Soil Use and Management (2004) **20**, 13–22 DOI: 10.1079/SUM2004214

Relative risk of surface water pollution by *E. coli* derived from faeces of grazing animals compared to slurry application

A.J.A. Vinten[1,*], J.T. Douglas[1], D.R. Lewis[1], M.N. Aitken[2] & D.R. Fenlon[3]

Abstract. This article examines some of the factors that influence the relative risk of *Escherichia coli* pollution of surface waters from grazing animals compared to cattle slurry application. Drainage water from pipe-drained plots grazed with sheep (16 sheep + lambs per hectare) from 29 May to 17 July 2002 had average *E. coli* counts of 11 c.f.u. mL^{-1} or 0.4% of estimated *E. coli* inputs over the grazing period. Drainage water from plots on the same site treated with cattle slurry (36 $m^3 ha^{-1}$ on 29 May 2002) had lower average *E. coli* counts of 5 c.f.u. mL^{-1} or 0.03% of estimated faecal input. Sheep (16 lambs per hectare) grazing under cooler, moister conditions from 24 September to 3 December 2001 gave drainage water with much higher average *E. coli* counts of 282 c.f.u. mL^{-1} or 8.2% of estimated input, which is more than twice the average *E. coli* counts previously reported under such conditions (Vinten *et al.* 2002 *Soil Use and Management* 18, 1–9). Laboratory studies of runoff from soil slabs after slurry application showed that the mobility of *E. coli* in surface soil decreased with time, suggesting that increased attachment to soil or migration to 'immobile' water also provides at least part of the physical explanation for the relatively higher risk of pollution from grazing animals compared with slurry. Sampling for *E. coli* in field drainflow and in streamwater during a storm event in the predominantly dairy Cessnock Water catchment, Ayrshire, Scotland supported the hypothesis that *E. coli* transport is linked to grazing animals. For a 7-mm rainfall event, roughly 14% of the estimated daily input from grazing livestock was transported to the river, even though little slurry spreading had occurred in the catchment in the previous month. Spot sampling of field drains in grazed fields and silage fields in the same catchment also showed that grazing animals were the principal source of *E. coli* and faecal streptococci.

Keywords: *E. coli*, runoff, drainage, bathing waters, risk assessment, slurry, grazing animals

INTRODUCTION

The presence of *Escherichia coli* and other faecal indicator organisms (FIOs), such as streptococci, in surface waters can indicate a human health hazard, because faecal contamination increases the risk of enteric pathogenic microorganisms being endemic. Transport of FIOs from land to bathing waters (Kay *et al.* 1999; SEPA 2002), to public or private water supplies (e.g. Fattal *et al.* 1988; Goss *et al.* 1998), and to river waters abstracted for irrigation of ready-to-eat vegetables (Beuchat 1995) are therefore of public concern. The regulation of such contamination is covered in the European Union by Directives such as the Bathing Waters Directive (Anon 1976), and more recently the Water Framework Directive (Anon 2000).

Two of the main non-human sources of waterborne FIOs are wastes from housed livestock, which are spread on land (slurries and manures), and fresh faeces from grazing animals (Kay *et al.* 1999; Tian *et al.* 2002). Some pathogens are also associated with non-livestock sources, for example, *Campylobacter* spp. derived from wild birds (Obiri-Dansok & Jones 1999). Where regular failure to comply with bathing water standards occurs, for example, on the Ayrshire coast in Scotland (SEPA 2002), it is important to quantify the relative risks from these two major sources of faecal contamination, so that rational mitigation strategies can be devised. Vinten *et al.* (2002) found that up to 5% of faecal *E. coli* inputs from slurry were leached in a viable state from drained plots in eastern Scotland, but there is little work comparing the relative risk of contamination of surface waters from field applications of slurry with that from grazing animals.

[1]SAC Environmental Research Group, Bush Estate, Penicuik, Midlothian EH26 0PH, UK. [2]SAC Environmental, Auchincruive, Ayrshire KA6 5HW, UK. [3]SAC Centre for Microbiological Research, Craibstone, Aberdeen AB2 9DR, UK.
*Corresponding author. Fax: +44 (0)131 535 3031. E-mail: a.vinten@ed.sac.ac.uk

A number of factors influence this relative risk. At a *soil profile* scale, these factors include the relative die-off rates, the relative strength of attachment to soil and to faecal surfaces (Thelin & Gifford 1983), the electrolyte concentration (M.J. Goss pers. comm.) and the relative filtration efficiency of FIOs from slurry and fresh faeces. At a *field* scale, slurry is spread relatively uniformly, whereas grazing animals deposit faecal material unevenly. If best practice advice on slurry spreading is followed (e.g. MAFF 1998; SOAEFD 1997), conditions which are prone to generate high losses will be avoided, so slope and soil type will be different from those of fields used for grazing (Fraser *et al.* 1998; Tian *et al.* 2002). At a *farm* scale, important factors include the relative size of FIO inputs to land from grazing animals and from slurry stores, and relative timing of slurry and fresh faecal inputs to fields. Time spent by livestock on hard-standing areas and tracks vulnerable to runoff will be longer where dairy animals are brought in from grazing to the milking parlour than if they are housed. This will lead to a higher risk of polluted water reaching streams. The direct access of grazing animals to streams rather than to drinking troughs is also an important consideration (Tiedemann *et al.* 1987). At a *catchment* scale, the efficiency of delivery of runoff and drainage water from field to stream may be different in grazed areas compared to slurry spreading areas, and connectivity with surface waters will also depend on livestock access to watercourses. Entrainment of river sediment containing protected *E. coli* (Milne *et al.* 1989) during storm events (Wilkinson *et al.* 1995; Wyer *et al.* 1996) may influence delivery of FIOs to coastal bathing waters. Larger inputs of sediment to rivers will tend to occur from fields poached during animal grazing than from slurry treated fields.

This article examines the hypothesis that, at a field plot scale, *E. coli* voided to soil by grazing animals is at least as significant a source of potential pollution of surface waters as *E. coli* applied in slurry. We explored three of the major factors that influence the risk of *E. coli* pollution from these two sources: input loads of *E. coli*; relative timing of inputs; and increasing strength of *E. coli* retention by soil with time. We also recorded that *E. coli* pollution of surface waters occurs from grazing animals in the Cessnock Water catchment, an intensive livestock farming area in southwest Scotland. Further work to extend and develop these results to farm and catchment scale, considering scaling and delivery issues more fully, is reported elsewhere (McGechan & Vinten 2003; Vinten *et al.* 2003; Lewis & Post 2003).

