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Dispersion and Transport of *Cryptosporidium* Oocysts from Fecal Pats under Simulated Rainfall Events

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The dispersion and initial transport of *Cryptosporidium* oocysts from fecal pats were investigated during artificial rainfall events on intact soil blocks (1,500 by 900 by 300 mm). Rainfall events of 55 mm h^{-1} for 30 min and 25 mm h^{-1} for 180 min were applied to soil plots with artificial fecal pats seeded with approximately 10^7 oocysts. The soil plots were divided in two, with one side devoid of vegetation and the other left with natural vegetation cover. Each combination of event intensity and duration, vegetation status, and degree of slope (5° and 10°) was evaluated twice. Generally, a fivefold increase ($P < 0.05$) in runoff volume was generated on bare soil compared to vegetated soil, and significantly more infiltration, although highly variable, occurred through the vegetated soil blocks ($P < 0.05$). Runoff volume, event conditions (intensity and duration), vegetation status, degree of slope, and their interactions significantly affected the load of oocysts in the runoff. Surface runoff transported from $10^{0.2}$ oocysts from vegetated loam soil (25-mm h^{-1}, 180-min event on 10° slope) to up to $10^{4.5}$ oocysts from unvegetated soil (55-mm h^{-1}, 30-min event on 10° slope) over a 1-m distance. Surface soil samples downhill of the fecal pat contained significantly higher concentrations of oocysts on devegetated blocks than on vegetated blocks. Based on these results, there is a need to account for surface soil vegetation coverage as well as slope and rainfall runoff in future assessments of *Cryptosporidium* transport and when managing pathogen loads from stock grazing near streams within drinking water watersheds.

Numerous studies have reported the presence of the parasitic protozoa *Cryptosporidium* and/or *Giardia* in raw surface waters (28, 33, 44). Sources of protozoan contamination of surface waters include sewage effluent overflows (19), wastewater discharges (42), abattoir waste (50), direct animal fecal deposition in waterways (22), indirect deposition via runoff from land grazed by livestock (24) and/or wildlife (10, 28), manure and effluent spreading (21), and storm water runoff (51).

Cryptosporidium oocysts present in animal fecal deposits on land have been qualitatively and causally linked to event-related increases in pathogen concentrations in streams and reservoirs (3, 4, 29). Pathogens from surface waters have been causally linked to waterborne disease outbreaks, including recent examples from developed-world metropolitan cities (27). There have been few attempts to quantify the relationship between pathogen dispersion and transport, rainfall, and surface water pathogen loads. Recent reviews (18, 20) identified that artificial buffer strips can be highly effective at retarding oocyst transport (7), with vegetated setbacks as little as 1 m being considered sufficient in one watershed. Models to estimate sediment and nutrient export from land and design criteria to predict the retardation of these pollutants by buffer strips (41) are available. However, studies undertaken to date do not provide an equivalent quantitative understanding for predicting pathogen export and for designing buffer strips to minimize pathogen transport.

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Infected calves are typically reported to shed between 10^5 and 10^7 oocysts (g of feces)$^{-1}$ (5, 39). Asymptomatic (adult) animals may also shed low concentrations of oocysts [up to 10^4 (g of feces)$^{-1}$] (6, 17), which has prompted investigations into the significance of the contribution of these animal inputs to watershed oocyst loads (40). However, there are difficulties associated with estimating the prevalence of pathogen infections in animals. These problems include whether to sample animals or their scats (25), how to calculate pathogen concentrations and prevalence from fecal pat data (12), and the detection limits of the methods used for enumeration (14, 40).

Most studies on the mobilization and transport of microbes from animal feces have focused on fecal coliform release from feces and/or manure (from poultry, cattle, and swine) under simulated rainfall conditions (11, 30, 32, 38, 47). Only more recently have studies considered the transport of nonbacterial microbes, such as the parasitic protozoan *Cryptosporidium parvum* (36, 46). Two studies have investigated the use of vegetated buffer strips to retard the surface transport of liquid suspensions of *Cryptosporidium* oocysts (7; J. R. Trask, P. K. Kalita, M. S. Kuhlenschmidt, R. D. Smith, and T. L. Funk, Abstr. 2001 ASAE Annu. Int. Meet., abstr. 01-2104, 2001). However, there are limited studies examining the factors that control the release of *Cryptosporidium* oocysts from the fecal matrix (8).

In general, fecal pathogen surface transport has been examined in test plots that are repacked with loose sieved soil and then vegetated from seed (7; Trask et al., Abstr. 2001 ASAE Annu. Int. Meet.) rather than the logistically more difficult acquisition of intact soil blocks with natural vegetation (36). However, since it has been reported that the vertical transport

of applied water is faster through intact soils than through disturbed soils (45), the use of intact soil blocks may provide a more realistic simulation of oocyst transport overland and by infiltration under natural conditions.

The main advantage of using simulated rainfall instead of natural rainfall events results from the elimination of the erratic and unpredictable variability of natural rainfall by providing controlled (in intensity and duration) rainfall in a reproducible manner, as used in a variety of agricultural studies (26). In addition, the design of the rainfall simulator used for the present study (P. Phillips, P. Hackney, S. Shrestha, and A. Panikkar, Abstr. 7th Int. Conf. Geosynthetics, p. 1241, 2002) enabled the block slope to be varied and allowed infiltrate samples, as well as surface runoff, to be collected.

