Applied and Environmental Microbiology, Mar. 2010, p. 1449–1455 Vol. 76, No. 5
0099-2240/10/$12.00 doi:10.1128/AEM.02335-09
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Long-Term Persistence and Leaching of *Escherichia coli* in Temperate Maritime Soils[▽]

Fiona P. Brennan,[1,2] Vincent O'Flaherty,[2] Gaelene Kramers,[1,3] Jim Grant,[4] and Karl G. Richards[1]*

Teagasc, Environmental Research Centre, Johnstown Castle, Wexford, Ireland[1]; Microbial Ecology Laboratory, Microbiology, School of Natural Sciences and Environmental Change Institute, National University of Ireland, Galway, Ireland[2]; School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Dublin, Ireland[3]; and Teagasc, Kinsealy Research Centre, Dublin, Ireland[4]

Received 28 September 2009/Accepted 16 December 2009

Enteropathogen contamination of groundwater, including potable water sources, is a global concern. The spreading on land of animal slurries and manures, which can contain a broad range of pathogenic microorganisms, is considered a major contributor to this contamination. Some of the pathogenic microorganisms applied to soil have been observed to leach through the soil into groundwater, which poses a risk to public health. There is a critical need, therefore, for characterization of pathogen movement through the vadose zone for assessment of the risk to groundwater quality due to agricultural activities. A lysimeter experiment was performed to investigate the effect of soil type and condition on the fate and transport of potential bacterial pathogens, using *Escherichia coli* as a marker, in four Irish soils ($n = 9$). Cattle slurry (34 tonnes per ha) was spread on intact soil monoliths (depth, 1 m; diameter, 0.6 m) in the spring and summer. No effect of treatment or the initial soil moisture on the *E. coli* that leached from the soil was observed. Leaching of *E. coli* was observed predominantly from one soil type (average, 1.11 ± 0.77 CFU ml^{-1}), a poorly drained Luvic Stagnosol, under natural rainfall conditions, and preferential flow was an important transport mechanism. *E. coli* was found to have persisted in control soils for more than 9 years, indicating that autochthonous *E. coli* populations are capable of becoming naturalized in the low-temperature environments of temperate maritime soils and that they can move through soil. This may compromise the use of *E. coli* as an indicator of fecal pollution of waters in these regions.

The contamination of groundwater, including potable water supplies, with microbial pathogens continues to be a global concern (52, 59). Of particular importance in developed countries are the high levels of contamination associated with small-scale and very-small-scale drinking water supplies (5, 19, 57), often groundwater, which serve an estimated 10% of the total population in the European Union (13). The high numbers of these water supplies found to be contaminated with fecal bacteria and thus considered to be unfit for human consumption are worrying because the water from them is often untreated or inadequately treated prior to consumption. Microbial pathogens are known to survive for considerable periods of time in groundwater (29), which increases the health risk due to utilization of contaminated supplies. There are various sources of contamination, but evidence suggests that contamination from the spreading of animal slurries and manures on land can be a significant contributor (3, 33, 53). Spreading of agricultural slurries and manures on land is used by the agricultural sector as a means of nutrient recycling. The health risks associated with the spreading of animal and human wastes containing enteric pathogens have been recognized for a long time (10, 18). Animal manure and wastewaters may contain a broad range of pathogenic microorganisms, including *Escherichia coli* O157:H7, *Campylobacter*, *Cryptosporidium*, *Salmonella* spp., and pathogenic viruses, which are released into the environment during spreading (15, 22, 55). The levels and incidence of pathogens present in animal manures and slurries are influenced by a number of factors, including herd health, age demographics, stress factors, diet, season, and manure management and storage (37, 39).

Soils (and subsoils) often act as a zone for mitigating microbial contamination of groundwater associated with the spreading of animal slurries and manures on land. Some of the pathogenic microorganisms applied to agricultural soils have, however, been observed to leach through the soil into groundwater, which can affect drinking water quality and pose a risk to public health (16, 26, 28, 42, 50), confirming that soil is not always a sufficient obstruction for protection of groundwater (16, 53). Consequently, characterization of the movement of pathogens through the unsaturated soil and subsoil zone (vadose zone) has become critical for assessment of the risk to groundwater posed by agricultural activities (8, 14, 42). The soil and subsoil type is believed to be a major factor influencing the potential transfer of pathogens through soil to groundwater (3, 34, 41, 50). The preapplication moisture status of a soil, which may be influenced by the season, also impacts pathogen survival, fate, and transport (2, 11, 43, 54).

E. coli is widely used as an indicator of fecal contamination of water, and certain strains are known to be pathogenic (12). Thus, characterizing this organism's transport through soil is important because of the health risk posed by the organism itself and with regard to its validity as an indicator of the fate of enteropathogens in the environment. *E. coli* strains have diverse properties and

* Corresponding author. Mailing address: Teagasc, Environmental Research Centre, Johnstown Castle, Wexford, Ireland. Phone: 353(0) 539171261. Fax: 353(0)539142213. E-mail: Karl.richards@teagasc.ie.

▽ Published ahead of print on 28 December 2009.

TABLE 1. Major properties of lysimeter soils

Soil	Soil classification	Parent material	Drainage	Depth (cm)	pH	C/N ratio	% Organic matter	Cation exchange capacity (meq/liter)	% Sand	% Silt	% Clay	Texture
Oakpark (OA)	Haplic Cambisol	Fluvioglacial gravels	Very good	0–20	6.6	14.6	4.9	13.3	67	23	11	Sandy loam
				20–45	7.8	10.4	3.4	14.9	68	20	12	Sandy loam
				45–100								Gravelly sand
Clonroache (CL)	Haplic Cambisol	Glacial drift	Good	0–20	6.5	10.1	8.4	11.4	44	39	17	Loam
				20–45	7.3	11.8	4.1	5.2	38	37	25	Loam
				45–90	7.1		3.0	3.9	45	41	14	Loam
Elton (EL)	Cutanic Luvisol	Glacial drift	Good	0–20	6.2	8.4	6.9	12.5	48	35	17	Loam
				20–50	6.8	8.2	3.2	11.1	36	50	14	Silt loam
				50–90	6.9	10.4	1.9	7.9	47	30	23	Loam
Rathangan (RA)	Luvic Stagnosol	Glacial sea drift	Poor	0–15	6	10.1	5.8	10.1	44	37	19	Loam
				15–40	6.3	8.6	2.9	6.4	48	28	24	Sandy clay loam
				40–60	6.3		1.2	6.1	42	30	28	Clay loam
				60–90	6.7		1.2	8.9	32	39	29	Clay loam