MATERIALS AND METHODS

Faecal indicator bacterial analysis

Total coliform and *E. coli* numbers were determined in water and soil samples by the 'Colilert' defined substrate method (Edberg *et al.* 1990; IDEXX Laboratories Inc. 2001). This test uses the Most Probable Number method to determine FIO counts. IDEXX provides a customized 51 well tray in which to incubate samples at 35 °C for 24 hours. Detection of *E. coli* is based on its ability to produce β-glucuronidase, which hydrolyses a synthetic substrate to a fluorescent product. A count of the number of fluorescing wells in the tray can then be compared with standard Quanti-tray^{TM} Most Probable Number tables.

Field experiments on grazed plots

Autumn 2001. An experiment to measure the effect of sheep grazing on *E. coli* concentrations in drainage water and runoff was set up at the Glencorse site near Penicuik, Midlothian, Scotland, in autumn 2001. Details of this site and sampling methods are given in Vinten *et al.* (2002). The site consisted of four 0.25 ha paddocks in a second-year grass ley established during the late summer of 2000, which had been cut for silage once during summer 2001 and had received 20 kg ha^{-1} of fertilizer N in late summer. Within each paddock the volume of drainage and surface runoff from an area of approximately 300 m^2 was measured using tipping-bucket flow meters. Flow weighted sampling devices provided water samples which were collected once or twice per week.

The field storage of samples may lead to a systematic error due to differences in die-off in samples. However, incubations of *E.coli* in stream water at 6°C and 15°C (Fenlon *et al.* 2002) showed that little die-off occurred in the first four days, so we considered the effect of in-field sample storage on the relative values for treatments to have been slight.

Four 6-month-old Scottish blackface lambs grazed on two of the paddocks (16 sheep per hectare) from 24 September to 3 December 2001, and two were left ungrazed. On one of the grazed paddocks, one of the lambs had to be removed because of sickness shortly after the start of the experiment. Faecal samples from 5 of the lambs were taken on one occasion and the total *E. coli* counts were: 3.5, 33, 62, 3.6 and 2.5×10^6 c.f.u. g^{-1} fresh faeces, with a geometric mean of 9.2×10^6 c.f.u. g^{-1}.

Summer 2002. A second experiment was set up in summer 2002 to allow a direct comparison of *E. coli* survival and leaching following slurry application and during grazing. In this experiment, two paddocks were treated with 36 m^3 ha^{-1} cattle slurry, and four blackface ewes with lambs were introduced on each of the other two paddocks. Faecal samples were collected from the two grazing paddocks on 5 June, 17 June, 24 June, 1 July and 8 July. Soil samples (composites of 10 sample points, to a depth of 50 mm) and grass samples (composites of 4×0.25 m^2 samples) were taken on the same dates as the faecal samples, and also on 11 and 13 June. Water extracts were tested for *E. coli* by the Most Probable Number method (see Fenlon *et al.* 2000). *E. coli* counts in the faecal samples from grazing animals were highly variable, ranging from 1.5×10^4 c.f.u. g^{-1} on 5 June (discarded as being probably non-fresh material and therefore containing lower counts) to 2.2×10^7 c.f.u. g^{-1} on 20 June. There were also differences between the paddocks in the counts obtained, suggesting that *E. coli* numbers in faeces varied greatly among animals. Details of both experiments are given in Table 1.

Laboratory experiments on detachment/entrainment in runoff

To evaluate the effect of time of contact with soil on *E. coli* mobility, an intact slab of soil was collected using the technique of Douglas *et al.* (1999) from a grassland field

Table 1. Summary of grazing and slurry experiments at Glencorse drained plots.

	Cattle slurry [a]	Sheep grazing (16 store lambs ha^{-1})	Cattle slurry	Sheep grazing (16 ewes + lambs ha^{-1})
Experimental period	March–April 1999	Sep–Dec 2001	May–Sep 2002	May–Sep 2002
Dates of sampling	8/3/99	24/9–3/12/01	29/5/02	29/5–17/7/02
Waste inputs	40 $m^3 ha^{-1}$	11 kg ha^{-1} day^{-1}	36 $m^3 ha^{-1}$	33.6 kg ha^{-1} day^{-1}
Log [E. coli] in waste (c.f.u. g^{-1}) ± SD	4.7±0.25 $n = 5$ 1 sample date	7.0±0.64 $n = 5$ 1 sample date	6.1±0.64 $n = 4$ 1 sample date	6.1±1.2 $n = 14$ 7 sample dates
Estimated *E coli* inputs in waste	1.9×10^{12} ha^{-1}	7.2×10^{12} ha^{-1} over 70 days	4.6×10^{13} ha^{-1} on day 1	2.1×10^{12} ha^{-1} over 48 days

[a]This experiment was reported in Vinten *et al.* (2002), but is included here for comparison with three new experiments.

adjacent to the site of the grazed plot experiments described above. The slab, which comprised a 1.3×0.9 m block of the 0–25 cm layer, was positioned with a 5° slope beneath a rainfall simulator. Dairy cattle slurry (8% dry matter) was poured on to the soil surface at a rate equivalent to 50 $m^3 ha^{-1}$. Simulated rain (10 mm h^{-1}) was started 30 minutes later and after about 10 minutes surface runoff commenced and was collected via a gutter at the lower end of the slab. Five, 100-ml samples were collected by intercepting the runoff for 3 to 4 minutes at intervals of approximately 15 minutes. This process was repeated 1 and 2 weeks later on the same slab. Total coliform and *E. coli* numbers in the runoff were determined.

To investigate the amount of energy required to detach *E. coli* from soil as a function of contact time, cow slurry from a dairy unit was poured evenly (60 $m^3 ha^{-1}$) on to a 1 m^2 area in the same field from which the soil slab had been collected. The slurry (8% dry matter) contained *E. coli* at 3.9×10^4 c.f.u. mL^{-1} , while in the soil there were trace amounts only (<10 c.f.u. g^{-1}). The upper 25 mm of soil was sampled at 20 positions, using a 15 mm diameter corer, 8, 14 and 30 days after the slurry application. Rain between the day of application and the first two sampling occasions (23 and 38 mm, respectively) ensured that most of the slurry constituents were carried into the soil. *E. coli* was extracted in 100 ml of water from 5 replicate soil samples by 4 different methods. These methods were devised to expose progressively more of the soil to the water extractant, as follows: (i) a gentle wash of the intact core for 10 seconds; (ii) as (i) after breaking the core into <5 mm aggregates; (iii) 5 minutes on a reciprocating shaker after breaking, and (iv) 5 minutes in an ultrasonic bath after breaking.