The aim of this study was to quantify the dispersion and initial transport of *Cryptosporidium* oocysts from fecal pats as a function of watershed characteristics such as slope, presence of vegetation, and rain event intensity and duration. The quantitative data derived for *Cryptosporidium* oocyst dispersion and transport from fecal pats should facilitate the construction of models to predict source water quality and to better manage the factors that govern pathogen transport within watersheds.

MATERIALS AND METHODS

Rainfall simulator. The rainfall simulator used, based on the design of Loch et al. (34), was designed and built by Paul Hackney and coworkers (Phillips et al., Abstr. 7th Int. Conf. Geosynthetics) (University of Western Sydney, Penrith, Australia) and consisted of a frame that was 4.3 m long, 2.65 m wide, and 2.9 m high. From the tips of the nozzles to the surfaces of the soil blocks was approximately 2 m. Two downward-pointing nozzles were used, with each fitted with a pressure gauge, allowing water pressure and therefore rainfall intensity (25 and 55 mm h^{-1} for 180 and 30 min, respectively) to be controlled for each nozzle. A 12-V motor controlled the sweeping of the nozzles backward and forward over the soil blocks. The two slopes (either 5° or 10°) were achieved by tilting the whole frame by use of a jack. Gutters carefully positioned on each side of the frame allowed recycling of the rainwater that did not fall on the soil block. The whole unit was operated within a building to eliminate the effects of wind and other environmental factors.

Artificial rainwater. Deionized water was collected as needed in a 1,000-liter container and a series of 200-liter containers. The following inorganic salts were added per 1,000 liters of deionized water to prepare artificial rainwater (31): $NaNO_3$, 4.07 g; NaCl, 3.24 g; KCl, 0.35 g; $CaCl_2 \cdot 2H_2O$, 1.65 g; $MgSO_4 \cdot 7H_2O$, 2.98 g; and $(NH4)_2SO_4$, 3.41 g. The resulting solution had an ionic strength of 0.3 mM and a pH of 5.2. The composition of the artificial rainwater used was typical of many reported in the literature for studies carried out in both the northern and southern hemispheres.

Site location and characterization of soil. The site chosen for the harvesting of intact soil blocks was located approximately 200 km southwest of Sydney, New South Wales (NSW), Australia, at Arthursleigh Farm, Marulan (GPS readings, 6172730N and 229880E). The farm, owned by The University of Sydney, is located in the Sydney drinking water supply watershed. Livestock had not grazed the location for at least 6 months prior to harvesting of the first soil blocks. It may, however, have been accessible to native marsupial wildlife such as wombats (*Vombatus ursinus hirsutus*) and Eastern Grey kangaroos (*Macropus giganteus*).

The infiltration properties of the surface soil were measured in situ at five positions in an area of approximately 20 m^2 by using a tension disk permeameter as described by Geering (23). Two bulk density cores were collected from undisturbed soil at each of the five areas where infiltration was measured.

Collection of intact soil blocks. Intact soil blocks were harvested from a fenced-off area of approximately 50 by 80 m at Arthursleigh Farm. For each soil block, a level, flat area of ground of 900 by 1,500 mm was marked out with metal pegs and tape. A backhoe was used to excavate the surrounding soil, approximately 300 mm out from the marked area and to a depth of approximately 1 m. The soil was then trimmed manually to the marked size with spades and power tools. Plywood sheets (300 mm wide by 1,300 mm long and 300 mm wide by 900 mm long) lined with pieces of foam sponge to provide support and cushioning were secured around the cut block of soil by use of straps. Metal rods were driven through the soil block below the plywood to facilitate breaking of the block from the base. A sling was then used to lift and break the soil block from its base and to lift it into the support tray for transport by truck back to the laboratory. If necessary, the bases of the blocks were leveled off to ensure that they sat horizontally in the support box. The block depth was typically 300 to 350 mm.

Preparation of soil blocks. Upon arrival at the laboratory, the soil blocks were stored outside. Tap water was applied daily to each block 5 days a week in order to moisten the soil, which had been collected from a region that was experiencing severe drought. This was carried out for at least 3 weeks before the block was used in an experiment and also enabled the vegetation to regrow. An estimation of the percentage of vegetation cover was made for each block immediately prior to the experiment. After the experiments were completed, specimens of the most common plant types present on the blocks were removed for identification.

Any large gaps between the block and the support tray were filled with expanding polystyrene foam filler (Bostik Findley Australia Pty. Ltd., Thomastown, Victoria, Australia). Three to 4 days prior to the experiment, the vegetation was removed from the surface of half of the block (split lengthwise), while minimizing the removal of topsoil, by use of a spade. The side of the block to be devegetated was randomized to ensure that bare or vegetated conditions did not always occur on the same side of the block. A metal divider panel was placed into the soil block to a depth of about 50 mm where the block was split into bare and vegetated areas to prevent runoff crossing over between bare and vegetated strips. Any loose bare soil was compacted by repeatedly applying pressure to the surface of the bare side of the block with a brick. Immediately prior to the experiment, the vegetation was cut to a height of approximately 2 cm to simulate grazing by livestock, and any vegetation that had started to grow back on the bare side was completely removed by cutting, taking care not to introduce macropores into the surface of the soil. Side panels were placed into the soil (lengthwise) to a depth of about 20 mm to prevent loss of runoff over the block sides. Modeling clay strips and silicon sealant (Selleys Pty. Ltd., Padstow, NSW, Australia) were used to seal any small gaps between the central divider and side panels and the soil. Plastic cover sheets were taped to the side and central panels to prevent rainfall falling directly into the infiltrate and runoff collection channels.