capabilities that affect their survival and transport in soils (9, 36, 56, 60). Consequently, data obtained by using total *E. coli* rather than individual surrogate strains can be more representative of the fate and transport of *E. coli* present in animal slurries. *E. coli* O157 die-off in soils has been reported to be the same as or quicker than total *E. coli* die-off, suggesting that data for total *E. coli* provide a conservative estimate of the survival potential (38, 56). Although many field and laboratory studies have investigated *E. coli* transport through soil columns (4, 6, 16, 43, 46, 47, 50, 51), most studies have investigated transport through soil to a depth of less than 30 cm. For assessment of the risk of transport to groundwater, such studies may not take into account the variation in soil physical and chemical characteristics with depth (e.g., the frequency and continuity of macropores, organic matter, and moisture contents) that affect bacterial transport. Furthermore, rainfall was often simulated in previous studies, which allows experimental conditions to be controlled but may not be representative of the risk due to variable natural rainfall events over time. In this study, we used intact soil monoliths that were 1 m deep to assess the risk of leaching of total *E. coli* in four representative Irish soil types under natural rainfall and environmental conditions.

The objective of this study was to quantitatively investigate the impact of soil type and season (soil moisture content) on the fate and transport of *E. coli* spread on four different temperate maritime soil types under natural rainfall conditions. We hypothesized that there would be a greater microbial risk to underlying groundwater with better-drained soil types than with relatively poorly drained soil types following the application of animal slurry. In addition, we hypothesized that *E. coli* cells spread on wetter spring soils would be transported in greater numbers than *E. coli* cells spread on drier soils in the summer.

MATERIALS AND METHODS

Lysimeter unit. A lysimeter experiment was carried out using an established lysimeter unit in Johnstown Castle, Wexford, Ireland (6°30′W, 52°17′N), under maritime temperate climatic conditions. The establishment and experimental design of the lysimeter unit were described previously by Ryan and Fanning (45). Briefly, replicate undisturbed monoliths of soils (diameter, 0.6 m; depth, 1 m) encased in rigid fiberglass cylinders were removed from each of five grassland sites in the Republic of Ireland in 1990. The soils were chosen to encompass a representative range of soil types, drainage characteristics, and soil parent material. Following transport to the lysimeter unit at Johnstown Castle, the lysimeters were randomly installed on both sides of an open trench where, by gravity, pipes direct drainage water from each lysimeter into collecting vessels. The area surrounding the lysimeters was backfilled with soil and covered with loose gravel. The lysimeter unit was caged to prevent contamination by birds or small mammals. A sward of perennial ryegrass (*Lolium perenne*) was established on each of the lysimeters. The lysimeter unit was previously used to study nitrate leaching on Irish soils (45), but no fecal material had been spread on the lysimeters since February 1998.

Treatments and sampling. Four of the five soils in the lysimeter unit (named Clonroche [CL], Elton [EL], Oakpark [OA], and Rathangan [RA] after the localities from which they were taken) were used in this experiment, and there were nine replicate lysimeters for each soil. Major properties of the soils are shown in Table 1. Three lysimeters for each soil type received an application of cattle slurry at an average concentration of 34 tonnes per ha in August 2006 (summer, low soil moisture) (day 0), while another three lysimeters received a similar application in February 2007 (spring, high soil moisture) (day 184). The remaining three lysimeters for each soil type were maintained as controls (no treatment) throughout the experimental period. Slurry was applied by hand in a manner replicating that of a splash plate spreader. Subsamples of slurry were analyzed to determine the dry matter (DM) content and *E. coli* load prior to spreading. The DM content was analyzed by drying samples for 48 h (or until a constant weight was obtained) at 100°C. Samples were analyzed to determine the *E. coli* content using the Idexx Colisure most-probable-number methodology (35) after dilution with phosphate-buffered saline.

The experiment was performed under natural rainfall conditions, and sampling was carried out regularly during the drainage period for 488 days. The volume of leachate obtained from each lysimeter was measured and expressed as mass (kg) throughout the experiment for each sampling event, while an onsite weather station next to the lysimeters recorded weather data, including rainfall, solar radiation, soil temperature, air temperature, and humidity. The following two types of sampling regimens were used. Throughout the experimental period, a subsample of the total drainage for each lysimeter was taken after each leachate-producing rainfall event for *E. coli* analysis. In addition, two rainfall events that occurred shortly after the first leachate emerged after application of the slurry were selected for intensive sampling. During this intensive sampling approximately every 250 ml of drainage water was sampled. For *E. coli* analysis 100-ml subsamples were taken aseptically and processed within 2 h, and organisms were enumerated using Idexx Colisure following incubation at 35°C for 24 h. Isolates obtained from a selection of positive Colisure wells on a number of sampling days were verified to be *E. coli* isolates by positive confirmation on

TABLE 2. Solar radiation, humidity, soil temperature, rainfall, and air temperature data for the lysimeter site during the period from September 2006 to August 2007

Parameter	Avg	SD	Maximum	Minimum
Daily rainfall (mm)	3.1	5.4	33.5	0.0
Air temp (°C)	11.0	3.6	18.9	2.0
Solar radiation (J/cm^2 day^{-1})	938.9	672.6	2,563.9	29.3
Relative humidity (%)	85.1	7.0	98.3	7.0
Soil temp (°C) at:				
10 cm	11.9	4.3	19.2	3.2
20 cm	11.9	4.2	18.7	3.8
30 cm	11.9	4.1	18.6	4.1
50 cm	12.0	3.8	17.4	3.8
1 m	12.1	3.1	16.4	3.1

MacConkey plates (Oxoid), UTI chromogenic plates (Oxoid), and API 20E (bioMérieux, Paris, France) strips.