Studies on E.coli *transport in the Cessnock Water catchment, Ayrshire*

No field plot experiments were carried out in a catchment with a bathing water pollution problem. Instead, field drain and river samples were collected in the Cessnock Water catchment in Ayrshire, to assess the contribution of grazing animals to FIO load in the River Irvine. The Cessnock Water discharges into the river Irvine, and has been linked with bacterial contamination suffered by the beaches at Irvine (SEPA 2002). In one subcatchment (details withheld for reasons of confidentiality), two fields of grass for silage and two fields containing grazing animals were selected in June 2002. Field drains were sampled from 26 June to 31 July 2002 and total numbers of faecal coliforms and streptococci were determined. The instantaneous flow rate on each drain was measured at the time of sampling with a bucket and stopwatch.

A manual stage recorder was installed just downstream of the confluence of a group of subcatchments (31.7 km^2) into the Cessnock Water. A stage–discharge relationship was obtained by flow estimation using the velocity area method (Gordon *et al.*1992) on several days during the summer. On 12 and 13 June, manual water sampling, stage measurements to estimate discharge and rain gauge recordings were undertaken at this point (22 samples over 34 hours). Total and faecal coliforms, nitrate, ammonium and total organic carbon were determined on these water samples by standard methods. A weekly survey of livestock numbers and waste spreading activity was carried out across the whole catchment from April to July 2002. These data allowed the estimation of FIO inputs to catchments and subcatchments. More detail on this survey is reported elsewhere (Vinten *et al.* 2003; Lewis & Post 2003).

RESULTS

Drained plots

Outputs of *E. coli* from the drained plots are summarized in Table 2. The *E. coli* concentrations in drainage and runoff water are given in Figure 1 and soil concentrations are given in Figure 2.

Autumn 2001. Drainage from plots with sheep grazing (16 lambs per hectare) under cool, moist conditions from 24 September to 3 December 2001 (Figure 1) had mean *E. coli* counts of 282 c.f.u. mL^{-1} or 8.2% of estimated input over the grazing period. *E. coli* counts in the soil (Figure 2) built up over the first 10 days of grazing. The concentration of *E. coli* in drainage water was similar to that in runoff water, and amounts of runoff collected were highly variable, but averaged 115 c.f.u. mL^{-1}. The ungrazed plots gave *E. coli* counts which were an order of magnitude less.

Summer 2002. The results for summer 2002 in Table 2 have been split into two periods: onset to completion of

Table 2. Summary of outputs of *E. coli* (c.f.u. ha^{-1}) and water in drainage and runoff from plots, assuming 1.4 kg fresh faeces $ewe^{-1}\,day^{-1}$ and 0.7 kg $lamb^{-1}\,day^{-1}$ (Strachan *et al.* 2001).

Expt details and mean counts	Replicate	Drainage	Runoff	Total	% of total input
Slurry 8/3–26/4/99,[a]	1	6.7×10^{10}	No runoff	6.7×10^{10}	3.6
drainage = 74 mm,	2	1.2×10^{11}	No runoff	1.2×10^{11}	6.4
runoff = 0	mean	9.3×10^{10}		9.3×10^{10}	5.0
	GM[b]	9.0×10^{10}		9.0×10^{10}	4.8
Mean *E. coli* c.f.u. mL^{-1}		**127**		**127**	
Grazed 24/9–3/12/01,	1	8.2×10^{11}	2.0×10^{8}	8.2×10^{11}	11.4
drainage = 209 mm,	2	3.6×10^{11}	4.1×10^{9}	3.7×10^{11}	5.1
runoff = 1 mm	mean	5.9×10^{11}	2.2×10^{9}	5.9×10^{11}	8.2
	GM	5.4×10^{11}	9.0×10^{8}	5.5×10^{11}	7.6
Mean *E. coli* c.f.u. mL^{-1}		**282**	**115**	**261**	
Post-grazing 3/12/01–22/1/02,	1	4.7×10^{9}	7.0×10^{4}	4.7×10^{9}	0.1
drainage = 96 mm,	2	8.4×10^{8}	No data	8.4×10^{8}	<0.1
runoff = 1 mm	mean	2.8×10^{9}	3.5×10^{4}	2.8×10^{9}	<0.1
	GM	2.0×10^{9}		2.0×10^{9}	<0.1
Mean *E. coli* c.f.u. mL^{-1}		**3**	**0**	**2**	
Grazed 29/5–17/7/02,	1	6.3×10^{9}	2.8×10^{8}	6.6 x10^{9}	0.3
drainage = 85 mm,	2	1.2×10^{10}	No runoff	1.2×10^{10}	0.6
runoff = 5 mm	mean	9.2×10^{9}	2.8×10^{8}	9.3×10^{9}	0.4
	GM	8.7×10^{9}		8.9×10^{9}	0.4
Mean *E. coli* c.f.u. mL^{-1}		**14**	**6**	**13**	
Post-grazing 17/7–10/9/02,	1	7.1×10^{8}	No data	7.1×10^{8}	<0.1
drainage = 180 mm,	2	2.4×10^{10}	No data	2.4×10^{10}	1.1
runoff = 12 mm	mean	1.2×10^{10}		1.2×10^{10}	0.6
	GM	4.1×10^{9}		4.1×10^{9}	0.2
Mean *E. coli* c.f.u. mL^{-1}		**2**		**2**	
Slurry 29/5–17/7/02,	1	1.2×10^{8}	2.6×10^{5}	1.2×10^{8}	<0.1
drainage = 85 mm,	2	7.2×10^{9}	1.86×10^{10}	2.6×10^{10}	0.1
runoff = 19 mm	mean	3.7×10^{9}	9.3×10^{9}	1.3×10^{10}	<0.1
	GM	9.3×10^{8}	6.9×10^{7}	1.8×10^{9}	<0.1
Mean *E. coli* c.f.u. mL^{-1}		**9**	**48**	**25**	
Post-grazing 17/7–10/9/2002,	1	2.0×10^{9}	No data	2.0×10^{9}	<0.1
drainage = 180 mm,	2	2.4×10^{10}	1.37×10^{8}	2.4×10^{10}	0.1
runoff = 37 mm	mean	1.3×10^{10}	1.4×10^{8}	1.3×10^{10}	<0.1
	GM	7.0×10^{9}		7.0×10^{9}	<0.1
Mean *E. coli* c.f.u. mL^{-1}		**13**	**6**	**11**	

[a]This experiment was reported in Vinten *et al.* (2002), but is included here for comparison with three new experiments.
[b]Geometric mean.

grazing (26 May to 17 July 2002) and after removal of the grazing animals (17 July to 10 September 2002). The experimental period was unusually wet, with 85 mm of drainflow from 29 May to 17 July and 180 mm from 17 July to 10 September. In many summers virtually no drainflow occurs at this site in the period from May to September. Drainage from the grazed plots from 29 May to 17 July 2002 had average *E. coli* counts of 14 c.f.u. mL^{-1} or 0.4% of estimated total *E. coli* inputs over the grazing period. Drainage water during the same period from the plots treated with cattle slurry (36 m^3 ha^{-1} on 29 May 2002) had smaller average *E. coli* counts (9 c.f.u. mL^{-1} or 0.03% of estimated faecal input). However, the mean counts in the small amount of surface runoff were greater in the slurry treated plots (48 c.f.u. mL^{-1}) than in the grazed plots (6 c.f.u. mL^{-1}). Most of this was due to runoff shortly after slurry application. Losses varied widely between the two replicates, mainly due to little runoff from the first replicate. The fraction of applied *E. coli* lost from the slurry treated plots was smaller than the fraction lost in the previously reported March 1999 experiment (see Table 1).

