such high concentrations of *E. coli*, with average values of 9 CFU ml^{-1} and 6 CFU ml^{-1} within artificial drain and undrained composite pathways, respectively. This marked decline is evident in the surface draining pathway on the drained plots also, with around a 23 times reduction of cell numbers on day 131 in comparison with day 30, perhaps reflecting a combined effect of removal of cells from the upper soil profile and micro-organism die-off. The rain event on day 131 was also of higher intensity than that of day 30, and generated flows from the plots of a much higher energy thought likely to mobilise and promote cell transfer, yet the concentrations were much reduced. However, in contrast the total load of *E. coli* exported through two of the drainage routes is greater for the later date because of the increased flow generated in the hydrological pathways. Such an observation demonstrates the role played by water in acting as a carrier and vector facilitating indicator bacteria and hence potential pathogen transfer to surface waters. Under low rainfall conditions, cells counts were frequently below detection limits some 3 months after the end of grazing and of little significance to surface water contamination. This is likely to be a result of the combined effect of both organism die-off and wash-out of cells from within the matrix as discussed earlier and these criteria are currently being evaluated in follow-up work to that reported here.

Figure 1 shows the correlation ($r = 0.793$; $p < 0.001$) between flow and *E. coli* concentration for drainflow from the plots for the period October 2002 to March 2003. The persistence of *E. coli*, albeit in vastly reduced numbers, complements previous studies of Buckhouse and Gifford (1976), Fukushima *et al*. (1999) and Jiang *et al*. (2002) who have detailed faecal coliform and *E. coli* survival within the protective niche of faecal deposits. All were consistent in finding that microbial tolerance of environmental conditions within these favourable microsites enabled faecal deposits to act as a long-term potential source of environmental pollution.

Differences in hydrological pathways

Observations of cell export reveal that there is little difference between the concentration of *E. coli* transferred in drains on drained plots and those of composite pathways on undrained plots. However, reference to Table 1 suggests a trend in that under general drainage conditions and low rainfall, more cells are removed in drainage waters from the undrained plots, but, at higher levels of rainfall, increased concentrations are exported via the mole drains. These lower concentrations emerging via the mole drains may be associated with an improved filtering efficiency of drained plots hindering the transfer of cells at depth due to the greater vertical profile negotiated. However, given increased energy these cells may

bypass the matrix in preferential flow pathways resulting in the increased mole drain concentrations detected during high flows.