Statistics. An analysis of variance (ANOVA) was performed with log-transformed total lysimeter *E. coli* load data with Proc Mixed (SAS 9.1), using soil type as a blocking factor.

RESULTS

The slurry spread in the summer was found to have an average DM content of 8% ± 0.01% and an average *E. coli* load of $1.6 \times 10^5 \pm 2.4 \times 10^4$ CFU per g (wet weight) of slurry, while the slurry spread in the spring had an average DM content of 7.4% ± 0.2% and an average *E. coli* load of $1.2 \times 10^5 \pm 2.8 \times 10^4$ CFU per g (wet weight) of slurry. A summary of the climatic data recorded by the onsite weather station is shown in Table 2. The data show the low average temperature, as well as the frequent rainfall and relatively small variation in soil and air temperatures, which is characteristic of a temperate maritime climate. The daily rainfall data and the average cumulative amounts of leachate for the four soils are shown in Fig. 1. The RA and EL soils exhibited similar drainage patterns during the experimental period, while the OA and CL soils had similar drainage patterns.

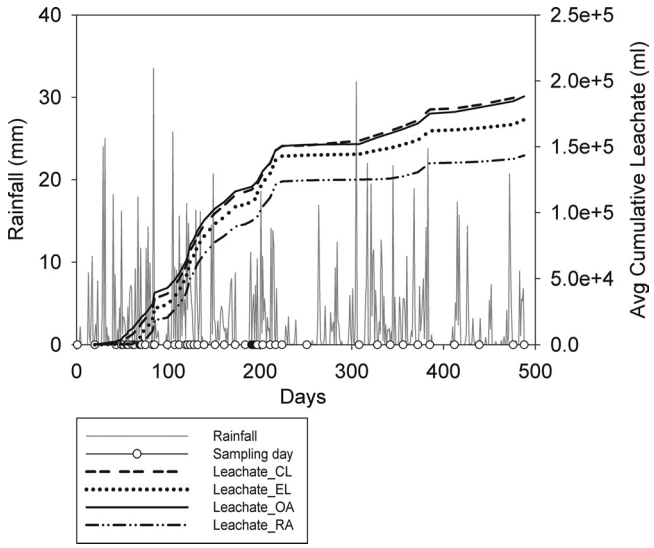

FIG. 1. Sampling days, rainfall, and average cumulative amounts of leachate ($n = 9$) for the 4 soils during the experimental period.

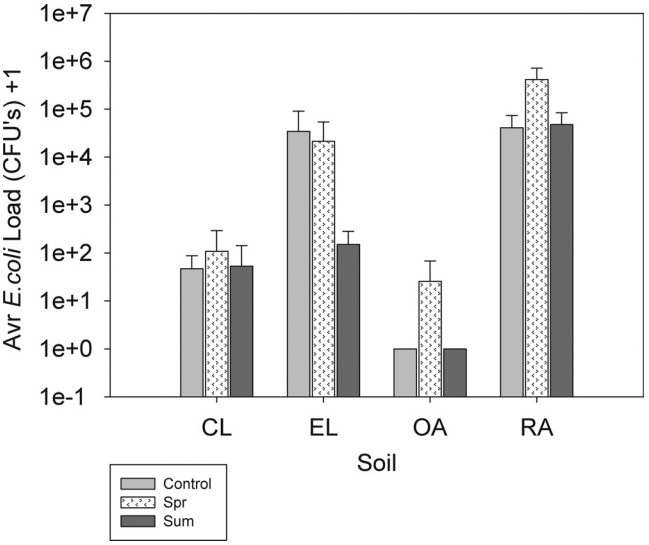

FIG. 2. Average ($n = 3$) total *E. coli* loads leached for the first 45 kg of leachate after application to soil by soil type (and treatment). Spr. Spring; Sum, summer.

Treatments. *E. coli* was detected in leachate from both lysimeters to which slurry was applied (spring and summer) and nontreated soil (control) lysimeters. As the slurry treatments were not applied at the same time, the comparison of treatments was based on the total amount of *E. coli* that leached in a fixed amount of leachate (45 kg) following application of the slurry or, in the case of the control treatment, after day 0 of the experiment. Although the time period required for this amount of drainage water to be leached varied between lysimeters and soils (from 49 to 297 days), this comparison was considered to be the most equitable comparison across all of the treatments. Box plots (not shown) suggested that the variance across soils was not equal. The heterogeneity was tested and modeled using the repeated statement in Proc Mixed. Residual checks showed that the assumptions of the tests were met. In addition, another analysis was carried out to compare the total amounts of *E. coli* leached from control lysimeters and from lysimeters to which slurry was applied in the summer for the whole experiment (488 days) and to compare the total amounts of *E. coli* leached from control lysimeters and from lysimeters to which slurry was applied in the spring for the experimental period after the spring application date (i.e., after day 184). All assumptions of the test were met. In both analyses treatment was not significant ($\alpha = 0.05$), while the block effect suggested that there was a strong association with soil type; the F statistic values were 104.96, 9.41, and 16.14 for the 45-kg leachate, summer-versus-control, and spring-versus-control comparisons, respectively.

Soils. Under natural rainfall conditions, *E. coli* was detected in the drainage water from all four soil types during the experimental period (Fig. 2). Leaching of the bacterium was observed predominantly from the RA soil, and low levels of bacterial contamination were frequently observed for the drainage water from all 9 replicate lysimeters for this soil, including water from the untreated soil in control lysimeters and water from other lysimeter soils prior to treatment (Fig. 3).

