Recently Planted Vegetation Strips Reduce *Giardia* Runoff Reaching Waterways

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Current methods for tracking pathogens across farmland and into surrounding waterways via runoff are limited and typically have been developed using artificially created landscapes. No studies have investigated how *Giardia* in farm runoff moves across the landscape, despite high prevalence rates in dairy cattle (*Bos taurus*) worldwide. Here, we report the development of a field-based tracking method specific for *Giardia* movement in runoff and use this technique to compare the pathogen reduction capability of recently planted vegetation strips with bare soil strips cleared of vegetation. Such scenarios represent typical events in schemes to plant vegetation barriers aimed at reducing waterway contamination. A significant treatment effect was identified, with 26% fewer *Giardia* detected in runoff collected from the planted strip (P = 0.006). These results highlight the immediate benefit of pathogen removal to be gained from vegetation planting. The successful discrimination of treatment effects by this new technique will enable the assessment of different vegetation types on runoff reduction and the effects of plant development over time.

Cysts of the protozoan pathogen *Giardia* have been detected from aquatic and terrestrial environments worldwide where large cattle populations reside (Barwick et al., 2003; Brookes et al., 2004; Brown et al., 1998; Thurman et al., 1998). Given significant levels of *Giardia* prevalence in dairy cattle, it is to be expected that cysts enter waterways in passive runoff from dairy farms (Learmonth et al., 2003; Nagels et al., 2005; Winkworth et al., 2008). Therefore, understanding how *Giardia* move across the landscape is central to the development of strategies to reduce waterway contamination. However, while the movement of water across the landscape is well studied (Adams et al., 2005; Davie, 2004), the movement of pathogens within that water is less so.

To date, research has focused on the movement of *Escherichia coli* (Ferguson et al., 2007; Muirhead et al., 2006b) and *Cryptosporidium* (Davies et al., 2004; Trask et al., 2004); studies of *Giardia* have not been reported. Arguably, results from studies of similar shaped, yet smaller organisms, like *E. coli* and *Cryptosporidium,* could be extrapolated to larger sized species such as *Giardia*. However, studies of microbial transport through soil have reported that soil grain and microbe particle sizes are not the only factors that determine movement. Adsorption/desorption effects, hydrological movement (Adams et al., 2005), and mechanical and biological features (Tufenkji et al., 2004) also seem to be influential and can depend on pollutant charge (Dai and Boll, 2003), size (Kim and Tobiason, 2004), structure and associated content of the runoff (Searcy et al., 2005). Accordingly, studies specific to *Giardia* are needed to properly understand cyst movement in runoff from dairy farms.

There have been several reports of waterway contamination by microbes as a result of surface runoff (Dosskey, 2001; Mawdsley et al., 1995), but previous studies aimed at understanding mechanisms that may reduce runoff contamination have focused on infiltration processes (Harter et al., 2008; Sullivan et al., 2007). However, surface runoff on agricultural land during the winter months in New Zealand is characterized by saturation excess overland flow, during which time infiltration exerts little influence (Muirhead et al., 2006c). Saturation excess overland flow occurs when pore spaces in the soil matrix are saturated from below such that any additional water applied from above has no available space to fill, and thus runs across the soil surface (Dingman, 2002). Not surprisingly, soil matrices that have been removed from their original location and the associated water table are altered, resulting in changes to macropore transport mechanisms and soil conductivity characteristics. Employing these artificial

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Abbreviations: PBS, phosphate buffered saline; RM, repeated measures.

landscapes could lead to the identification of runoff patterns that may not translate well to those observed in natural environments. On the other hand, by specifically maintaining original soil characteristics, and thus the natural runoff mechanisms associated with saturation excess overland flow, accurate *Giardia* movement across the landscape could be determined. With this in mind, the aims of our study were:

1. To develop a method capable of tracking *Giardia* cysts across a landscape unmanipulated except for the presence or absence of vegetation, and
2. To compare cyst-reduction capabilities of freshly cleared, yet unplanted, riparian sites with those recently planted with native vegetation, thus simulating actual riparian planting initiatives.