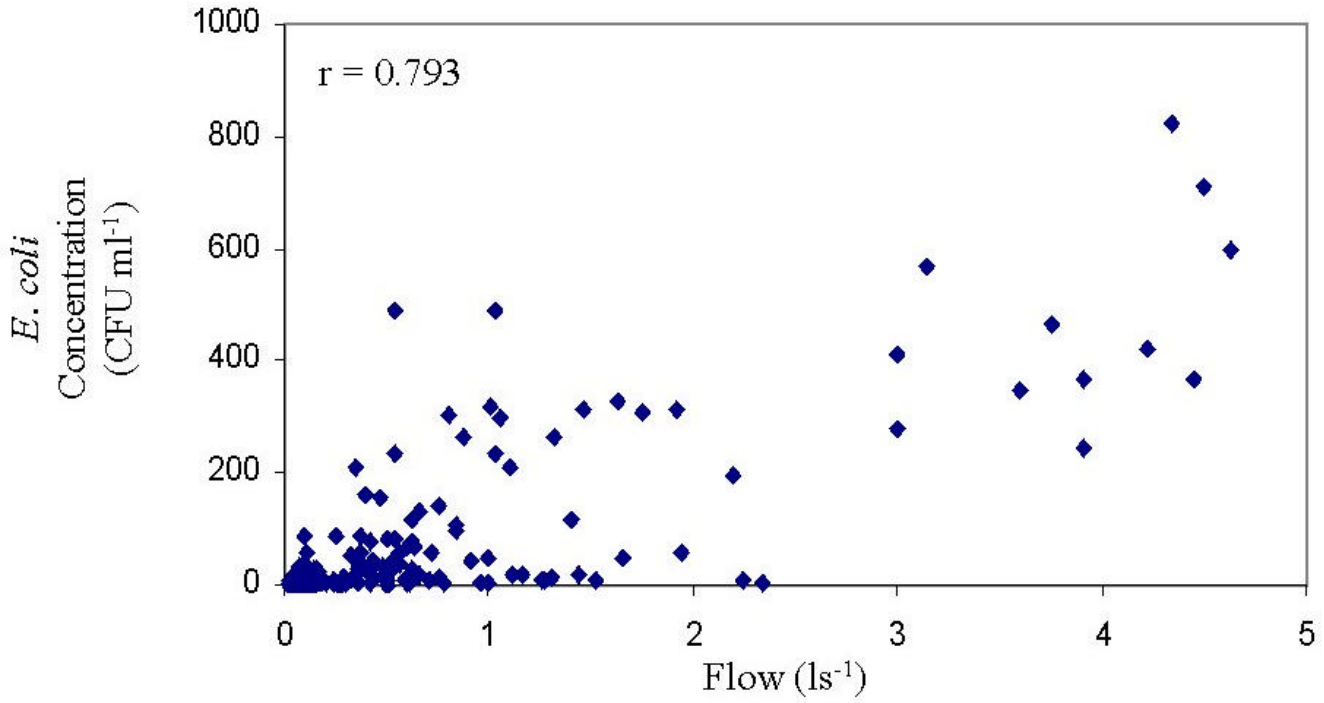

Figure 1*: Relationship between flow from artificial drain pathway and* E. coli *concentration*

During increased rates of rainfall, undrained plots experience extreme waterlogging in contrast to their drained counterparts. Soil erosion is promoted through rainsplash (Van Dijk *et al*., 2003) by the impaction of falling raindrops and this may not have had such an erosive effect in mobilising soil particulates and in releasing cells from the protective niche of faecal material due to the increased depth of pooled water on undrained plots. The drained plots, in comparison, accommodate less surface-accumulating water and so the incoming kinetic energy is not dispersed as much in a layer of water on the soil surface, but is instead dissipated to the soil surface itself. Consequently the transferred momentum of raindrops may have dislodged soil particles along with sorbed microbes, freed attached micro-organisms into the overlying water and physically broken down and transferred faecal matter. In any case, the hydrological energy has acted to initiate the movement and transfer of cells through the upper soil regions and perhaps prompted colloidal movement into soil pores.

Klein and Casida (1967) have drawn attention to the fact that under conditions of excessive moisture, as experienced in the undrained plots, there exists the potential for considerable dilution of organic carbon thus perhaps imposing unfavourable conditions for *E. coli* survival and promoting a more rapid die-off on these plots. Lower numbers in emerging drainage waters may then simply relate to conditions unfavourable to these micro-organisms within these plots.

During heavy storms, the surface composite pathway is activated on drained plots and this acts as an overflow pathway analogous to true overland flow conditions. The concentrations in this pathway were high and perhaps reflect the lateral splash transportability and surface runoff processes, such as 'wash in' of faecal material, acting at the soil surface. The importance of the overland flow pathway has been acknowledged in the earlier study of Abu-Ashour and Lee (2000). However, the cell concentrations were not as high on the undrained plot under the same conditions. This perhaps suggests that the more waterlogged undrained plots, more susceptible to overland flow, have already experienced a more severe depletion of their surface supply of faecal material through wash-in processes. On drained plots this is a less frequent occurrence. Alternatively, as noted by Stephenson and Street (1978), if the source of faecal contamination is deposited in a location where overland flow is unlikely to capture waste associated cells then the sporadic positioning of faecal waste may explain the differences in concentrations observed between drained and undrained plots. However, as noted by Camper *et al*. (1993), it is not only porous medium hydro-dynamics that govern microbial transport; in addition bacterial characteristics such as size and motility and properties of the soil itself such as soil hydrophobicity all interact to determine microbial fluxes.