In the period after the grazing animals were removed (17 July), elevated *E. coli* levels in the drainage water continued to be evident, both in slurry treated and grazed plots. Average counts in drainage from slurry treated plots (13 c.f.u. mL^{-1}) were larger than from grazed plots (2 c.f.u. mL^{-1}). Losses during this period were similar to losses in the autumn period. This is hard to explain, particularly in the slurry treated plots where soil *E. coli* counts declined steadily to a near background level after 40 days. However, we note that the high counts in slurry and grazed plot drains occurred in the first flush after 3 weeks of no flow. Soil counts in the grazed plots increased by 1–2 orders of magnitude over the first 20 days of grazing, but the values were strongly influenced by one count of 110 000 c.f.u. g^{-1}, which may be a sample containing a large proportion of fresh faecal material. After this there was a decline in counts until the animals were removed.

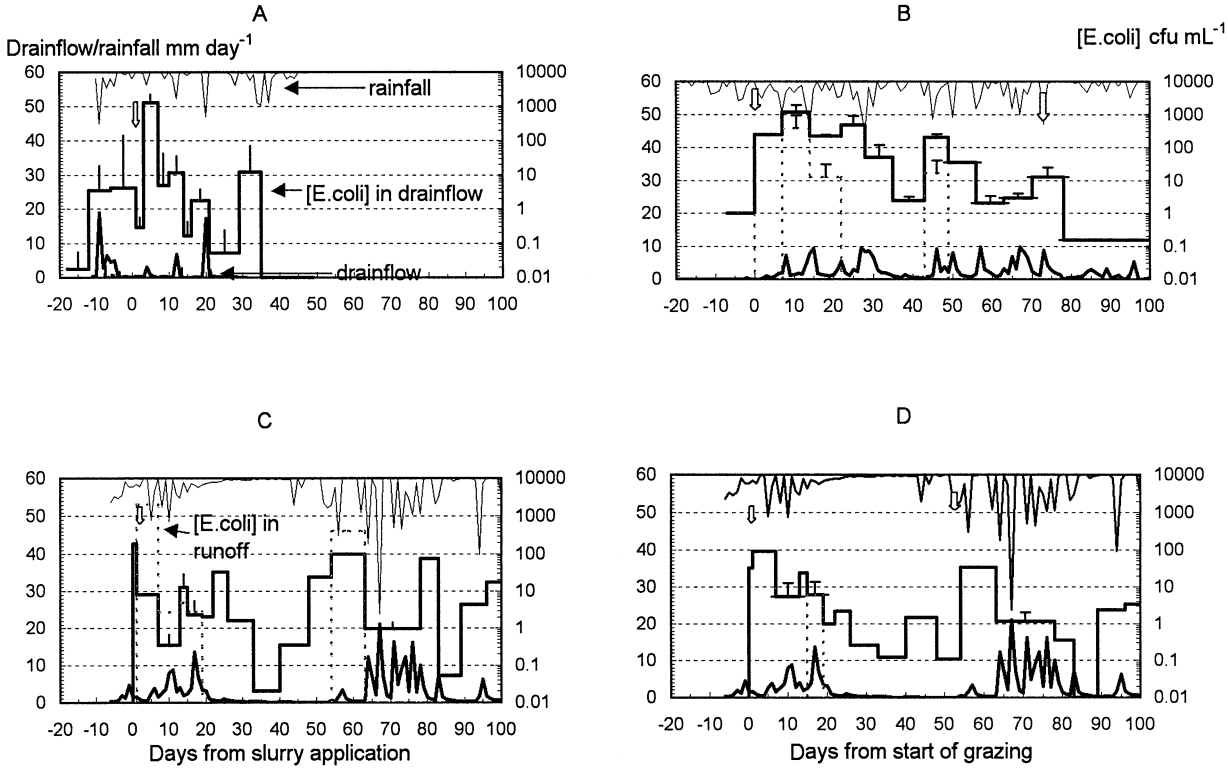

Figure 1. *E. coli* concentrations in drainage water. A, 40 m^3 ha^{-1} slurry application on 8 March 1999; B, grazing 24 September–3 December 2001; C, 36 m^3 ha^{-1} slurry application on 29 May 2002; D, grazing from 29 May–17 July 2002.