Materials and Methods

Study Area

The field site was located along a farm field edge adjacent to an undisturbed waterway. During times of heavy rainfall, the waterway facilitates drainage of surrounding fields by enabling runoff to be transported away passively by gravity. Soil at the site is classified as recent Clutha soils from schist alluvium (Series: Pomahaka, depth: deep, texture: sandy loam, slope: gently undulating; Series: Momona, depth: deep, texture: loamy silt, slope: gently undulating) (New Zealand classification; Leamy and Leslie, 1985). A 30-m strip, 3 m wide and running parallel to the waterway, was prepared by applying a commercially available all purpose weed killer (Round Up, Monsanto Company, Marysville, OH) to kill the pastoral vegetation, followed a month later by mechanically removing the resulting dead vegetation. One meter wide individual plots were created perpendicular to the waterway by inserting stainless steel sheets (2.5 m long, 0.2 m wide, 1.3 mm thick) lengthwise along each of the 2.5-m long sides to a vertical depth of approximately 150 mm, or to the base of the A horizon (Fig. 1). On average, plot slope toward the waterway edge from the top of the plot was 5°. Of the six plots tested, three were left without further manipulations while the other three were each planted with eight New Zealand native sedges (*Carex secta;* Boott in Hook. f. Flora of New Zealand 1, 1853, 281) in a 2–1–2–1–2 configuration lengthways down the plot (Fig. 1). *Carex secta* is a grass-like plant in a genus comprising more than 1000 species, 70 of which are native to New Zealand (Cave and Paddison, 2005). Growing up to 1 m high, *C. secta* is commonly found beside water and in swampy conditions (Cave and Paddison, 2005).

Giardia Cyst Generation

Nonviable cysts were prepared by encysting laboratory-adapted strains of *Giardia* grown in tissue culture following previously described protocols (Kane et al., 1991). The prepared cysts were heat-treated for 20 min at 60°C to render them nonviable; this treatment yields 100% nonviable cysts, as illustrated by multiple viability stains, 0% excystation (next life cycle stage), and no infections when presented to mice (*Mus musculus*) (Taghi-Kilani et al., 1996). Five samples of the pooled heat-treated cyst suspension were screened using diamidinophenylindole (DAPI) and propidium iodine (PI), two fluorescent dyes that differentiate viable from nonviable cysts, to confirm 100% cyst nonviability. Prepared cysts were maintained at 4°C until use.

Runoff Simulation and Collection

A ground-level drip irrigation system was used to generate overland flow evenly across individual plots using water from an on-site bore. The application of water directly to the plot surface was to create runoff broadly comparable to movement down a hillside and was not intended to represent rainfall of a particular magnitude (Collins et al., 2004). Water was pumped at a constant rate of 2 L per minute, generating the saturated soil conditions typically observed during late autumn, winter, and early spring months in New Zealand (Muirhead et al., 2006c). Plots were deemed saturated when identical and constant flow rates were measured using V-notch weirs at the upper and lower edges of the plot, the points of water application and water collection, respectively. Runoff experiments were performed during August 2006 (late winter/early spring) when soil and air temperatures were greater than 4°C, ensuring the applied water could infiltrate the soil, generate saturated soil conditions, and thus run off.

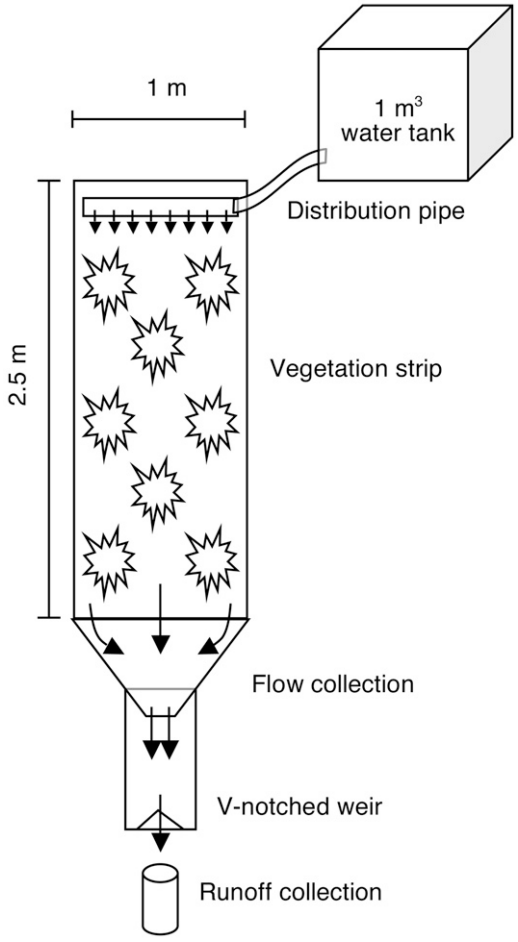

Fig. 1. Schematic of field-based experimental setup showing the plot arrangement, water supply, and runoff collection.