Cleaning and disinfection procedures. Between each experiment, the block dividers and sample collection components were dismantled, and soil particles were removed by scrubbing followed by disinfection with sodium hypochlorite solution (1 in 10 dilution of 12% [wt/vol] solution). Sodium thiosulfate solution (30% [wt/vol]) was used to neutralize the residual chlorine, and all components were rinsed in tap water and air dried.

Preparation of artificial fecal pats. Fresh adult cow feces (<2 h old) were collected from Corstorphine Farm, Camden, NSW, Australia. The feces were gamma irradiated in sealed 20-liter plastic containers by a ^{60}Co source at a dose of approximately 120 kGy and then were stored at 4°C. The purpose of this was to stabilize the feces in terms of biotic activity, thereby enabling storage of the feces for the duration of the experiments, a period of 4 months, with minimal changes to the matrix composition. The containers of feces were only opened in a laminar-flow cabinet, and a large sterile stainless steel spoon was used to mix the contents prior to the preparation of fecal pats. The *Cryptosporidium*-negative status of the mixed feces in each container was confirmed by processing triplicate 0.5-g portions of the mixed feces withdrawn from different locations within the container as described below.

Cryptosporidium oocysts used for spiking were purified from defatted fresh calf feces by density gradient flotation in sucrose solution (49). Oocysts from a single purification of one fecal sample were used for all experiments.

For each pat, 1,030 g of irradiated cow feces was weighed into a 2-liter plastic beaker. Approximately 3 g of this was removed and mixed with 20 ml of deionized water to form a slurry. A volume (1 ml) of an oocyst stock suspension containing approximately 10^7 oocysts was added to the slurry. The seeded slurry was mixed thoroughly with a sterile tongue depressor and added back to the bulk of the weighed fecal material. Thorough mixing with a plastic spatula was carried out for 10 min. A springform tin (19 cm in diameter) was assembled with a piece of polyethylene (a stomacher bag cut open to form a single sheet) lining the base of the tin and a mesh disk (19 cm in diameter) placed on the bottom of the tin on top of the polyethylene. One thousand grams of mixed feces spiked with oocysts was weighed into the tin and the top was smoothed flat with a spatula. The tin was placed onto a flat tray and the spring was released so that the sides of the tin could be removed. The base of the tin was removed by sliding it out from underneath, leaving the pat sitting on the mesh disk, plastic sheet, and tray. The pat was "crusted" by drying in an incubator at 20°C for 24 h. The remaining fresh feces were used for moisture determination. For each pat, triplicate portions of 5 to 10 g of feces were immediately dried in preweighed crucibles at 105°C for 48 h.

For determination of whether the oocysts were uniformly distributed within

TABLE 1. Physicochemical characteristics of cow feces

Fecal sample	Ammonia nitrogen (mg kg^{-1})	Total nitrogen (mg kg^{-1})	Conductivity (mS cm^{-1})	pH	Total solids (%)	Total organic carbon (%)
Fresh 1[a]	261	17,700	1.80	8.38	16.35	38.18
Fresh 2[a]	40	17,200	ND[c]	8.5	10.92	41.18
Fresh 3[a]	27	17,500	1.55	8.37	10.92	38.64
Stored unirradiated feces (6 months)	2,050	17,500	1.50	7.83	14.32	41.57
Gamma-irradiated[b] feces (immediately following irradiation)	82	17,900	ND[c]	ND[c]	10.47	38.64
Gamma-irradiated[b] feces (after storage for 6 months)	1,050	19,100	1.93	7.88	16.83	41.61

[a] Collected on different dates.
[b] Gamma irradiated at 120 kGy.
[c] ND, not determined.

the feces by the mixing procedure outlined above, five replicate 0.5-g portions of the mixed, seeded feces (with approximately 10^7 oocysts) from different parts of the springform tin were processed for two of the pats.

After 6 months of storage, the gamma-irradiated cow feces, as well as nonirradiated stored and fresh cow feces, were analyzed for a range of physicochemical parameters by standard methods (1) (Table 1).

Analysis of samples. Where necessary, runoff and infiltrate samples were concentrated by ultrafiltration with Hemoflow hollow fiber cartridges (HF80S; Fresenius Medical Care AG, Bad Homburg, Germany) as described by Simmons et al. (43). All samples were processed by immunomagnetic separation (IMS) (Dynal Biotech, Oslo, Norway) and were stained on membranes (diameter, 3 mm; pore size, 0.8 μm) (Millipore Australia Pty. Ltd., North Ryde, NSW, Australia) with fluorescein isothiocyanate-labeled monoclonal antibody (immunofluorescent antibody staining) (EasyStain; BTF Decisive Microbiology, North Ryde, NSW, Australia), used according to the manufacturer's instructions, and with 4′,6′-diamidino-2-phenylindole (DAPI) (Sigma Aldrich Pty. Ltd., Castle Hill, NSW, Australia). The membranes were mounted on glass slides and scanned at ×250 magnification as described by Davies et al. (14). Runoff samples from the pat experiments generally did not require concentration by ultrafiltration, but portions (10 ml) of these samples were assayed directly by IMS.

Fecal and soil samples were analyzed for *Cryptosporidium* oocysts by a method described previously by Davies et al. (14). Briefly, this involved a desorption step using 0.002 M sodium pyrophosphate and separation of the oocysts from the fecal particles by IMS, followed by immunofluorescent antibody staining and staining with DAPI. An internal control in the form of 100 ColorSeed *C. parvum* oocysts (BTF Decisive Microbiology) was used to determine the percent recovery of *Cryptosporidium* from each sample type, i.e., feces, soil, and runoff or infiltrate, for each experiment.