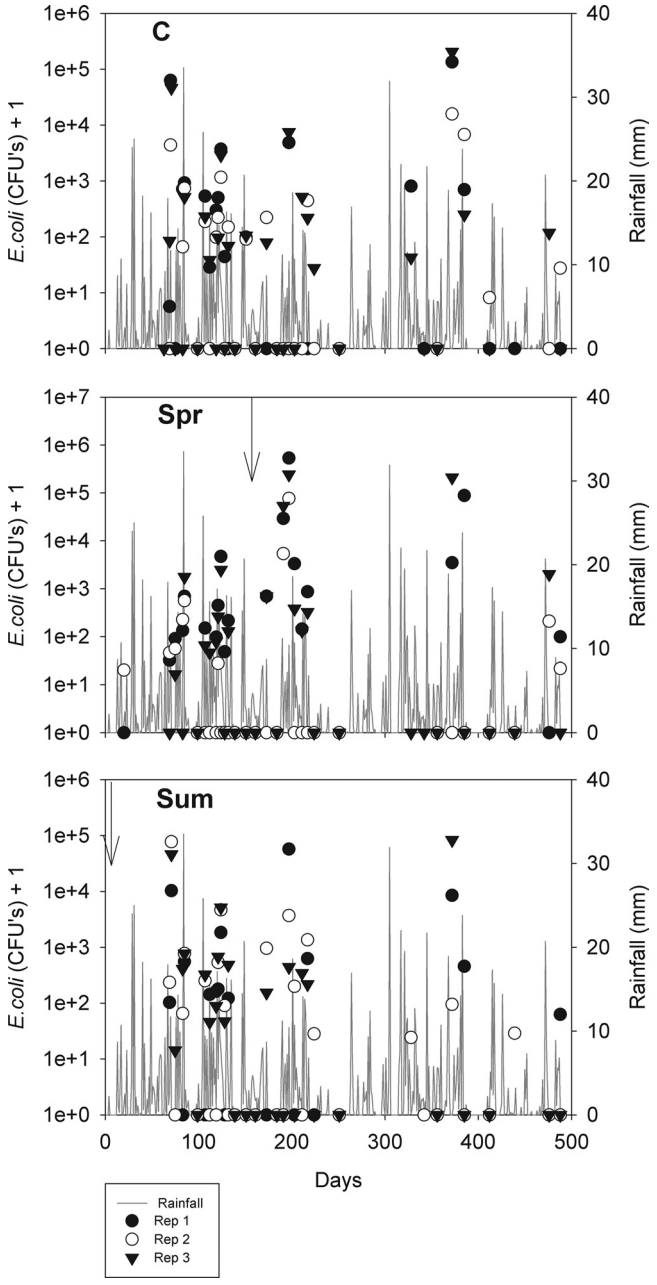

FIG. 3. *E. coli* loads leached for the 3 replicates for each RA treatment (C, control; Spr, treated in the spring; Sum, treated in the summer) and daily rainfall over the experimental period. The arrows indicate slurry application dates.

The total amount of *E. coli* leached from the RA soil lysimeters during the experiment was 9.5, 368, and 2,863 times the total amounts leached from the EL, CL, and OA soil lysimeters, respectively. *E. coli* also leached more frequently from the RA soil lysimeters than from the other soil lysimeters; during the experimental period 138 leachate samples from the RA soil were positive for *E. coli* (Fig. 3), while 46, 11, and 7 of the leachate samples from the EL, CL, and OA soils, respectively, were positive for *E. coli*. The results indicated that positive samples comprised 48, 16, 3, and 2.0% of the total leachate

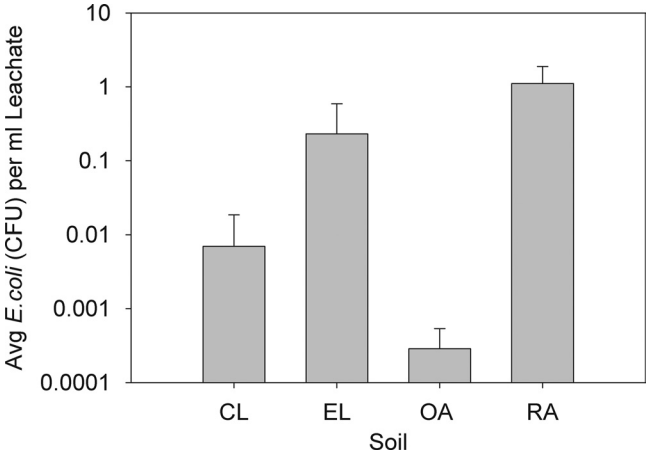

FIG. 4. Average amounts of *E. coli* leached per ml of leachate from the control lysimeters ($n = 3$) for each soil type. The error bars indicate standard deviations.

samples collected for the RA, EL, CL, and OA soils, respectively. When the risk of *E. coli* leaching from the soil types in the control lysimeters was assessed, the largest amount per gram of leachate was found to leach from the RA soil (Fig. 4).

The presence of *E. coli* in the drainage water of the soils was often found to be related to rainfall events, and high numbers of bacteria were often associated with high-intensity or prolonged rainfall. For the RA soil, this was often associated with a change in the color (to brown) of the leachate. Variability in the occurrence and temporal variation of the *E. coli* leached was observed between replicate lysimeters. An example of this is the EL soil (Fig. 5). On some occasions, all replicate lysimeters of a treatment leached simultaneously, and this was often associated with a rainfall event. In other cases, leaching was more sporadic, with *E. coli* leaching from different replicates at different times. With the other soil types *E. coli* leaching was often confined to the drainage water of specific lysimeters, and *E. coli* never leached from other lysimeters (e.g., replicate 2 EL control soil [Fig. 5]).

DISCUSSION

This study was designed to quantitatively investigate the impact of soil type and application season on the fate and transport of *E. coli* spread on four different temperate maritime soils under natural rainfall conditions. We hypothesized that *E. coli* transport would be different in different soil types and that *E. coli* spread on land would be transported more quickly through better-drained soils. In addition, we hypothesized that *E. coli* spread on land in the spring would be transported in greater numbers than *E. coli* spread on land in the summer due to wetter soil conditions that facilitated transport and survival. The results demonstrate that there was a soil type effect on the transport of *E. coli* in our experiments. Contrary to our hypothesis, however, the lowest numbers of *E. coli* cells leached from the best-drained soil (OA), while the greatest numbers of *E. coli* cells leached from the most poorly drained soil (RA). The results indicate that the soil moisture at the time of application had no effect on the numbers of *E. coli* cells leached.