Bromide was used as a nonreactive, nonsorbing, conservative tracer at a concentration of 2326 mg/L. Water sourced from the on-site bore exhibited a constant bromide concentration of no more than 0.7 mg/L, which was insufficient to influence the results.

When each plot was tested, 1-min-runoff samples were collected in separate 2-L containers at the lower edge of the plot for 20 min (20 samples). An instantaneous spike containing the bromide tracer and inactivated *Giardia* cysts was applied at ground level 5 cm in front of the irrigation apparatus after 1 min had elapsed. Cyst presence on the plots before spike addition was theoretically possible as 5% of cattle excrete cyst numbers comparable to those used in the experiment (Learmonth et al., 2003). However, the adjacent farm field had been used for crop generation continuously during the previous year and had not contained cattle. Nevertheless, the first runoff sample collected before application of the tracer-cyst spike was important for estimating naturally present cyst concentrations. The applied spike contained 7×10^6 cysts suspended in 200 mL of on-site bore water and adjusted to the required bromide concentration. Logistical constraints ruled out running all six replicates on the same day. Thus, pairs of each treatment (planted and bare) were tested every other day over a period of 6 d (two plots per day). Samples were stored at 4°C in the dark until processed.

Tracer and Cyst Analysis

Bromide measurements occurred within 24 h of sample collection, involved the entire sample, and used a selective ion probe (Mettler Toledo Inc., Columbus, OH).

Cyst measurements were completed within 2 wk of sample collection and involved microscopy on subsamples. Specifically, from the original 2 L individual runoff sample collected per minute, 250 mL was pelleted using centrifugation at 5000 × *g* for 5 min at 4°C. The supernatant was discarded, the pellet resuspended in 2 to 5 mL phosphate buffered saline (PBS), and transferred to a 15 mL conical centrifuge tube for storage at 4°C. Finally, cyst concentrations were enumerated from 10 μL of each concentrated runoff sample.

Using the MeriFluor *Cryptosporidium/Giardia* kit (Meridian Bioscience Incorporated, Cincinnati, OH), a direct immunofluorescence assay, 10-μL subsamples were transferred into 1.5-mL snap-lock tubes containing 25-μL detection reagent and incubated in the dark for 30 min at room temperature. To reduce detection costs per sample, but with no observable difference in reagent efficiency, the manufacturer's supplied detection reagent was diluted fivefold in PBS immediately before use. Following incubation, samples were pelleted at 16, 000 × *g* for 1 min and the supernatant discarded. Pellets were washed twice to remove unbound detection reagent by resuspension in 50 μL PBS and repelleted at 16, 000 × *g*. Pellets were then counterstained in 50 μL PBS containing 0.2% Evans Blue (bioMérieux, Durham, NC) and 1% Tween-20 (Sigma-Aldrich NZ Ltd, NZ) for 10 min at room temperature in the dark. Following counterstaining, samples were again washed twice with PBS, resuspended in 50 μL PBS, transferred onto pretreated three-well microscope slides (Dynal Biotech Pty Ltd, Australia), and air-dried. Each air-dried sample was mounted using Fluroprep (bioMérieux, Durham, NC) and covered with a cover slip. Microscopic analysis was performed using a Zeiss Axiophot Fluorescent microscope, equipped with a filter system specific for fluorescein isothiocyanate (FITC; excitation wavelength 490–500, barrier filter 510–530). Each microscope slide well corresponded to one runoff sample and was scanned in its entirety, with all observed cysts counted. Three 10-μL subsamples of each concentrated runoff sample were enumerated using this method.

Calculating Concentrations

Mean bromide and *Giardia* concentrations were calculated for every time point of each treatment. The bromide mg/L concentrations reported were measured using the method described above and adjusted by the dilution factor observed during the experiment. Proportional recovery of bromide was calculated by dividing the concentration recovered (C) for each of the two treatments by the original concentration applied (Co) in the spike for individual time points, as well as for the overall totals recovered. Breakthrough curves were generated by plotting the proportional recoveries of bromide against time.

As subsampling was performed for *Giardia* cyst enumeration, the resulting raw counts were back calculated to reflect the number of cysts present in the entire 2-L sample collected. Therefore, the reported mean numbers, and subsequent recoveries of *Giardia*, represent numbers extrapolated to the entire sample using the following equation:

$$\text{No. cysts/2 L} = (\text{no.cysts/well})(\text{concentrate volume}/10\ \mu\text{L}) \times (2000\ \text{mL}/250\ \text{mL})$$

Assuming a minimum concentration volume of 2 mL, the detection limit for individual samples screened using the reported technique was 10^3 cysts/2 L.