The filter paper method (16) was used to determine the soil moisture content and soil moisture potential in triplicate samples of surface soil collected from each soil block immediately prior to and after the rainfall event.

Simulated rainfall experiments. The rainfall intensities and durations of the simulated events were generated from event average recurrence interval (ARI) curves provided by Sydney Catchment Authority for a wide geographical spread of locations within the Sydney watershed. In addition, a search of available literature confirmed that the intensities and durations selected were also relevant to North American conditions according to those employed in previous similar rainfall simulation studies (7, 47; Trask et al., Abstr. 2001 ASAE Annu. Int. Meet.). The intensities employed in the simulated rainfall events were 25 and 55 mm h^{-1} for 180 and 30 min, respectively. A 55-mm h^{-1}, 30-min event represented a 1 in 1 year ARI for the majority of locations selected from the Sydney watershed, and a 25-mm h^{-1}, 180-min event represented a range of ARIs depending on location, from 1 in 5 years to 1 in >100 years. Slopes of 5° and 10° were also selected as being typical of the Sydney watershed for areas that may be grazed by livestock.

Duplicate control and pat experiments were run for each combination of conditions of intensity and duration of rainfall and slope of the soil block. Since only one set of conditions could be tested at one time, the pairs of experiments were run randomly. The control events (no pats) were run approximately 24 h before the experiments using pats. The purpose of the control was threefold, as follows: first, to determine if there were significant numbers of oocysts already present in or on the soil blocks; second, to assess the efficacy of the between-experiment cleaning and disinfection procedures for the sample collection channels and divider panels; and third, to moisten the soil.

A crusted artificial fecal pat was placed on each side of the soil block such that the edge of the pat was approximately 13 cm from the top edge of the soil block and, therefore, approximately 1,370 cm from the runoff collection channels at the bottom end of the sloping block. The polyethylene sheet was slid out from under the pat and discarded, but the mesh support was left in place. A rainfall event was then applied to the block, using artificial rainwater. The runoff and infiltrate from each side of the block were collected in 10-liter dairy bladders (clean room manufactured) (EB407; Entapak Pty. Ltd., Dandenong, Victoria, Australia). The infiltrate continued to be collected for 24 h after the event. When runoff was no longer being produced, triplicate soil cores were collected at distances of 10 and 30 cm downhill of each pat, to the right, middle, and left of the particular side of the block. This was achieved by using sterile 10-ml syringes that had been modified by cutting off the tips. The top 5 mm of these cores was analyzed for *Cryptosporidium* oocysts.

Data analysis. Data generated in the fecal pat experiments were analyzed by analysis of variance and analysis of covariance with the SAS generalized linear model procedure (version 6.12; SAS Institute Inc., Cary, N.C.). The Student-Newman-Keuls test was used to test for significant differences between means at the level of an α of 0.05.

When the factors of interest were intensity and duration of rainfall (25 mm h^{-1} for 180 min or 55 mm h^{-1} for 30 min), slope (5° or 10°), and vegetative state (bare or vegetated), the model used was as follows:

$$y = \text{constant} + \text{intensity and duration of rainfall} + \text{slope} + \text{vegetation} + \text{intensity and duration} \times \text{slope} + \text{intensity and duration} \times \text{vegetation} + \text{slope} \times \text{vegetation} + \text{intensity and duration} \times \text{slope} \times \text{veg} + \varepsilon \quad (1)$$

where $\varepsilon \sim N(0, \sigma^2)$, with σ representing the standard deviation and N representing the number of oocysts.

Given that both the runoff and infiltration rates were affected to some degree by factors in equation 1, runoff and infiltrate volumes were used as covariates for analysis of oocyst loads by using similar factors, resulting in equation 2:

$$y = \text{constant} + \text{volume} + \text{intensity and duration of rainfall} + \text{slope} + \text{vegetation} + \text{intensity and duration} \times \text{slope} + \text{intensity and duration} \times \text{vegetation} + \text{slope} \times \text{vegetation} + \text{intensity and duration} \times \text{slope} \times \text{vegetation} + \varepsilon \quad (2)$$

where volume is the covariate (either runoff or infiltrate).

The soil oocyst concentration data included an extra level (replication) that did not occur for the volume and load data. For inclusion of this structure into the analysis, an extra term [rep(intensity and duration of rainfall × slope × vegetation)] was included in the model, where "rep(. . .)" represents a nested factor (equation 3):

$$y = \text{constant} + \text{intensity and duration of rainfall} + \text{slope} + \text{vegetation} + \text{intensity and duration} \times \text{slope} + \text{intensity and duration} \times \text{vegetation} + \text{slope} \times \text{vegetation} + \text{intensity and duration} \times \text{slope} \times \text{veg} + \text{rep(intensity and duration} \times \text{slope}$$

TABLE 2. Mean oocyst loads (unadjusted for treatment factor) in bare and vegetated soil block runoff and infiltrates