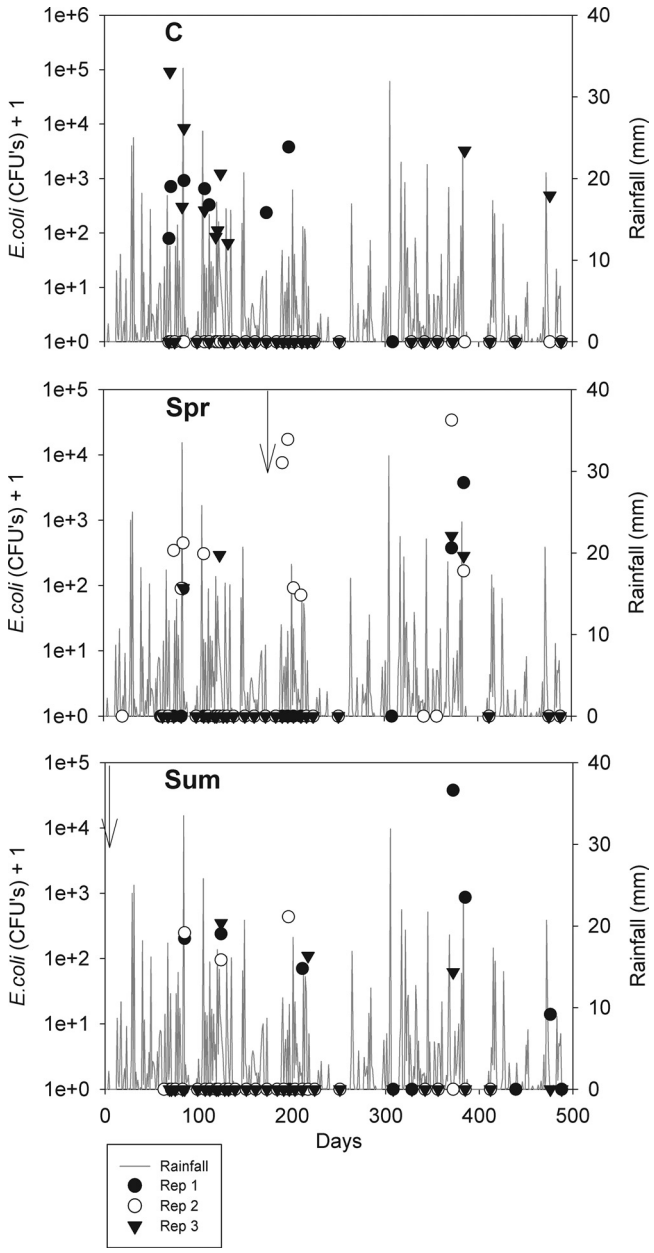

FIG. 5. Amounts of *E. coli* leached from the 3 replicates for each EL treatment (C, control; Spr, treated in the spring; Sum, treated in the summer) and daily rainfall over the experimental period. The arrows indicate slurry application dates.

Filtration is believed to be the principal factor influencing the transport of bacteria through soil (17). The similar drainage patterns of the RA and EL soils, from which the highest numbers of *E. coli* cells leached during the experimental period, suggest that the physical properties of these soils may be similar, and this has been reported previously for these soils (31). In both soils the clay content increases with depth, and this may favor the formation of macropores, which are known to be important pathways for bacterial transport and to reduce the filtration capacity of soils (1, 6, 53). Aislabie et al. (4) reported that differences in soil structure between soils resulted in differences in microbial movement, with the coarse subsoil structure of poorly drained soils favoring preferential flow through macropores and the fine soil structure of well-drained soils favoring matrix flow. Smith (50) also found that the extent of *E. coli* transport in soils was related to soil structure, and transport through macropores was believed to be an important transport mechanism. Water travels at a higher velocity through macropores than through the soil matrix, and this may shear off bacteria attached to soil particles in pore channels. Guber et al. (20) asserted that differences in bacterial transport in soil could be attributed to variations in pore water velocity caused by the spatial variability of the soil structure, while Abu-Ashour and Abu-Zreig (1) reported that desorption of biotracer cells was favored at a higher interstitial velocity, which resulted in greater shear forces. The leaching of greater numbers of *E. coli* cells from the more poorly drained soils in this experiment suggests that bacterial transport occurs by means of preferential flow routes. The observation that *E. coli* leaching occurred only in certain lysimeters within soil types further supports the hypothesis that there is structural variation in soils between lysimeters, and this variation may include the number and continuity of preferential routes.

The increases in the levels of bacterial leaching associated with high-intensity or prolonged rainfall events indicated that microorganisms were flushed through the soils. The change in the color of the leachate for the RA soil accompanying these rainfall events supports this view. This was not unexpected as a number of studies have related numbers of bacteria and the hydraulic loading rate associated with major rainfall events (21, 23, 51). The leaching of *E. coli* between intensive rainfall events or during lower-intensity rainfall events may have occurred because the threshold value required for saturation of the lysimeter base necessary for leaching of the drainage water was reached. Saini et al. (46) found that the length of time between application of manure and the first rainfall event was the most important factor influencing the leaching of *E. coli*. Due to the large background amounts of *E. coli* that leached throughout the experimental period in our study, *E. coli* leaching could not be directly attributed to application of slurry. However, in both the RA and EL soils large amounts of *E. coli* leached shortly after the spring application of slurry, which may have been indicative of preferential flow events. The reduced moisture content of the soils during the summer application may have precluded transport after application due to the reduction in the number of conducting transport pathways.

The leaching of *E. coli* from control soils, and from amended soils prior to treatment, more than 9 years after the last application of fecal material indicates that this organism can survive for prolonged periods in the lysimeter soils. The survival of enteric bacteria in soils is dependent on a number of factors (3, 17). A principal factor is the moisture content of the soil environment, which is strongly influenced by the soil particle distribution and organic matter content of the soil (27). The clay content of soils is particularly important with respect to survival as clay particles provide a larger ecological niche that is protected against predators and greater availability of substrates and moisture than sand particles (32). Survival of enteric bacteria in soil is, therefore, strongly dependent on the soil type. The OA soil, from which the lowest numbers of *E. coli* cells leached, has a low water-holding capacity and the

lowest clay content of the soils studied. Survival of enteric bacteria in this soil type would be expected to be limited and perhaps confined to organic material hotspots. In contrast, the RA soil is clay rich and has a compact soil structure below a depth of 50 cm, above which there are likely to be saturated soil conditions for a considerable portion of the year. The survival of *E. coli*, a facultative anaerobe, may be favored by the resulting anaerobic environment. Protozoan grazing, which is known to reduce *E. coli* populations in soils (44), may also be decreased under anaerobic conditions.