To enable comparisons between total numbers of *Giardia* recovered from the two treatment types, the mean number of cysts collected over the entire 20-min runoff period was calculated using two independent enumeration methods. Statistical analysis (outlined below) was used to determine whether values calculated using the two different methods were comparable, and thus interchangeable as appropriate representations of the number of total cysts recovered from the two treatment types. For the first method, all cysts in each of the 20 consecutive samples collected from the same plot were enumerated (using three subsamples per sample, see above). The resulting means of cyst numbers per individual sample were then added to reflect the total number of *Giardia* cysts recovered over the entire period from that plot. The second method, which required additional time and effort after the individual subsampling enumeration, involved pooling the 20 individual samples from the same plot then enumerating 10 subsamples from this combined sample to determine the total number of recovered cysts. Lastly, for both methods, the total number of cysts recovered (C) from each treatment was divided by the original total number of cysts applied (Co) to determine the proportion of cysts recovered (C/Co). As with the bromide breakthrough curves, proportional

recoveries of *Giardia* were plotted against time for both treatments. Thus, the reduction of *Giardia* cyst numbers could be compared between the two different plot treatments and also between the 20 consecutive time points.

Data Analysis

Statistical analyses were performed using SPSS version 11 for Mac OS X (SPSS Inc., Chicago, IL). A nested repeated-measures (RM) ANOVA was used to compare the concentration of *Giardia* cysts recovered from individual "time-point" samples collected over the 20 min runoff period between the two experimental treatments (within-subjects factor "time" [1–20]; between-subjects factors "plot type" [planted or bare] and "subsample" [1–3] nested within plot type).

Bromide concentrations in the time-point samples were also analyzed with RM-ANOVA. Here, plot type was the only between-subjects factor because a single bromide value was determined per experimental plot (see above). In cases where the assumption of data sphericity was violated, the overall results of the within-subjects analyses were corrected with the Greenhouse-Geisser method (Quinn and Keough, 2002).

A nested ANOVA was used to compare the total number of *Giardia* between treatments, as calculated from enumerating the combined samples. In this analysis, the between-subjects factor "subsample" (1–10) was nested within the main factor plot type.

Average relative *Giardia* to bromide (G/B) ratios plotted against time for the two treatments were calculated by dividing the *Giardia* recovery proportion (C/Co) by the bromide recovery proportion (C/Co) following a previously described protocol (Harvey and Garabedian, 1991; Muirhead et al., 2006a).

Results

Prespike, background bromide concentrations recorded in runoff were 0.64 (± 0.07 SE) and 0.50 (± 0.04 SE) mg/L for the bare soil and planted treatments, respectively. No *Giardia* cysts were detected from either treatment before addition of the spike.

Both methods used to enumerate the total number of recovered *Giardia* cysts yielded significant differences between the two experimental treatments (Tables 1a and 1b). No differences between individual subsamples (three for each of the 20 time-point samples or 10 for the pooled sample) were found for either enumeration method (Tables 1a and 1b).

The between-subjects results of the RM-ANOVAs indicate that more bromide and more *Giardia* cysts were recovered from bare plots than from newly planted plots (Table 1; Fig. 2). Similarly, when assessing overall changes with time (Table 2) and overall changes of treatment effects with time (Table 2, time × plot type interaction), significant differences were detected for both bromide and *Giardia*. In the bare soil treatment, an elevated bromide concentration was detected in the first minute after addition of the spike (time 2) and peaked 2 min later at time 4 (Fig. 3). Concentrations comparable to prespike levels had been regained 16 min after spike application (at time 17; Fig. 3). Similarly, *Giardia* was detected by time 2 and peaked at time 4 in the bare soil treatment (Fig. 3). In contrast, both bromide and *Giardia* were delayed in the planted treatment; elevated bromide concentrations were first detected at time 3, 2 min after spike application, though bromide still peaked at time 4 (Fig. 3). *Giardia* was also first detected at time 3 and peaked at time 4 (Fig. 3).

The calculated G/B ratios showed similar patterns in the two treatments, with ratios <1 and an initial peak followed by a gradual decline (Fig. 4). The G/B ratio peaked during the first minute after spike addition in the bare soil treatment and 2 min after spike addition in the planted treatment (time 2 and time 3, respectively in Fig. 4).