Experiment type	Soil block no.	Rainfall intensity (mm h^{-1})	Event duration (min)	Slope (°)	Estimated vegetation cover (%)[b]	No. of oocysts in runoff or infiltrate[a] (vol collected [liters])			
						Bare runoff	Bare infiltrate	Vegetated runoff	Vegetated infiltrate
Control	1	55	30	5	75	0 (1.3)	0 (1.6)	ND[c] (0)	0 (1.5)
	5	55	30	5	65	0 (1.5)	0 (0.05)	0 (0.4)	0 (0.05)
	6	55	30	10	65	0 (0.6)	0 (0.6)	0 (0.4)	0 (1.7)
	4	55	30	10	70	0 (3.4)	0 (0.4)	44 (0.01)	21 (1.3)
	2	25	180	5	80	0 (0.3)	1 (0.4)	0 (0.2)	0 (5.6)
	8	25	180	5	55	0 (4.0)	0 (0.2)	0 (1.8)	0 (9.0)
	7	25	180	10	60	0 (1.8)	0 (0.3)	0 (1.8)	0 (4.6)
	3	25	180	10	70	0 (4.3)	0 (4.6)	0 (0.002)	0 (9.9)
Fecal pat	1	55	30	5	75	3,554 (1.9)	246 (2.6)	ND[c] (0)	0 (2.0)
	5	55	30	5	65	11,617 (1.4)	0 (0.1)	0 (0.3)	18 (0.1)
	6	55	30	10	65	17,292 (0.5)	0 (0.6)	500 (0.4)	1,570 (1.7)
	4	55	30	10	70	64,167 (4.4)	713 (0.8)	104 (0.05)	4,029 (2.8)
	2	25	180	5	80	84,643 (7.9)	31 (0.3)	0 (0.05)	141 (11.4)
	8	25	180	5	55	18,000 (4.5)	290 (1.0)	0 (1.1)	865 (15.4)
	7	25	180	10	60	6,759 (1.4)	10 (0.3)	0 (1.6)	414 (6.2)
	3	25	180	10	70	235,185 (12.7)	297 (8.6)	0 (0.002)	348 (15.5)

[a] Adjusted for recovery.
[b] On vegetated side of block.
[c] ND, not determined, as no runoff was produced.

$$\times \text{ vegetation}) + \varepsilon \quad (3)$$

By using the sampling distance (10 or 30 cm) from the pat as a factor, a fourth model was constructed (equation 4):

$$\begin{aligned} y = {} & \text{constant} + \text{intensity and duration of rainfall} + \text{slope} + \text{vegetation} \\ & + \text{depth} + \text{intensity and duration} \times \text{slope} + \text{intensity and duration} \\ & \times \text{vegetation} + \text{intensity and duration} \times \text{distance} + \text{slope} \\ & \times \text{vegetation} + \text{slope} \times \text{distance} + \text{vegetation} \times \text{distance} \\ & + \text{intensity and duration} \times \text{slope} \times \text{vegetation} \\ & + \text{intensity and duration} \times \text{slope} \times \text{distance} + \text{distance} \times \text{slope} \\ & \times \text{vegetation} + \text{intensity and duration} \times \text{slope} \times \text{vegetation} \\ & \times \text{distance} + \text{rep(intensity and duration} \times \text{slope} \times \text{vegetation} \\ & \times \text{distance)} + \varepsilon \end{aligned} \quad (4)$$

RESULTS

Efficiency of mixing procedure for preparation of fecal pats. The concentrations of oocysts in 0.5-g portions sampled at five different parts of each of two artificial cow fecal pats (uncrusted) were $1.4 \times 10^7 \pm 1.3 \times 10^6$ and $1.1 \times 10^7 \pm 1.6 \times 10^6$. The coefficients of variation were 9.7 and 14.0%, respectively. These concentrations have been adjusted for recovery according to the measured recovery of 40% of a ColorSeed internal control (100 oocysts) added to one of the 0.5-g fecal replicates (14). The results indicate that the procedure used for preparing fecal pats provided evenly distributed oocysts throughout the pat.

Intact soil block experiments. The eight intact soil blocks were predominantly (>70%) vegetated by the introduced grass *Phalaris aquatica* L. The grasses *Avena barbata* Pott. Ex Link. and *Carex inversa* R. Br. were also present, but at much lower densities (<10%). An unidentified daisy species also made up approximately 10% of the vegetative cover. Less abundant species were not collected for identification.

The soil was classified as loam in texture (49% sand, 27% silt, and 24% clay). The mean hydraulic conductivity (K_o) of the surface soil (five replicates) and the mean bulk density (10 cores comprising duplicates from each of five areas) for the location from which the soil blocks were collected were 15.0 ± 2.1 mm h^{-1} and 1.032 ± 0.11 g cm^{-3}, respectively.

Given that the moisture contents of each of the fecal pats before crusting were similar (mean percent moisture, 85.8% ± 0.41%; $n = 16$) and that crusting was carried out consistently at 20°C for 24 h, it was assumed that all pats exposed to the rainfall events were similar in moisture content at the time of the experiments. The measured mean number of oocysts spiked into the fecal pats was $7.2 \times 10^6 \pm 2.7 \times 10^6$ ($n = 16$).

Table 1 shows the physicochemical characteristics of fresh and stored, gamma-irradiated and nonirradiated cow feces. Storage appeared to result in a decrease in pH (>0.4 pH units) but an increase in ammonium-*N*, independent of gamma irradiation. For the parameters examined, gamma irradiation appeared not to result in significant changes in the chemistry of the feces and appeared to retard the production of ammonia, although the latter observation is based on very limited data.

Runoff and infiltrate oocyst loads for control and pat experiments (unadjusted for treatment factor, but adjusted for recovery), as well as volumes of runoff and infiltrate collected, are presented in Table 2. The recoveries of 100 ColorSeed oocysts from runoff samples ranged from 20 to 49%, with a mean of 34% ± 11%, and from infiltrate samples ranged from 20 to 57%, with a mean of 35% ± 13%. The recoveries of oocysts from soil samples ranged from 18 to 55%, with a mean of 39% ± 11%.