Die-off rates for *E. coli* in soil have been investigated often, and the majority of studies have reported a survival time of 2 to 4 months for enteric bacteria (27). Ohtomo et al. (40) reported that *E. coli* could survive for at least several months in grassland soils. Avery et al. (7) observed that *E. coli* O157:H7 could survive for at least 8 weeks in pasture. Sjogren (49) investigated *E. coli* survival in soils and produced model die-off curves that estimated probable survival times ranging from 20.7 to 23.3 months. Very few studies have shown long-term persistence of *E. coli* in grassland soils. Sjogren (48) reported that an antibiotic-resistant *E. coli* strain applied to Podzol field plots survived for 13 years after it was applied at high loading rates in a nutrient broth. In most studies, however, it has been observed that the majority of *E. coli* cells die rapidly once they are introduced into soil, and a key factor in this may be *E. coli*'s inability to step down its metabolic rate to cope with the low availability of usable carbon in the soil environment (30). While in general the size of an *E. coli* population declines rapidly after this organism enters soil, elements of the population may exhibit enhanced survival due to advantageous physiological properties or colonization of more favorable sites (38). It has been proposed for a long time that bacteria can survive under inhospitable environmental conditions by entering a viable but nonculturable (VBNC) state (58). However, isolates recovered from lysimeter soils in this study were readily cultured and so were considered physiologically active.

The physiological status of the organisms, combined with the length of time since the last application of fecal material and the amount and frequency of *E. coli* leaching from all nine lysimeters with the RA soil, indicates that *E. coli* not only survives in this soil but also likely grows. Autochthonous, or naturalized, *E. coli* populations have previously been reported for soils in tropical and subtropical regions and, more recently, for soils in temperate and northern temperate regions (25), but to our knowledge this is the first report of the presence of autochthonous *E. coli* populations in relatively low-temperature maritime temperate soils. This raises interesting questions about how these organisms grow and compete for niche space with indigenous soil organisms at temperatures which are suboptimal for *E. coli* growth and demonstrates the importance of long-term survival studies. Ishii (24) demonstrated that naturalized *E. coli* was present and grew in northern temperate soils but observed that during laboratory incubation *E. coli* could grow only at higher temperatures and growth was followed by rapid die-off. This suggests that soilborne isolates survive in soils until the temperature rises enough to facilitate growth. In maritime temperate soils *E. coli* growth would have to occur at lower temperatures. This suggests that organisms may have phenotypic characteristics favorable for growth in this environment or that the soil environment is favorable in terms of substrate availability and protection from predation. Outside tropical and subtropical environments, where high temperatures and nutrient availability favor growth, autochthonous *E. coli* strains have been found mainly in wet or submerged environments (25), indicating that survival of *E. coli* in the environment may be favored by anaerobic or microaerobic conditions. *E. coli* may have the capacity to grow in anaerobic zones or in micropores (where the formation of biofilms may provide protection against predation) in the RA soil. Similar favorable sites in the EL soil may allow the presence of autochthonous *E. coli* populations.

In conclusion, the greatest numbers of *E. coli* cells leached from the poorly drained Luvic Stagnosol soil in our lysimeter study, while the smallest numbers of *E. coli* cells leached from the freely drained Haplic Cambisol soil over the experimental period. For all soil types, spatial variability in the soil structure was important in the transport of the bacterium. No effect of the soil moisture status prior to application was observed in this study. In this trial *E. coli* was found to leach from lysimeters for all four soil types at a wide range of concentrations under natural rainfall conditions, but the public health consequences of this finding are unclear. The potential for bacterial transport through subsoil below a depth of 1 m, in which there may be reduced macropore conductivity, is poorly understood and is an important consideration in assessing the risk to groundwater. In more poorly drained soil types, it is likely that artificial soil drainage schemes may facilitate the transport of *E. coli* in soil to surface water. The high levels of *E. coli* leaching from control soils that had not been amended with fecal material in over 9 years indicates that there is long-term persistence of *E. coli* in Irish soils, implying that the characteristics of a soil that influence survival are at least as important as the characteristics that influence transport in predicting potential risk. The high frequency of *E. coli* leaching, particularly in the Luvic Stagnosol soil, suggests that autochthonous *E. coli* populations are capable of becoming naturalized in the low-temperature environments of temperate maritime soils. This may compromise use of *E. coli* as the sole indicator of fecal pollution in waters in these regions.

ACKNOWLEDGMENTS

This work was funded by the Irish Research Council for Science, Engineering and Technology (IRCSET) and was supported by Teagasc, Johnstown Castle.

REFERENCES

1. **Abu-Ashour, J., and M. Abu-Zreig.** 2005. Effect of interstitial velocity on the adsorption of bacteria onto soil. Adsorp. Sci. Technol. **23:**535–542.
2. **Abu-Ashour, J., D. M. Joy, H. Lee, H. R. Whiteley, and S. Zelin.** 1998. Movement of bacteria in unsaturated soil columns with macropores. Trans. ASAE **41:**1043–1050.
3. **Abu-Ashour, J., D. M. Joy, H. Lee, H. R. Whiteley, and S. Zelin.** 1994. Transport of microorganisms through soil. Water Air Soil Pollut. **75:**141–158.
4. **Aislabie, J., J. J. Smith, R. Fraser, and M. McLeod.** 2001. Leaching of bacterial indicators of faecal contamination through four New Zealand soils. Aust. J. Soil Res. **39:**1397–1406.
5. **Artz, R. R. E., and K. Killham.** 2002. Survival of *Escherichia coli* O157:H7 in private drinking water wells: influences of protozoan grazing and elevated copper concentrations. FEMS Microbiol. Lett. **216:**117–122.
6. **Artz, R. R. E., J. Townend, K. Brown, W. Towers, and K. Killham.** 2005. Soil macropores and compaction control the leaching potential of *Escherichia coli* O157:H7. Environ. Microbiol. **7:**241–248.
7. **Avery, L. M., P. Hill, K. Killham, and D. L. Jones.** 2004. *Escherichia coli* O157 survival following the surface and subsurface application of human pathogen contaminated organic waste to soil. Soil Biol. Biochem. **36:**2101–2103.