Discussion

The degradation of waterways that traverse farming areas has been reported worldwide and is caused by both point (direct) and nonpoint (passive) source pollution containing chemical and biological contaminants (Dosskey, 2001; Mawdsley et al., 1995). To date, approaches for reducing contamination have typically focused on direct causes and the physical removal of contaminant sources from water. For example, the regional authority charged with protecting the environment throughout the Otago Province of the South Island of New Zealand (Otago Regional Council) has recently implemented strategies to reduce direct causes of farm pollution. One such strategy requires all farm waterways to be fenced, ensuring stock cannot defecate directly into them (ORC, 2003). However, the impact of such strategies on passive contamination, like runoff from farm fields containing pathogens transmitted in feces, is unclear. To date, few techniques exist for assessing the passive contamination of waterways by pathogens in runoff traveling across unmanipulated saturated soils (Muirhead et al., 2006c). Rather, most techniques have been developed to investigate fluxes under the influence of infiltration in either unmanipulated landscapes (Atwill et al., 2006; Fiener and Auerswald, 2005; Sullivan et al., 2007) or artificially created landscapes, such as soil matrices that have been removed from their original location (Davies et al., 2004; Harter et al., 2008;

Table 1. Between-subjects results of the ANOVAs comparing the reduction in absolute *Giardia* and bromide values between bare soil and recently planted *Carex secta* treatments. Results for *Giardia* are presented for (a) the nested repeated-measures ANOVA on the 20 individual time points; and (b) the nested ANOVA on the 10 subsamples [= nested factor "sample"] counted for each "combined sample" [= all time-point samples from each replicate plot pooled before counting], while (c) contains the results of the repeated-measures ANOVA for bromide concentrations on the 20 individual time points.

	df	F	*P*	Power
(a) *Giardia*: individual samples				
Plot type	1	5.66	0.04	0.59
Subsample (plot type)	4	0.17	0.95	0.07
Error	12			
(b) *Giardia*: combined samples				
Plot type	1	5.88	0.02	0.66
Subsample (plot type)	18	0.53	0.93	0.29
Error	40			
(c) Bromide: individual samples				
Plot type	1	15.31	0.02	0.83
Error	4			

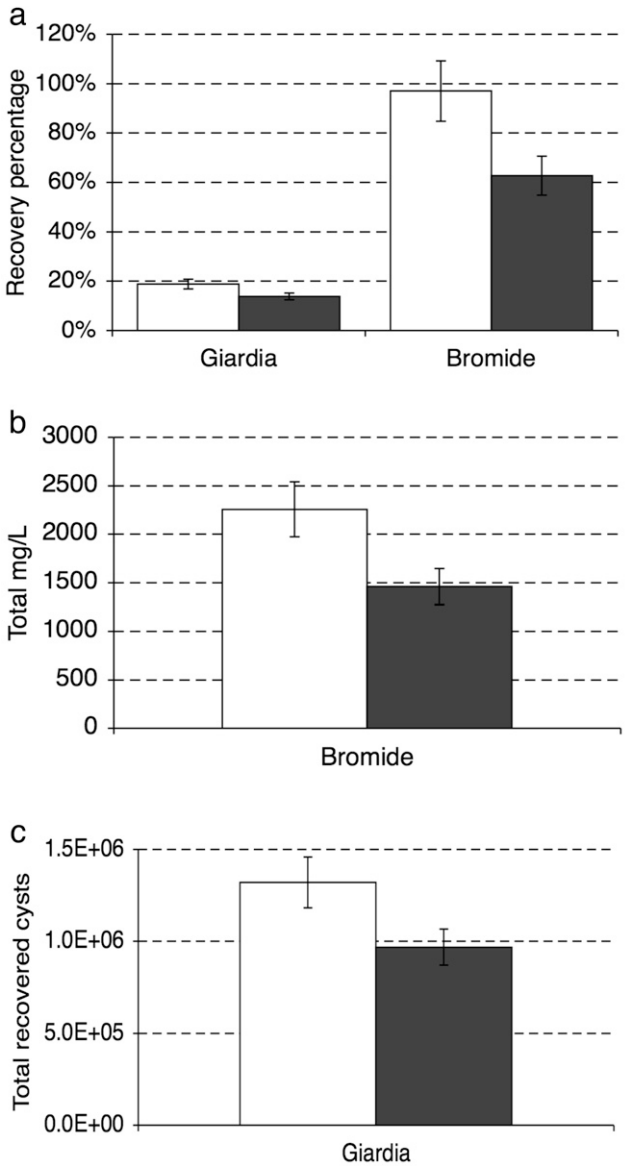

Fig. 2. (a) Mean total percentage recoveries for bromide and *Giardia* (± SE); total recovery values for (b) bromide (mg L^{-1}; ± SE) and (c) *Giardia* cysts (exponential scale; ± SE) collected from the bare treatment (white bars) and planted treatment (black bars).