No oocysts were detected in the runoff or infiltrate samples collected from the control experiments, with the exception of samples of runoff and infiltrate collected from the vegetated side of soil block 4 (used for a rainfall event of 55 mm h^{-1} for 30 min with a 10° slope) and a single oocyst from the bare side of soil block 2. Given that no oocysts were detected in samples from the bare side of block 4, it is likely that some oocysts were

already present on the vegetated surface soil block when collected from the field. The results indicate that the procedure used to disinfect the sample collection trays and channels between experiments was effective and also that, generally, few detectable oocysts were present on the soil blocks prior to the experiments.

Mean surface soil oocyst concentrations following each rainfall event are given in Table 3. These values are adjusted for recovery but not for treatment factor. Percent moisture and moisture potential determinations are also given in Table 3. The initial and final percentages of moisture for the different soil blocks were similar. Initial moisture potential results were variable but relatively similar for the different soil blocks following the rainfall events.

Factor effects on data from pat experiments. Looking at the differences in runoff volume produced with equation 1 [$y = \log_{10}(\text{runoff volume} + 1)$], there was a significant effect of vegetated state (bare versus vegetated) on the runoff volume produced, with bare areas generating approximately five times more runoff ($\log_{10}$ mean bare runoff = 0.620; $\log_{10}$ mean vegetated runoff = 0.130). None of the other factors appeared to significantly affect runoff volume. In contrast, infiltrate volumes (assessed by using equation 1) were significantly affected by rainfall intensity and duration as well as by the vegetation status of the soil blocks. An interaction between the intensity and duration of rainfall and the vegetated state was also a possibility, although it was not significant at an α value of 0.05. A significantly larger volume of infiltrate (approximately double) was generated by the 25-mm h^{-1}, 180-min event ($\log_{10}$ mean infiltrate = 0.737) than during the event with a rate of 55 mm h^{-1} for 30 min ($\log_{10}$ mean infiltrate = 0.323). The volume of infiltrate appeared to be significantly reduced on bare soil ($\log_{10}$ mean infiltrate = 0.321) compared with vegetated soil ($\log_{10}$ mean infiltrate = 0.739). Plots of the residuals and fitted values appeared to be reasonable in terms of the normality and homogeneity of variances for the relatively small sample sizes involved.

When the runoff volume was used as a covariate in equation 2 [$y = \log_{10}(\text{runoff load} + 1)$], the runoff volume, intensity and duration of rainfall, vegetation status, and slope were all shown to have significant effects on oocyst loads in runoff. Furthermore, the covariate volume was significant, and therefore the means were adjusted for the value of the covariate (runoff volume). This adjustment was achieved by using the least-squares option in the SAS software, and least-squares mean oocyst loads for runoff and infiltrate and oocyst concentrations in soil are provided in Table 4.

The high intensity, short duration event produced significantly larger runoff oocyst loads ($\log_{10}$ mean = 3.055) than the low intensity, long duration event ($\log_{10}$ mean = 2.298), and the 10° slope produced significantly larger runoff oocyst loads ($\log_{10}$ mean = 2.871) than the 5° slope ($\log_{10}$ mean = 2.400). Runoff oocyst loads were significantly larger on bare soil ($\log_{10}$ mean = 4.381) than on vegetated soil ($\log_{10}$ mean = 0.674) (Table 3) (some of the vegetated sides of the blocks generated no runoff [Table 2]). For the low intensity, long duration event, the difference was approximately 15- to 16-fold (on a $\log_{10}$ scale). This difference was similar for the high intensity, short duration event and lower degree of slope (5°). However, when a high intensity, short duration event was applied on a higher

TABLE 3. Mean oocysts concentrations (unadjusted for treatment factor) in surface soils of bare and vegetated soil blocks

Experiment (intensity/duration/slope)[a]	Soil block no.	Mean soil moisture content before event (%)[b]	Mean soil moisture content after event (%)[b]	Mean soil moisture potential before event (kPa)[b]	Mean soil moisture potential after event (kPa)[b]	Mean oocyst concentration in downhill surface soil after rainfall event [no. of oocysts (g of dry weight of soil)$^{-1}$][c]			
						Bare side (10 cm)	Bare side (30 cm)	Vegetated side (10 cm)	Vegetated side (30 cm)
55/30/5A	1	16.9 ± 0.2	25.1 ± 1.2	−13.8 ± 2.1	−3.0 ± 1.8	281 ± 29	45 ± 57	9 ± 10	0 ± 0
55/30/5B	5	10.7 ± 0.9	24.0 ± 0.3	−1,029.7 ± 401	−2.0 ± 0.5	45 ± 27	31 ± 36	29 ± 25	3 ± 1
55/30/10A	6	14.1 ± 0.4	23.4 ± 0.2	−172.6 ± 38.9	−1.9 ± 1.1	110 ± 78	5 ± 2	34 ± 25	5 ± 2
55/30/10B	4	15.8 ± 0.5	23.2 ± 0.4	−30.2 ± 1.5	−2.9 ± 0.5	85 ± 60	23 ± 19	11 ± 7	4 ± 2
25/180/5A	2	19.5 ± 0.3	22.7 ± 0.4	−6.0 ± 1.1	−5.3 ± 1.7	0 ± 0	4 ± 2	0 ± 0	0 ± 0
25/180/5B	8	16.4[d]	21.3 ± 1.1	−101.3[d]	−5.9 ± 0.8	34 ± 30	6 ± 4	34 ± 29	8 ± 7
25/180/10A	7	15.5 ± 0.5	18.4 ± 0.9	−49.7 ± 1.0	−9.9 ± 1.3	43 ± 29	48 ± 54	5,151 ± 8,868	27 ± 35
25/180/10B	3	22.8 ± 0.1	30.0 ± 0.7	−4.6 ± 0.9	−1.3 ± 0.2	74 ± 61	22 ± 13	6 ± 2	14 ± 17

[a] A and B are replicate experiments. Intensity is given in millimeters per hour, duration is given in minutes, and slope is given in degrees.
[b] Data are means of three replicates ± 1 standard deviation.
[c] Adjusted for recovery.
[d] One replicate only.