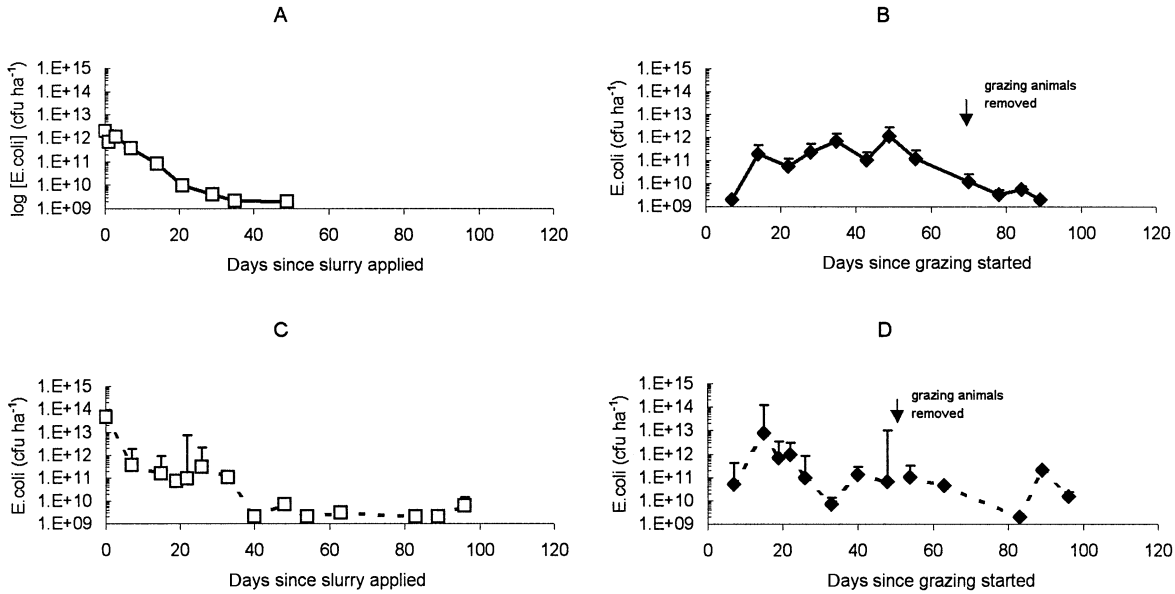

Figure 2. *E. coli* numbers per hectare of soil (0–5 cm). A, slurry application on 8 March 1999; B, grazing 24 September–3 December 2001; C, slurry application on 29 May 2002; D, grazing from 29 May–17 July 2002. Y-axis gives numbers in scientific notation, for example, 1.E+14 = 10^{14}.

Laboratory studies

The *E. coli* counts in runoff from the slurry-treated soil slab varied with amount of rain and between-rain events. The *E. coli* counts in runoff generated within hours of slurry application declined during the course of the event, probably as a result of dilution. In contrast, 1 week later, counts increased during the course of a similar rain event, which indicated progressive release of bacteria from the slurry remnants and/or from the soil surface (Figure 3).

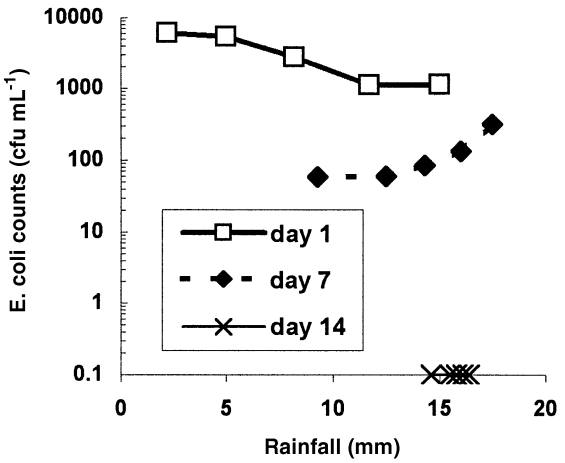

Figure 3. Pattern of *E. coli* concentrations in surface runoff from a soil slab showing that the bacteria become more firmly attached to soil with time.

Reasons for this observation were further investigated by estimating numbers of *E. coli* extracted from field soil samples with increasing intensity of soil disruption during extraction (Figure 4a). The improved extraction with increasing soil disruption was less pronounced in the soil sampled at 14 and 30 days after slurry application than in that sampled 8 days after application, as shown by the data normalized relative to release from the lowest intensity 'washed' treatment (Figure 4b). This trend indicates that, with time, slurry-derived *E. coli* become either more firmly attached to soil particles or entrapped in relatively inaccessible small pores.

Monitoring of the Cessnock Water catchment

Figure 5 summarizes the results of sampling at four drains in grass fields in the Cessnock Water catchment. Estimates of *E. coli* loads from these data are not possible, because only single samples were taken each week. However, it is clear that the *E. coli* counts in water draining from grazed fields, especially the 'large drain' sample, were greater than water draining from silage fields. Moreover, the concentrations in the drains from the grazed fields related well with *E. coli* counts in the Cessnock Water, into which these fields drain. Figure 6 gives the total coliforms, *E. coli*, nitrate, ammonium, and total organic carbon, rainfall and discharge at our main sampling point in Cessnock Water for a 7.6 mm rainfall event on 12–13 June 2002. The cumulative load of *E. coli* for this event was 1.4×10^{13} c.f.u. Based on observations of livestock made for the week beginning Monday 10 June 2002, we estimated faecal coliform inputs from grazing animals were 10.2×10^{13} c.f.u. per day over the whole catchment, with no slurry spreading observed owing to the wet conditions. Only two observations of recent slurry spreading were recorded in the weekly surveys from 23 April to 11 June 2002, whereas later in the year clear evidence of slurry spreading was observed (e.g. 13 out of 317 fields, or 4% of catchment, showed evidence of recent spreading on 8 July). The *E. coli* load in the Cessnock Water would appear to be mainly linked to grazing and represents about 14% of the daily input of faecal coliforms to the land from grazing animals.

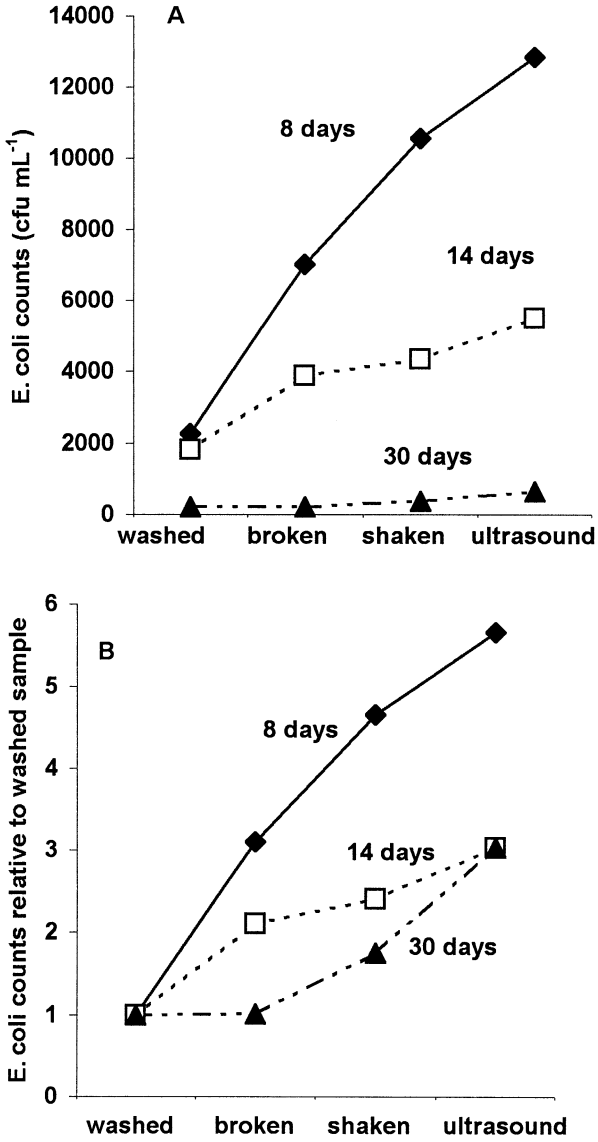

Figure 4. A, absolute *E. coli* counts in water from soils treated with slurry after 8,14 and 30 days, extracted by four methods of increasing intensity. B, data in 4A normalized to the least intensive extraction method (washed) for each sampling time.