Roodsari et al., 2005; Trask et al., 2004). Currently no such technique is available for *Giardia* despite recent studies reporting up to 100% prevalence in some age groups of cattle (Trout et al., 2007). Our successful development of a tracking technique employing an unmanipulated natural landscape that is not under the influence of infiltration is a significant advance in understanding contamination by pathogens, in particular *Giardia*,

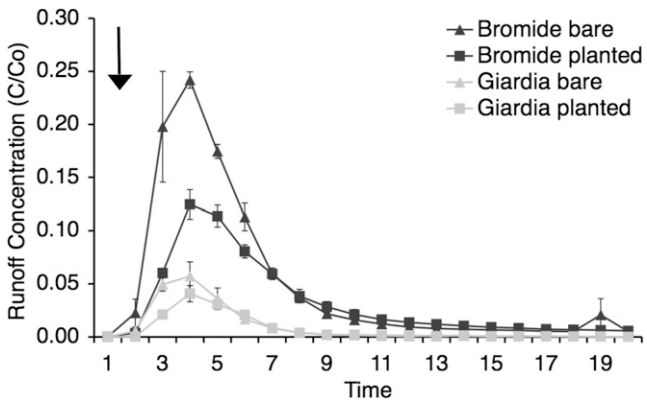

Fig. 3. Mean breakthrough curves (± SE) for bromide and *Giardia* in runoff collected from the two treatments over the experiment's 20-min duration (1 min before spike application and 19 min following). Arrow indicates timing of spike addition.

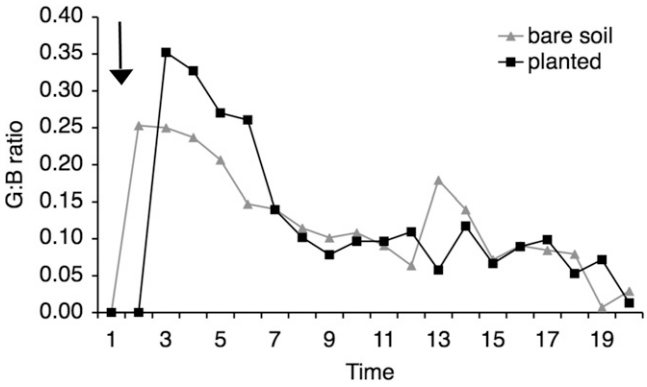

Fig. 4. Relative *Giardia* to bromide (G/B) ratios calculated from the breakthrough curves of the two treatments over the experiment's 20-min duration (1 min before spike application and 19 min following). Arrow indicates timing of spike addition.

from passive farm-based sources. Further, our results demonstrate the discriminatory power of the tracking technique for assessing different riparian planting regimes.

Technique Development

Several factors were key to the successful development of a tracking technique for *Giardia*. First, given the scale of dilution occurring during the 20-min experiment, the concentration of *Giardia* cysts in the spike had to ensure sufficient quantities were not only detectable in the collected runoff, but also in adequate concentrations so as to enable comparison between different treatment conditions. Employing spike concentrations of 10^6 cysts/mL, a minimum of 10^3 cysts/2 L (0.8 cysts/mL) or approximately 0.1% of the original spike concentration was

Table 2. Within-subjects results (overall effects) of the repeated-measures ANOVAs comparing bromide and *Giardia* reduction capabilities between bare soil and recently planted *Carex secta* treatments. All *P*-values were corrected with the Greenhouse-Geisser adjustment.

	Bromide				*Giardia*			
	df	F	*P*	Power	df	F	*P*	Power
Time	1.40	61.99	<0.001	1.00	2.19	80.47	<0.001	1.00
Time × plot type	1.40	8.99	0.02	0.76	2.19	4.56	0.02	0.75
Time × subsample (plot type)					8.78	0.18	0.99	0.09
Error (time)	5.60				26.33			

detectable given the proportion of each sample screened. Bovine fecal matter spiked with known cyst concentrations would have been preferable to using a water-based spike; however, compared with other microbes, the release and transport of *Giardia* cysts from bovine feces remains poorly understood. Furthermore, extrapolating the mechanisms responsible for the release of other microbes from fecal spikes to *Giardia* would have likely proved unreliable because microbial characteristics vary considerably (Yeghiazarian et al., 2006). For example, different mechanisms have been reported to govern the movement of *Escherichia coli*, *Cryptosporidium,* and viral phages from bovine feces (Ferguson et al., 2007). Therefore, while our spike reflected the average concentration of *Giardia* to be found in fecal matter of infected cattle (Xiao, 1994), the application of cysts in a water-based spike eliminated uncertainty surrounding the release of cysts from feces. Though somewhat artificial in nature, a technique for tracking *Giardia* in runoff across the landscape was successfully developed by initially employing a water-based spike, enabling comparisons both within and between treatments over time. Future research identifying the key factors relating to the mechanisms of release of *Giardia* from fecal matter would further expand the value of this new tracking technique.