TABLE 4. Least-squares means for oocyst loads adjusted for runoff and infiltrate volumes and surface soil oocyst concentrations from bare and vegetated soil blocks

Event intensity/ duration (mm h^{-1} min^{-1})	Slope (°)	Vegetation status	Least-squares mean (log_{10} runoff oocyst load + 1)	Least-squares mean (log_{10} infiltrate oocyst load + 1)	Least-squares mean (log_{10} oocyst concentration + 1) 10 cm downhill	Least-squares mean (log_{10} oocyst concentration + 1) 30 cm downhill
25/180	5	Bare	4.087	2.379	0.680	0.763
25/180	5	Vegetated	0.273	1.575	0.687	0.431
25/180	10	Bare	3.978	1.746	1.546	1.386
25/180	10	Vegetated	0.241	1.884	1.003	1.099
55/30	5	Bare	3.932	1.516	1.913	1.287
55/30	5	Vegetated	0.311	0.991	1.120	0.304
55/30	10	Bare	4.536	1.816	1.875	1.002
55/30	10	Vegetated	2.681	3.624	1.217	0.716

degree of slope (10°), runoff oocyst loads on the vegetated side increased considerably so that the difference in runoff loads between bare and vegetated soil was only approximately twofold (on a log_{10} scale). Runoff loads on bare soil were increased slightly for the high intensity, short duration, and large degree of slope combination.

When we used the equation $y = \log_{10}(\text{infiltrate load} + 1)$ (equation 2), the covariate infiltrate volume was not significant, nor were the intensity and duration of rainfall, the slope, the vegetation status (bare versus vegetated), or their interaction terms. This suggests that the variability in infiltrate oocyst loads was high and that differences were small relative to the variation. Given the issue of the low power of tests with small degrees of freedom (i.e., df = 1), it is difficult to be conclusive about potential differences in infiltrate oocyst loads. However, Table 4 shows that the highest adjusted mean infiltrate oocyst load was generated on the vegetated soil at the highest degree of slope. All other loads were reasonably similar. It should be noted that the infiltrate oocyst load was zero for two of the bare plots and for one of the vegetated plots.

Oocyst concentrations in surface soil. As with runoff and infiltrate oocyst loads, the soil oocyst concentrations required a log_{10} transformation [$\log_{10}(\text{concentration} + 1)$] to normalize the pattern of the residuals and to reduce the heterogeneity of the variances. As calculated by use of equation 3, the adjusted least-squares mean soil oocyst concentrations collected 10 and 30 cm downhill of the fecal pats are given in Table 4. The results of the analysis showed that the intensity and duration of rainfall and the vegetation status had significant effects on oocyst concentrations in soil collected 10 cm downhill of the fecal pat. None of the other factors or interactions were significant. The high intensity, short duration rainfall yielded a significantly higher soil oocyst concentration 10 cm downhill of the pat (log_{10} mean = 1.518) than that (log_{10} mean = 0.979) for the low intensity, long duration event. Bare soil at 10 cm had a significantly higher mean oocyst concentration (log_{10} mean = 1.489) than did vegetated soil (log_{10} mean = 1.008).

Similar to oocyst concentrations at 10 cm, a log_{10} transformation [$\log_{10}(\text{concentration} + 1)$] improved the pattern of residuals (in terms of homogeneity of variances) for oocyst concentrations in soil collected 30 cm downhill of the fecal pats, although the residuals were not fully normally distributed. An analysis of the square root transformed oocyst concentrations also improved the pattern of residuals relative to the unadjusted data, although there were still some unusual patterns (not shown). For continuity, only results of the analysis of the log_{10}-transformed soil oocyst concentration data collected 30 cm downhill of the pat are included here.

The effects of slope and vegetation status were significant for oocyst concentrations at 30 cm, whereas the effect of the intensity and duration of rainfall was not significant. The interaction between intensity of rainfall and slope was also significant. With regard to slope, the higher degree of slope (10°) yielded a significantly higher oocyst concentration (log_{10} mean = 1.051) at 30 cm than did the lower slope (5°) (log_{10} mean = 0.696). Bare soil had a significantly higher oocyst concentration (log_{10} mean = 1.110) than did vegetated soil (log_{10} mean = 0.638).

Equation 4 added complexity to the statistical model by incorporating the distance from the pat (10 or 30 cm) as a factor. An assessment of residuals from the analysis using raw oocyst data indicated that variances were heterogeneous and residuals were not normal. Again, analysis of the log_{10}-transformed data [$\log_{10}(\text{concentration} + 1)$] improved the pattern of residuals somewhat, but the distribution of residuals showed some departure from a normal distribution.