DISCUSSION

The foremost factor influencing the potential pollution of surface waters by *E. coli* from animal faeces is the relative farm scale inputs from fresh faeces and from slurry. *E. coli* inputs per livestock unit from fresh faeces are expected to be larger than from stored slurries, because of opportunity for die-off during storage. Mawdsley *et al.* (1995) state that *E. coli* counts of fresh faeces can be up to $10^9\,g^{-1}$ and unpublished data from a survey of cattle in Inverness-shire showed *E. coli* counts of $6 \pm 9 \times 10^6$ mL^{-1} in fresh cattle faeces (D.R. Fenlon, pers. comm.). In our 2002 experi-

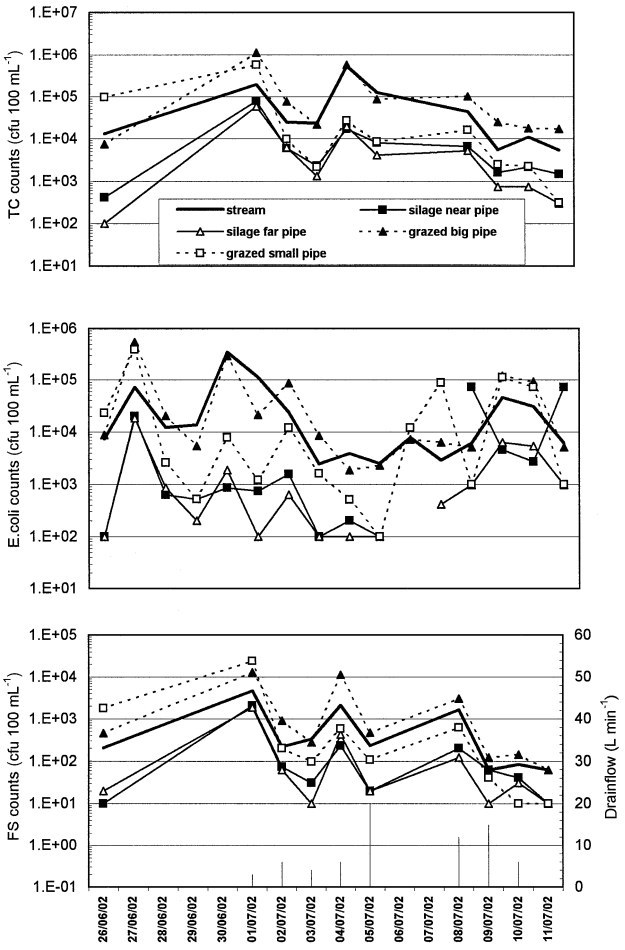

Figure 5. Total coliform (TC), *E. coli* and faecal streptococci (FS) counts in drainage water samples from grazed and silage fields in the Cessnock catchment, 26 June–12 July 2002. Instantaneous flow shown is for a large pipe draining a grazed grass field. Y-axis gives numbers in scientific notation, for example, 1.E+04 = 10^4.

ments, the *E. coli* content in the cattle slurry was similar to that in fresh sheep faeces (see Table 1), but in the 1999 experiment and in previous work (Vinten *et al.* 2002) we found smaller *E. coli* counts in slurry ($5.3 \times 10^4\,g^{-1}$ to $5.7 \times 10^5\,g^{-1}$ over 4 experiments). Larsen & Munch (1983), reported in Kearney *et al.* (1993), found die-off half-lives for *E. coli* in slurry of 4 and 18 days at 20 °C and 7 °C, respectively. If we consider a typical dairy unit with 50% of faecal material managed as slurry and 50% deposited in fields during grazing, die-off during slurry storage, possibly for several months, will clearly lead to much smaller total inputs of *E. coli* to the fields in slurry than as fresh faeces.

For a given field input of *E. coli*, a second factor that would influence surface water pollution is probably the timing of the input. In our experiments the proportion of *E. coli* lost to drainage water was greater in spring and autumn than in summer, irrespective of the input source. This suggests longer survival in the cooler soil conditions. In previous work we found the die-off half-life of *E. coli* in soil decreased from 2.6 days to 1.2 days with increase in temperature from 6 to 15 °C (Vinten *et al.* 2002). Drying and exposure to ultraviolet light may also be important. Moreover, under lowland UK conditions there is less drainage during the summer, and grazing inputs of *E. coli* occur mainly in the summer months, when soils are on average drier and therefore more able to absorb and delay *E. coli* transport to water. These seasonal considerations favour greater losses from slurry derived *E. coli.* However, the risk of losses of slurry *E. coli* during the bathing water season (May to September) will be lower. In a survey in Ayrshire, Scotland, it was found that the majority of slurry spreading occurred in January to April, with only 24% (Girvan catchment) and 26% (Irvine catchment) occurring from May to September (Aitken 2003). Moreover, at a farm scale, the management of slurry spreading to avoid high risk sites and weather conditions (MAFF 1998; SOAEFD 1997) will lead to further reduction of the risk, relative to grazing animals.

Our results show that for a given season and a given input of *E. coli* to field plots, the proportion of *E. coli* transported to drains from grazing is at least as high as that from slurry, even though inputs to grazing are spread over the whole grazing period rather than concentrated at the start of the period. It can be shown theoretically that with equal total inputs, the risk of leaching of *E. coli* to water is lower with daily grazing input than with a single slurry input (see Appendix 1). It may be that the particular rainfall distributions in our experiments favoured leaching from grazing compared with slurry, but our results could also indicate greater overall *E. coli* mobility from grazing input than from slurry input. Our laboratory runoff experiments suggest an explanation by showing that *E. coli* removal from soil becomes more difficult with time, possibly because of increasing strength of adsorption of surviving *E.coli* to soil surfaces, or because of migration to smaller soil pores. This reduces the relative longer term risk of transport from slurry spreading compared with grazing, as continuous fresh inputs of faeces will contain *E. coli* that are more readily mobilized. Thelin & Gifford (1983) also found that detachment and mobilization of FIOs from faecal pats of cattle takes longer and requires more rainfall as faecal material ages. A third possibility is that the uncertainty of input *E. coli* numbers may be responsible.

The drain sampling from grazed and silage fields and the streamflow event in the Cessnock Water catchment on 12–13 June 2002 confirm the potential for large losses of *E. coli* from grazing animals. Very little slurry spreading had occurred in the catchment since mid-May, although farm steading runoff and stream sediment entrainment may also have contributed to the stream *E. coli* levels. These data confirm that an important part of any pollution mitigation strategy needs to focus on the grazing animal as well as on slurry management.