8. **Balkwill, D. L., E. M. Murphy, D. M. Fair, D. B. Ringelberg, and D. C. White.** 1998. Microbial communities in high and low recharge environments: implications for microbial transport in the vadose zone. Microb. Ecol. **35:**156–171.
9. **Bolster, C. H., B. Z. Haznedaroglu, and S. L. Walker.** 2009. Diversity in cell properties and transport behavior among 12 different environmental *Escherichia coli* isolates. J. Environ. Qual. **38:**465–472.
10. **Burton, C. H., and C. Turner.** 2003. Health risks from pathogens in livestocks manures, p. 109–157. *In* C. H. Burton and C. Turner (ed.), Manure management. Treatment strategies for sustainable agriculture, 2nd ed. Silsoe Research Institute, Bedford, United Kingdom.
11. **Chu, Y., Y. Jin, T. Baumann, and M. V. Yates.** 2003. Effect of soil properties on saturated and unsaturated virus transport through columns. J. Environ. Qual. **32:**2017–2025.
12. **Committee on Indicators for Waterborne Pathogens.** 2004. Indicators for waterborne pathogens. National Academy of Sciences Press, Washington, DC.
13. **Cortvriend, J., and A. Hulsmann.** 2006. Europe paves the way for the revision of the drinking water directive. Water 21 **August:**17–19.
14. **Darnault, C. J. G., T. S. Steenhuis, P. Garnier, Y.-J. Kim, M. B. Jenkins, W. C. Ghiorse, P. C. Baveye, and J. Y. Parlange.** 2004. Preferential flow and transport of *Cryptosporidium parvum* oocysts through the vadose zone: experiments and modelling. Vadose Zone J. **3:**262–270.
15. **Duffy, G.** 2003. Verocytoxigenic *Escherichia coli* in animal faeces, manures and slurries. J. Appl. Microbiol. **94:**94S–103S.
16. **Gagliardi, J. V., and J. S. Karns.** 2000. Leaching of *Escherichia coli* O157:H7 in diverse soils under various agricultural management practices. Appl. Environ. Microbiol. **66:**877–883.
17. **Gerba, C. P., and G. Bitton.** 1984. Microbial pollutants: their survival and transport to groundwater, p. 65–88. *In* G. Bitton and C. P. Gerba (ed.), Groundwater pollution microbiology. John Wiley & Sons Inc., New York, NY.
18. **Gerba, C. P., and J. E. Smith, Jr.** 2005. Sources of pathogenic microorganisms and their fate during land application of wastes. J. Environ. Qual. **34:**42–48.
19. **Goss, M. J., D. A. J. Barry, and D. L. Rudolph.** 1998. Contamination in Ontario farmstead domestic wells and its association with agriculture. 1. Results from drinking water wells. J. Contam. Hydrol. **32:**267–293.
20. **Guber, A. K., D. R. Shelton, and Y. A. Pachepsky.** 2005. Transport and retention of manure-borne coliforms in soil. Vadose Zone J. **4:**828–837.
21. **Hagedorn, C., D. T. Hansen, and G. H. Simonson.** 1978. Survival and movement of fecal indicator bacteria in soil under conditions of saturated flow. J. Environ. Qual. **7:**55–59.
22. **Hutchison, M. L., L. D. Walters, S. M. Avery, B. A. Synge, and A. Moore.** 2004. Levels of zoonotic agents in British livestock manures. Lett. Appl. Microbiol. **39:**207–214.
23. **Huysman, F., and W. Verstraete.** 1993. Water-facilitated transport of bacteria in unsaturated soil columns: influence of inoculation and irrigation methods. Soil Biol. Biochem. **25:**91–97.
24. **Ishii, S., W. B. Ksoll, R. E. Hicks, and M. J. Sadowsky.** 2006. Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. Appl. Environ. Microbiol. **72:**612–621.
25. **Ishii, S., and M. J. Sadowsky.** 2008. *Escherichia coli* in the environment: implications for water quality and human health. Microbes Environ. **23:**101–108.
26. **Jackson, S. G., R. B. Goodbrand, R. P. Johnson, V. G. Odorico, D. Alves, K. Rahn, J. B. Wilson, M. K. Welch, and R. Khakhria.** 1998. *Escherichia coli* O157:H7 diarrhoea associated with well water and infected cattle on an Ontario farm. Epidemiol. Infect. **120:**17–20.
27. **Jamieson, R. C., R. J. Gordon, K. E. Sharples, G. W. Stratton, and A. Madani.** 2002. Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: a review. Can. Biosyst. Eng. **44:**1.1–1.9.
28. **Jiang, G.** 2005. Transport and deposition of *Bacillus subtilis* through an intact soil column. Aust. J. Soil Res. **43:**695–703.
29. **John, D. E., and J. B. Rose.** 2005. Review of factors affecting microbial survival in groundwater. Environ. Sci. Technol. **39:**7345–7356.
30. **Klein, D. A., and L. E. Casida, Jr.** 1967. *Escherichia coli* die-out from normal soil as related to nutrient availability and the indigenous microflora. Can. J. Microbiol. **13:**1461–1470.
31. **Kramers, G.** 2009. Preferential flow in Irish grassland soils. Ph.D. thesis. University College Dublin, Dublin, Ireland.
32. **Lang, N. L., and S. R. Smith.** 2007. Influence of soil type, moisture content and biosolids application on the fate of *Escherichia coli* in agricultural soil under controlled laboratory conditions. J. Appl. Microbiol. **103:**2122–2131.
33. **Mawdsley, J. L., R. D. Bardgett, R. J. Merry, B. F. Pain, and M. K. Theodorou.** 1995. Pathogens in livestock waste, their potential for movement through soil and environmental pollution. Appl. Soil Ecol. **2:**1–15.
34. **Mawdsley, J. L., A. E. Brooks, and R. J. Merry.** 1996. Movement of the protozoan pathogen *Cryptosporidium parvum* through three contrasting soil types. Biol. Fertil. Soils **21:**30–36.