CONCLUSIONS

The period immediately following the grazing season has a significant impact on bacteriological quality of drainage waters when combined with elevated surface and subsurface runoff from experimental plots driven by storm events. These storm waters mobilise faecal bacteria at a much higher concentration than during base flow conditions. This demonstrates the importance of the physical driving force of the wetting front moving through the soil as opposed to the diluting effect often attributed to large volumes of water. Although storm events may be of a relatively short duration, the bacterial loads exported from agricultural fields are greatest during these events. The study has demonstrated that different hydrological routes contribute to the loading of surface waters with bacterial contaminants and shown that artificial drainage has the potential to enhance the export of cells under periods of high rainfall. These data provide a basic framework upon which to develop a bacterial transfer model that can incorporate pathogenic strains of bacteria. The characteristic survival curves of gut-derived bacteria are currently being determined for a suite of environmental conditions to predict the relative contributions of precipitation events and organism die-off with respect to the decline of faecally-derived bacteria in managed grasslands.

ACKNOWLEDGEMENTS

We wish to acknowledge the assistance of Patricia Butler and Adrian Joynes in gathering field data and also thank Kirsten Skot and Leif Skot at IGER Aberystwyth for sequencing partial 16S rRNA genes. This research was funded by a University of Sheffield Research Studentship.

REFERENCES

Abu-Ashour, J. and Lee, H. (2000). Transport of bacteria on sloping soil surfaces by runoff. *Environmental Toxicology*, **15**(2), 149-153.

Aitken, M.N. (2003). Impact of agricultural practices and river catchment characteristics on river bathing water quality. *Water Science and Technology*, In press.

Armstrong, A.C. and Garwood, E.A. (1991). Hydrological consequences of artificial drainage of grassland. *Hydrological Processes*, **5**, 157-174.

Bates, J., Jordens, J.Z. and Griffiths, D.T. (1994). Farm-Animals as a Putative Reservoir for Vancomycin-Resistant Enterococcal Infection in Man. *Journal of Antimicrobial Chemotherapy*, **34**(4), 507-514.

Bryan, F.L. (1977). Diseases transmitted by foods contaminated by wastewater. *Journal of Food Protection*, **40**(1), 45-56.

Buckhouse, J.C. and Gifford, G.F. (1976). Water quality implications of grazing on a semi-arid watershed in southeastern Utah. *Journal of Range Management*, **29**, 109-113.

Camper, A.K., Hayes, J.T., Sturman, P.J., Jones, W.L. and Cunningham, A.B. (1993). Effects of Motility and Adsorption Rate Coefficient on Transport of Bacteria through Saturated Porous-Media. *Applied and Environmental Microbiology*, **59**(10), 3455-3462.

Fenlon, D.R., Ogden, I.D., Vinten, A. and Svoboda, I. (2000). The fate of *Escherichia coli* and *E-coli* O157 in cattle slurry after application to land. *Journal of Applied Microbiology*, **88**, 149S-156S.

Findlay, D.C., Colborne, D.W., Cope, D.W., Harrod, T.R., Hogan, D.V. and Staines, S.V. (1984). *Soils and their use in South West England. Soil Survey of England and Wales*. Bull **14**. Harpenden, UK. Whitstable Litho Ltd, Whitstable, UK.

Fukushima, H., Hoshina, K. and Gomyoda, M. (1999). Long-term survival of Shiga toxin-producing *Escherichia coli* O26, O113, and O157 in bovine feces. *Applied and Environmental Microbiology*, **65**(11), 5177-5181.

Haygarth, P.M., Hepworth, L. and Jarvis, S.C. (1998). Forms of phosphorus transfer in hydrological pathways from soil under grazed grassland. *European Journal of Soil Science*, **49**, 65-72.

Heinonen-Tanski, H. and Uusi-Kamppa, J. (2001). Runoff of faecal microorganisms and nutrients from perennial grass ley after application of slurry and mineral fertiliser. *Water Science and Technology*, **43**(12), 143-146.