Microbial pathogens act as biocolloids, with colloid-colloid interactions governed by mechanisms that include Lifshitz–van der Waals, electrostatic, and acid-base interactions (Dai et al., 2004; Ferguson et al., 2003). The deposition of pathogenic colloids in soil involves attachment, mechanical filtration (retention of colloids at the soil surface) and straining (entrapment of colloids within the soil) and depends on environmental parameters such as soil type, pH, and saturation, as well as the colloid's size, surface charge, and degree of hydrophobicity (Bradford et al., 2006; Mawdsley et al., 1995). Investigations of the way organisms move passively across the landscape must ensure the experimental parameters employed have not inadvertently compromised associated colloid-colloid interactions and resulted in unrealistic processes. Inactivating pathogens using heat-based treatment, like that employed in our study, can affect such colloid-colloid interactions by altering surface properties. For example, recent studies of *Cryptosporidium* have reported changes to oocyst effective surface charges, or zeta-potentials, when treated for 1 h at 80°C, while changes to *Bacillus* spore hydrophobicity have been observed following incubation for 1 h at 85°C (Byrd and Walz, 2007; Furukawa et al., 2005). Given the impact heat inactivation of pathogens can have on colloid adhesion, caution must be used when extrapolating results to untreated pathogens.

The effects of our heat treatment (20 min at 60°C) on *Giardia* zeta-potential and hydrophobicity are unknown. However, disparities in zeta-potentials at neutral pH between *Giardia* and *Cryptosporidium* (–17 and –38 mV, respectively), together with their contrasting isoelectric points (pH 2.2 and 3.3, respectively), suggest the two organisms have different surface properties and that heat-induced responses may also differ (Hsu and Huang, 2002). For instance, though hydrophobicity and surface charge are evident in the adhesion of both organisms, hydrophobicity displays a greater influence on *Giardia* adhesion, while surface charge is more important for *Cryptosporidium* (Dai et al., 2004). Nevertheless, the influence of any attachment mechanism depends not only on the characteristics of the microbes, but also the environmental context under investigation. Thus, while cyst surface properties may have been altered as a result of heat treatment in the current experiment, opportunities for cyst-soil adhesion were limited due to the minimal duration of cyst-soil contact under the saturation excess overland flow conditions (Muirhead et al., 2006c).

Two key hydrological features indicate that we achieved saturated soil conditions and overland flow in the current experiment: the volume of water collected as runoff was equal to that applied at the upper edge of the plots and, furthermore, almost 100% of the conservative tracer applied in the spike was collected in the runoff. However, the same patterns might be explained by infiltration excess overland flow, otherwise known as Hortonian overland flow, where the rate of water application to the soil surface is greater than the infiltration rate into the soil (Ward and Robinson, 2000). Although Hortonian overland flow cannot entirely be discounted, this possibility is countered by our observation that plot surfaces tended to display ponding before the application of water. Further, it should be noted that the bromide-*Giardia* spikes were not applied to plots until stable applied-to-collected volumes were achieved, indicating saturation excess was reached and the experiment could commence.

Turning now to the breakthrough curves of proportional recovery across time on the bare soil, both bromide and *Giardia* showed similar patterns (Fig. 3). Highlighting the limited influence of infiltration, a tight association of the cysts with the bromide front indicates *Giardia* cysts moved across the landscape unimpeded. This pattern also suggests that movement occurred as single organisms rather than as clumps of cysts. Microscopic analysis confirmed *Giardia* did not clump together, echoing a previous study that found *Giardia* did not attach to natural soil particles and traveled freely in the water (Dai and Boll, 2003). Studies involving *E. coli* have also reported a tendency under saturated soil conditions for organisms to move as single entities, neither attached to soil or to each other (Muirhead et al., 2006c).