35. **McFeters, G. A., S. C. Broadaway, B. H. Pyle, M. Pickett, and Y. Egozy.** 1997. Comparative performance of Colisure. J. Am. Water Works Assoc. **89:**112–120.
36. **Morrow, J. B., R. Stratton, H. H. Yang, B. F. Smets, and D. Grasso.** 2005. Macro- and nanoscale observations of adhesive behavior for several *E. coli* strains (O157:H7 and environmental isolates) on mineral surfaces. Environ. Sci. Technol. **39:**6395–6404.
37. **Nicholson, F. A., S. J. Groves, and B. J. Chambers.** 2005. Pathogen survival during livestock manure storage and following land application. Bioresour. Technol. **96:**135–143.
38. **Ogden, I. D., D. R. Fenlon, A. J. A. Vinten, and D. Lewis.** 2001. The fate of *Escherichia coli* O157 in soil and its potential to contaminate drinking water. Int. J. Food Microbiol. **66:**111–117.
39. **Ogden, I. D., M. MacRae, and N. J. C. Strachan.** 2004. Is the prevalence and shedding concentrations of *E. coli* O157 in beef cattle in Scotland seasonal? FEMS Microbiol. Lett. **223:**297–300.
40. **Ohtomo, R., K. Minato, and M. Saito.** 2004. Survival of *Escherichia coli* in a field amended with cow feces slurry. Soil Sci. Plant Nutr. **50:**575–581.
41. **Paterson, E., J. S. Kemp, S. M. Gammack, E. A. Fitzpatrick, M. S. Cresser, C. E. Mullins, and K. Killham.** 1993. Leaching of genetically modified *Pseudomonas fluorescens* through intact soil microcosms: influence of soil type. Biol. Fertil. Soils **15:**308–314.
42. **Powelson, D. K., and C. P. Gerba.** 1995. Fate and transport of microorganisms in the vadose zone, p. 123–135. *In* L. G. Wilson, L. G. Everett, and S. J. Cullen (ed.), Handbook of vadose zone characterization and monitoring. CRC Press, Boca Raton, FL.
43. **Powelson, D. K., and A. L. Mills.** 2001. Transport of *Escherichia coli* in sand columns with constant and changing water contents. J. Environ. Qual. **30:** 238–245.
44. **Recorbet, G., C. Steinberg, and G. Faurie.** 1992. Survival in soil of genetically engineered *Escherichia coli* as related to inoculum density, predation and competition. FEMS Microbiol. Lett. **101:**251–260.
45. **Ryan, M., and A. Fanning.** 1996. Effects of fertiliser N and slurry on nitrate leaching—lysimeter studies on 5 soils. Irish Geogr. **29:**126–136.
46. **Saini, R., L. J. Halverson, and J. C. Lorimor.** 2003. Rainfall timing and frequency influence on leaching of *Escherichia coli* RS2G through soil following manure application. J. Environ. Qual. **32:**1865–1872.
47. **Saini, R., J. C. Lorimor, and L. Halverson.** 2001. Effect of manure application and rainfall timing on the leaching of labeled bacteria through soil columns, paper no. 2195. *In* Proceedings of the ASAE Annual International Meeting, California. American Society of Agricultural and Biological Engineers, St. Joseph, MI.
48. **Sjogren, R. E.** 1995. 13-Year survival study of an environmental *Escherichia coli* in field mini-plots. Water Air Soil Pollut. **81:**315–335.
49. **Sjogren, R. E.** 1994. Prolonged survival of an environmental *Escherichia coli* in laboratory soil microcosms. Water Air Soil Pollut. **75:**389–403.
50. **Smith, M. S.** 1985. Transport of *Escherichia coli* through intact and disturbed soil columns. J. Environ. Qual. **14:**87–91.
51. **Stevik, T. K., G. Ausland, J. F. Hanssen, and P. D. Jenssen.** 1999. The influence of physical and chemical factors on the transport of *E. coli* through biological filters for wastewater purification. Water Res. **33:**3701–3706.
52. **Theron, J., and T. E. Cloete.** 2002. Emerging waterborne infections: contributing factors, agents, and detection tools. Crit. Rev. Microbiol. **28:**1–26.
53. **Unc, A., and M. J. Goss.** 2004. Transport of bacteria from manure and protection of water resources. Appl. Soil Ecol. **25:**1–18.
54. **Van Elsas, J. D., J. T. Trevors, and L. S. Van Overbeek.** 1991. Influence of soil properties on the vertical movement of genetically-marked *Pseudomonas fluorescens* through large soil microcosms. Biol. Fertil. Soils **10:**249–255.
55. **Vernozy-Rozand, C., M. P. Montet, F. Lequerrec, E. Serillon, B. Tilly, C. Bavai, S. Ray-Gueniot, J. Bouvet, C. Mazuy-Cruchaudet, and Y. Richard.** 2002. Prevalence of verotoxin-producing *Escherichia coli* (VTEC) in slurry, farmyard manure and sewage sludge in France. J. Appl. Microbiol. **93:**473–478.
56. **Vinten, A. J. A., D. R. Lewis, D. R. Fenlon, K. A. Leach, R. Howard, I. Svoboda, and I. Ogden.** 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 Manag. **18:**223–231.
57. **Vinten, A. J. A., J. Potts, L. Avery, and N. J. C. Strachan.** 2009. Microbial pollution of water by livestock: approaches to risk assessment and mitigation. Animal **3:**744–752.
58. **Winfield, M. D., and E. A. Groisman.** 2003. Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. Appl. Environ. Microbiol. **69:**3687–3694.
59. **World Health Organization.** 2006. Microbial aspects, p. 121–144. *In* Guidelines for drinking water quality, 3rd ed., 1st addendum. World Health Organization, Geneva, Switzerland.
60. **Yang, H.-H., J. B. Morrow, D. Grasso, R. T. Vinopal, and B. F. Smets.** 2006. Intestinal versus external growth conditions change the surficial properties in a collection of environmental *Escherichia coli* isolates. Environ. Sci. Technol. **40:**6976–6982.