Heuer, H. and Smalla, K. (1997). Application of denaturing gradient gel electrophoresis and temperature gradient gel electrophoresis for studying soil microbial communities. In: Modern soil microbiology, J.D. van Elsas, J.T. Trevors, andE.M.H. Wellington (ed.), Marcel Dekker Inc., New York, N.Y. p. 353-373.

Jiang, X., Morgan, J. and Doyle, M.P. (2002). Fate of *Escherichia coli* 0157:H7 in manure amended soil. *Applied and Environmental Microbiology*, **68**(5), 2605-2609.

Klein, D.A. and Casida, L.E. (1967). *E. coli* die-off from normal soil as related to nutrient availability and the indigenous microflora. *Canadian Journal of Microbiology*, **13**(11), 1461-1470.

Nagels, J.W., Davies-Colley, R.J., Donnison, A.M. and Muirhead, R.W. (2002). Faecal contamination over flood events in a pastoral agricultural stream in New Zealand. *Water Science and Technology*, **45**(12), 45-52.

Natvig, E.E., Ingham, S.C., Ingham, B.H., Cooperband, L.R. and Roper, T.R. (2002). *Salmonella enterica Serovar Typhimurium* and *Escherichia coli* contamination of root and leaf vegetables grown in soils with incorporated bovine manure. *Applied and Environmental Microbiology*, **68**(6), 2737-2744.

Nicholson, F.A., Hutchinson, M.L., Smith, K.A., Keevil, C.W., Chambers, B.J. and Moore, A. (2000). *A study on farm manure applications to agricultural land and an assessment of the risks of pathogen transfer into the food chain*. A report to: The Ministry of Agriculture Fisheries and Food.

Ogden, I.D., Fenlon, D.R., Vinten, A.J.A. and Lewis, D. (2001). The fate of *Escherichia coli* O157 in soil and its potential to contaminate drinking water. *International Journal of Food Microbiology*, **66**(1-2), 111-117.

Patni, N.K., Toxopeus, H.R. and Jui, P.Y. (1985). Bacterial quality of runoff from manured and non-manured cropland. *Transactions of the ASAE*, **28**(6), 1871-1884.

Roser, D., Skinner, J., LeMaitre, C., Marshall, L., Baldwin, J., Billington, K., Kotz, S., Clarkson, K. and Ashbolt, N. (2002). Automated event sampling for microbiological and related analytes in remote sites: a comprehensive system. *Water Science and Technology: Water Supply*, **2**(3), 123-130.

Smith, K.A. and Frost, J.P. (2000). Nitrogen excretion by farm livestock with respect to land spreading requirements and controlling nitrogen losses to ground and surface waters. Part 1: Cattle and sheep. *Bioresource Technology*, **171**, 173-181.

Solomon, E.B., Yaron, S. and Matthews, K.R. (2002). Transmission of *Escherichia coli* 0157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalisation. *Applied and Environmental Microbiology*, **68**(1), 397-400.

Stephenson, G.R. and Street, L.V. (1978). Bacterial variations in streams from a Southwest Idaho Rangeland Watershed. *Journal of Environmental Quality*, **7**(1), 150-157.

Talman, A.J. (1983). A Device for Recording Fluctuating Water Levels. *Journal of Agricultural Engineering Research*, **28**(3), 273-277.

Van Dijk, A., Bruijnzeel, L.A. and Eisma, E.H. (2003). A methodology to study rain splash and wash processes under natural rainfall. *Hydrological Processes*, **17**(1), 153-167.

Vinten, A.J.A., Lewis, D. R., Fenlon, D. R., Leach, K. A., Howard, R., Svoboda, I., and Ogden, I. (2002). Fate of *Escherichia coli* and *Escherichia coli* O157 in soils and drainage water following cattle slurry application at 3 sites in southern Scotland. *Soil Use and Management*, **18**(3), 223-231.

Wight, J.P., Rhodes, P., Chapman, P.A., Lee, S.M. and Finner, P. (1997). Outbreaks of food poisoning in adults due to *Escherichia coli* O111 and *Campylobacter* associated with coach trips to northern France. *Epidemiology and Infection*, **119**(1), 9-14.