Delivery to surface waters from farm steadings

Hard-standing areas of steadings, uncovered farmyard middens and access tracks are highlighted in Aitken (2003) as high-risk farm scale sources of organic waste pollution to surface waters. We can draw no conclusions from our drained plot data concerning the importance of these at a catchment scale, relative to field sources. However, we note

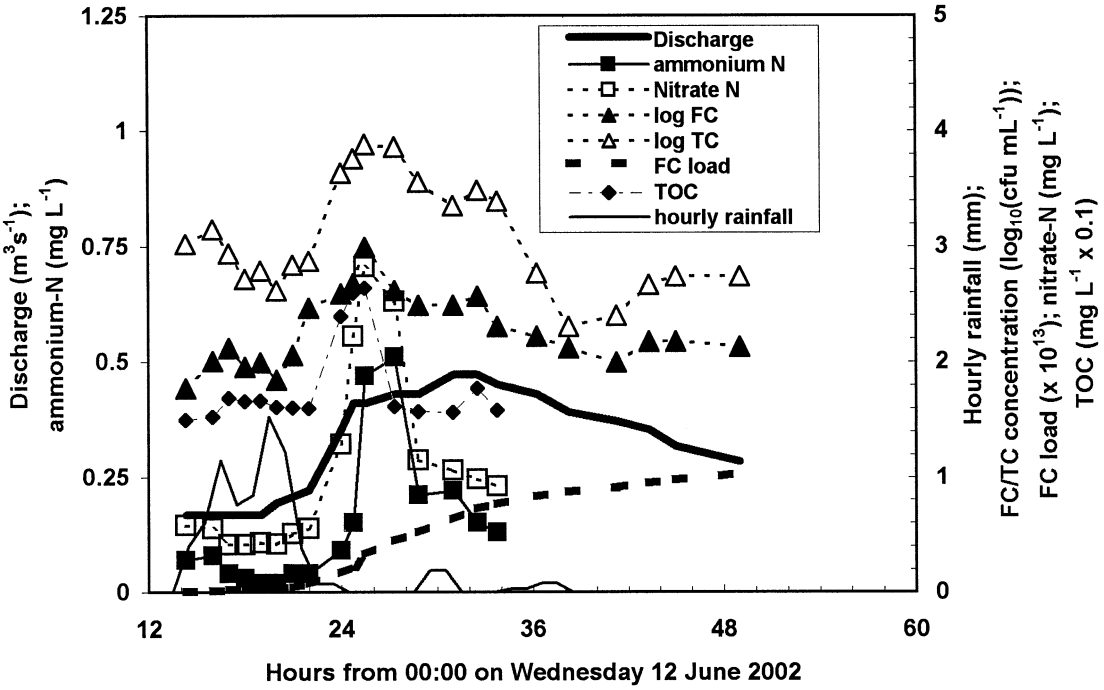

Figure 6. Total coliform (TC) and *E. coli* counts, nitrate, ammonium, and total organic carbon (TOC) concentrations, rainfall and discharge into Cessnock Water, 12–13 June 2002.

that total coliform and *E. coli* counts in the Cessnock Water event (Figure 6) tracked each other closely, with total coliforms approximately an order of magnitude higher. If we assume that the non-*E. coli* coliforms are soil derived (Edberg *et al.* 2000), this observation suggests that soil bacteria are being transported together with faecal bacteria, implying that fields rather than farm steadings were the major source of pollution on this occasion. This inference is also supported by the observation that both nitrate and ammonium concentrations increase with the *E. coli* counts. If farm steadings were the principal source of pollution, then nitrate levels would not change so markedly, as most of the inorganic N would be in the ammonium form, given that response time of the watercourse is only a few hours so little nitrification would occur.

CONCLUSIONS

Results from drained plots showed that the risk of leaching *E. coli* to field drains under grazing sheep exceeds that from slurry under both autumn/spring and wet summer conditions. Laboratory work showed that over a period of several weeks, remaining live soil *E. coli* from an application of slurry become increasingly difficult to entrain into water, an observation consistent with these field results. Risk of *E. coli* leaching was smaller during summer than in spring or autumn. Stream event monitoring in an intensively grazed livestock catchment also showed high *E. coli* loading (14% of daily input for a 7-mm rainfall event) at a time when little or no recent slurry spreading had occurred. The chemistry and microbiology of the event suggest a field source rather than steading source for the pollution on that occasion.

This study shows that mitigation strategies for faecal indicator pollution need to focus at least as much on the losses from grazing animals as on losses from slurry spreading, and on losses from field drains as well as from surface runoff and direct livestock inputs, particularly where new and efficient drainage systems have been installed.

ACKNOWLEDGEMENTS

The financial support of SEERAD and the technical support of C. Crawford, R. Ritchie,R. Howard and F. Wright are gratefully acknowledged.

REFERENCES

Aitken MN 2003. Impact of agricultural practices and river catchment characteristics on river and bathing water quality. Water Science and Technology 2003 (in press).

Anon 1976. Council Directive 76/160/EEC concerning the quality of bathing water. Official Journal of the European Communities, L31 (5.2.1976), pp 1–7.

Anon 2000. Council Directive 2000/60/EC establishing a framework for the Community action in the field of water policy. Official Journal of the European Communities, L327, pp 1–152.

Beuchat LR 1995. Pathogenic micro-organisms associated with fresh produce. Journal of Food Protection 59, 204–216.

Douglas JT Ritchie RM Takken I Crawford CE & Henshall JK 1999. Large intact soil slabs for studying the effects of soil and plant properties on surface runoff. Journal of Agricultural Engineering Research 73, 395–401.

Edberg SC Allen MJ Smith DB & Kriz NJ 1990. Enumeration of total coliforms and *Escherichia-coli* from source water by the defined substrate technology. Applied and Environmental Microbiology 56, 366–369.

Edberg SC Rice EW Karlin RJ & Allen MJ 2000. *Escherichia coli*: the best biological drinking water indicator for public health protection. Journal of Applied Microbiology 88, 106S–116S.

Fattal B Guttman-Bass Agursky N & Shuval HI 1988. Evaluation of health risk associated with drinking water quality in agricultural communities. Water Science and Technology 20, 409–415.

Fenlon DR Vinten AJA & Lewis DR 2002. Survival of *E. coli* O157 in Scottish soils and private water supplies. Final report of SEERAD funded project SAC/204/98.

Fraser RH, Barten PK & Pinney DAK 1998. Predicting stream pathogen loading from livestock using a Geographical Information System-based delivery model. Journal of Environmental Quality 27, 935–945.