Focusing now on total percentage recoveries from our tracking technique on the bare soil treatment, the high recovery rate for bromide (97%) indicated the majority of the generated runoff was collected at the outflow. Conversely, the lower recovery of *Giardia* (19%) indicated the majority of cysts were either retained on the plots and not collected at the outflow, or were present in concentrations below that of the detection limit for individual time samples. However, assuming a maximum of 10^3 cysts had gone undetected for every minute of the experiment's duration (the technique's minimum detection limit), only 2×10^4 of the missing cysts or 0.28% of the total concentration initially applied would have been accounted for, indicating the latter interpretation of this result is highly unlikely. Consequently, despite the discrepancies observed for the bromide and *Giardia* recovery rates, the individual rates were considered sufficient for reliable data collection, as well as the detection of treatment effects over time. Finally, the summation of cyst totals from the individual time points proved sufficient in establishing the

total concentration recovered from a plot, without the need to combine the 20 samples before enumerating the total concentration. Therefore, additional enumeration of the combined sample for each treatment to determine the overall recovery percentage should not be necessary in future research.

Practical Application

A practical application of our new technique was to compare the *Giardia* retention capabilities of two treatments along a waterway edge recently prepared for riparian planting, thus simulating real-life situations on dairy farms. We performed the experiment during the spring calving season, a time typically characterized by saturated soils, runoff as a result of saturation excess, and high numbers of newborn calves excreting *Giardia* cysts onto the landscape. Our planted treatment involved a species of New Zealand sedge often chosen when establishing riparian barriers along waterway edges (Anonymous, 2007a, 2007b). Our aim, in comparing bare soil and the recently planted sedges, was to assess the potential immediate benefits of riparian planting before significant plant development, under conditions representing a worst case scenario, and in the absence of any confounding influence of weed invasion over time (Muirhead et al., 2006c).

Significant differences in recovery rate were observed in the two treatments for both bromide and *Giardia*; 35% less bromide and 26% fewer *Giardia* cysts were released from the planted treatment compared to the bare soil treatment at this early stage in vegetation barrier development. Although the area covered by the eight plants at ground level was relatively small, the presence of sparsely distributed vegetation has previously been reported to promote surface roughness, decrease water velocity, and though not a contributing factor in the current experiment, increase infiltration (Hsieh and Bolton, 2007). Our results demonstrated an immediate, if small, benefit of riparian planting in reducing pathogens reaching the waterway edge, even under saturated soil conditions. What is more, it is conceivable that a 26% reduction in *Giardia* cysts reaching waterways may equate to a reduction in disease risk, though this remains to be quantified.

Several patterns observed in the current experiment indicated the presence of vegetation altered hydrological processes at the soil surface. Focusing on the details of the breakthrough curves and *Giardia* to bromide (G/B) ratios, at all but one of the 20-min time points both bromide and *Giardia* recovery percentages were lower from the planted treatment than the bare soil (Fig. 3). Brief delays were observed for both bromide and *Giardia* in the planted treatment, though both peaked 3 min after addition of the spike, matching the peak timings observed in the bare soil treatment. Similarly, the calculated G/B ratios also highlighted differences between the two treatments, with peak timings offset by a minute (Fig. 4). Taken together, the breakthrough curves and G/B ratios strongly suggest that the presence of vegetation affected hydrological processes, even though saturated soil conditions limited the influence of infiltration. Furthermore, the results also indicate fine powers of discrimination of pathogen response using the new technique.

Turning now to the G/B ratio plots from the two treatments, overall they were remarkably similar across time, differing only in the timing and maxima of the peaks. Focusing on the specific movement of *Giardia* in relation to bromide across the soil surface, if identical transport mechanisms had been involved in their movement, then the resulting G/B ratios would have equaled one (Muirhead et al., 2006a). Our findings of G/B ratios of <1 for both the bare and planted treatment indicate differences between *Giardia* and bromide movement; for instance, while most of the applied bromide was recovered from each treatment, unknown factors prevented similar recovery rates for *Giardia*. Examining the changing G/B ratios over time (an initial peak followed by a gradual decline), the data indicated *Giardia* was retained more than bromide on both the bare and planted treatments, echoing findings from previous studies involving saturated soils (Muirhead et al., 2006a). As such, our G/B ratios plotted against time suggest bromide diffused in and out of the soil matrix, while any *Giardia* that had entered the matrix were probably trapped and unlikely to be released during any subsequent runoff events, accounting for G/B ratios of <1.

In conclusion, our field-based technique for tracking *Giardia* across the landscape allows comparison of both individual time points and total recovery percentages under saturated soil conditions. Comparative studies can now be undertaken to evaluate the reduction capabilities of different vegetation types. After evaluating planting strategies for success in reducing passive *Giardia* contamination, we expect to make recommendations that expand and complement existing initiatives of regional authorities.

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