Gordon ND McMahon TA & Finlayson BL 1992. Stream hydrology: an introduction for ecologists. J Wiley & Sons Chichester UK p157.

Goss MJ Barry DAJ & Rudolph DL 1998. Contamination in Ontario farmstead domestic wells and its association with agriculture: 1. Results from drinking water wells. Journal of Contaminant Hydrology 32, 267–293.

IDEXX Laboratories Inc. 2001. http://www.idexx.com/Water/Products/Colilert/index.cfm

Kay D Wyer MD Crowther J O'Neill JG Jackson G Fleisher JM & Fewtrell L 1999. Changing standards and catchment sources of faecal indicators in near shore bathing waters. In: Water quality: process and policy, eds ST Trudgill DE Walling & BW Webb, John Wiley & Sons Chichester UK pp 47–64.

Kearney TE Larkin MJ & Levett PN 1993. The effect of slurry storage and anaerobic digestion on survival of pathogenic bacteria. Journal of Applied Bacteriology 74, 86–93.

Larsen HE & Munch B 1983. Practical applications of knowledge on the survival of pathogenic and indicator bacteria in aerated and non-aerated slurry. In: Hygienic problems of animal manures, ed D Strach, University of Hohenheim Stuttgart pp 20–34.

Lewis DR & Post B 2003. PAMIMO-C. A catchment scale model of Faecal Indicator Organism (FIO) transport from agricultural land to surface waters. International Water Association Conference on Diffuse Pollution and Basin Management, Dublin, August 2003 (in press).

MAFF 1998. The Water Code (code of good agricultural practice for the protection of water) PB0587, MAFF Publications London.

Mawdsley J L Bardgett R D Meny R J Pain B F & Theodorou M K 1995. Pathogens in livestock waste, their potential for movement through soil and environmental pollution. Applied Soil Ecology 2, 1–15.

McGechan MB & Vinten AJA 2003. Simulation of transport through soil of *E. coli* derived from livestock slurry using the MACRO model. Soil Use and Management 19, 321–330.

Milne DP Curran JC Findlay JS Crowther JM & Wallis SG 1989. The effect of estuary type suspended solids on survival of *E. coli* in saline waters. Water Science & Technology 24, 133–136.

Obiri-Dansok & Jones K 1999. Distribution and seasonability of microbial indicators and thermophylic campylobacters in two freshwater bathing sites on the River Lune in northwest England. Journal of Applied Microbiology 87, 822–832.

SEPA 2002. A study of Bathing Waters Compliance with EC Directive 76/160/EEC: The relationship between exceedance of standards and antecedent rainfall. http://www.sepa.org.uk/data/bathingwaters/rainfall_and_bathing_water_quality.pdf Scottish Environmental Protection Agency.

SOAEFD 1997. Prevention of environmental pollution from agricultural activity: Scottish Office Agriculture Environment and Fisheries Department Edinburgh.

Strachan NJC Fenlon DR & Ogden ID 2001. Modelling the vector pathway and infection of humans in an environmental outbreak of Escherichia coli O157. FEMS Microbiology Letters 203, 69–73.

Tiedemann D Higgins A Quigley TM Sanderso HR & Marx DB 1987. Responses of fecal coliform in streamwater to four grazing strategies. Journal of Range Management 40, 322–329.

Thelin R & Gifford GF 1983. Fecal coliform release patterns from fecal material of cattle. Journal of Environmental Quality, 12,57–63.

Tian Y Q Gong P Radke JD & Scarborough J 2002. Spatial and temporal modelling of microbial contaminants on grazing farmlands. Journal of Environmental Quality 31, 860–869.

Vinten AJA Lewis DR Fenlon DR Leach KA Howard Svoboda RI & Ogden ID 2002. Fate of *E. coli* and *E. coli* O157 in soils and drainage water following cattle slurry application at three sites in southern Scotland. Soil Use and Management 18, 1–9.

Vinten AJA McGechan MB Duncan A Aitken M Crawford C & Lewis DR 2003a. Achieving microbiological compliance of bathing waters influenced by livestock inputs: reduce stocking levels or improve mitigation measures? International Water Association Conference on Diffuse Pollution and Basin Management, Dublin, August 2003 (in press).

Vinten AJA Duncan A Hill C Crawford C Aitken M Lewis DR & McGechan MB 2003b. A simple model for predicting microbiological compliance of bathing waters influenced by diffuse riverine inputs of faecal indicators. Water Science.[update?]

Wilkinson J Jenkins A Wyer M & Kay D 1995. Modelling faecal coliform dynamics in streams and rivers. Water Research 29, 847–855.

Wyer MD Kay D Dawson HM Jackson GF Jones F Yeo J & Whittle J 1996. Delivery of microbial indicator organisms to coastal waters from catchment sources. Water Science and Technology 33, 37–50.

Received February 2003, accepted after revision August 2003.

APPENDIX 1

Proof that the risk of *E. coli* leaching from soil is always higher from a single step input at time zero (as in slurry application) than the same input spread over a fixed period of time, T (as in grazing).

Slurry case

Soil content of *E. coli* ($C_s(T)$) after slurry application is given by:

$$(C_s(T)) = C_s(0)e^{-kT} \tag{A1}$$

where:

k = first order loss rate constant from soil pool (= k_{leach} + k_{dieoff}) (day^{-1})

T = fixed time period (i.e. grazing period) (days)

$C_s(0)$ = dimensionless soil *E. coli* content after slurry application (–)

Grazing case

Soil content of *E. coli* ($C_g(T)$) during period of grazing with total *E. coli* inputs the same as from slurry:

$$\frac{dC_g(T)}{dT} = \frac{C_s(0)}{T} - kC_g(T) \tag{A2}$$

where:

$C_s(0)/T$ = daily input rate from grazing *E. coli.*

For boundary conditions $C_g(0) = 0$ at $t = 0$, (A3)

$$C_g(t) = C_g(T) \text{ at } t = T \tag{A4}$$

the solution is $$C_g(T) = C_s(0)\left[\frac{1 - e^{-kT}}{kT}\right] \tag{A5}$$

The ratio of the losses from grazing to those from slurry during the period from $t = 0$ to $t = T$ can now be compared:

$$R = \frac{\text{losses from grazing}}{\text{losses from slurry}} = \frac{C_s(0) - C_g(T)}{C_s(0) - C_s(T)}$$
$$= \frac{\left[1 - \left(\frac{1-e^{-kT}}{kT}\right)\right]}{1 - e^{-kT}} = \frac{1}{1 - e^{-kT}} - \frac{1}{kT} \tag{A6}$$

As $kT \to 0$, $R \to 0$ and as $kT \to \infty$, $R \to 1$, so over the possible range of values for kT, 1 is the maximum value, R.