As shown with the variable $\log_{10}(\text{concentration} + 1)$ in equation 4, the effects of slope, vegetated state, and distance (downhill from the pat) significantly affected soil oocyst concentrations. The intensity and duration of rainfall were close to being significant (but were above the α level of 0.05). The interaction terms "intensity and duration of rainfall $\times$ slope" and "intensity and duration of rainfall $\times$ distance" were also significant. The significance of the interactions between factors meant that interpretations should really be limited to separate mean distances (10 and 30 cm), as was undertaken above. However, in general, the 10-cm distance had significantly higher oocyst concentrations (log_{10} mean = 1.249) than did the 30-cm distance (log_{10} mean = 0.874). The higher degree of slope resulted in significantly higher soil oocyst concentrations (log_{10} mean = 1.236) than did the lower degree of slope (log_{10} mean = 0.878), and bare soil had significantly higher oocyst concentrations (log_{10} mean = 1.295) than did vegetated soil (log_{10} mean = 0.819).

DISCUSSION

Given the complexities and our current lack of understanding of oocyst interactions with environmental surfaces (13),

empirical studies with well-controlled rainfall events were considered an effective way to increase our understanding of initial oocyst release from fecal pats and their immediate retardation on soil. Based on the estimated pH of the fecal pats (Table 1) and the isoelectric point of purified oocysts (15), it is expected that the oocysts may be negatively charged and thus readily detachable from fecal matter.

In summary, the results show that the runoff oocyst load was significantly affected by the vegetation status, the slope of the soil, and the event characteristics in terms of rainfall intensity and duration. The same factors significantly affected the concentrations of oocysts retarded on the surface soil a short distance (10 or 30 cm) downhill of the pat. Furthermore, slope, vegetation status, and event intensity and duration had an interactive effect on runoff oocyst loads. Based on our observations, sloping land (10° or more) with little or no vegetation cover and a short burst of rainfall of significant intensity would represent a significant risk factor for the dispersion of oocysts from recent animal fecal deposits and their transport into nearby surface waters. Although we found evidence of the retardation of oocysts on the soil surface, the concentrations represented an insignificant proportion compared to those transported in the runoff across 1 m of bare soil. In addition, the oocysts that are retained on the soil surface may be further transported with the next flush of runoff. It has been hypothesized that because of their lower density compared to soil particles of similar size, microorganisms are not deposited on the soil surface unless they are attached to more dense soil particles, but instead remain free floating or attached to less dense fecal matter (48).

Once oocysts are released from fecal pats, heavily grazed or devegetated soils clearly represent a higher risk for surface transport than vegetated ones. Though this would seem obvious, previous work appears not to have reported the quantitative effect of heavily grazed (zero) vegetation on oocyst transport, with the exception of a study by Trask et al. (Abstr. 2001 ASAE Annu. Int. Meet.). Furthermore, given that statistically significant interactions occurred between experimental factors (runoff volume, rainfall intensity and duration, slope, and vegetative cover), it was important to compare adjusted rather than raw means. Infiltration through the soil blocks varied widely, and the analysis of covariance results indicated no significant interactions between the effects of the factors studied. This is not surprising given the differences between soil blocks in terms of moisture content, macropore size and density, and percent vegetation cover. An estimation of the impact of macropores could have been achieved if the experiments had been replicated with repacked soils of the type used by Trask et al. (Abstr. 2001 ASAE Annu. Int. Meet.) and Atwill et al. (7).

The data did indicate a trend of less infiltration through bare soil than through vegetated soil. Such a result could be explained by the increased plant root and invertebrate activity in the vegetated blocks opening up channels and macropores (2, 9). However, for the present study, the bare side of the block (recently devegetated) would also contain macropores from roots and invertebrate activity. It is more likely, therefore, that the higher level of infiltration on the vegetated side was a result of the vegetation impeding the horizontal flow of water, thereby promoting vertical flow of the runoff. This has implications for watershed management practices in that artificial systems for retarding water movement, such as swales of straw, may provide effective temporary solutions to water pollution problems in areas where vegetation recovery will take considerable time or is impractical. A number of authors have reported that vertical flow through soil macropores may result in the rapid transport of microorganisms and other pollutants through soil because it circumvents the adsorptive or retentive properties of the bulk of the soil matrix (45). Atwill et al. (7) repacked soil with different bulk densities to simulate different densities of soil micro-, macro-, and mesopores and hypothesized that as the soil bulk density decreases, the ability of a buffer strip to retain oocysts from runoff increases.

Mawdsley et al. (35) reported that the majority of oocysts (72.8%) remained in the top 2 cm of an intact soil column during infiltration. *Cryptosporidium* oocysts would, at least in part, be expected to be physically strained in soil pores due to their size (4 to 5 μm in diameter) when water passes through soil under gravity (37). However, McGechan also reported that if the soil is close to saturation, oocysts may move without restriction, potentially releasing previously soil-trapped oocysts. Therefore, modeling of the soil sorption and desorption of oocysts should account for mass flow and direction.

Regarding the role of vegetative ground cover, in contrast to our findings with runoff oocyst loading, the bare surface soil at 10 and 30 cm contained significantly higher numbers of oocysts than did vegetated soil. Since significantly smaller volumes of runoff were produced on the vegetated side, these results suggest that the initial dispersion and transport of oocysts from the vicinity of the pat were less efficient than on the bare side due to the presence of vegetation.

Notwithstanding oocyst inactivation within fecal pats (currently under investigation in our laboratory), the present study implies that freshly crusted cow pats containing some 10^7 oocysts transported from $10^{0.2}$ oocysts on vegetated loam soil (25-mm h^{-1}, 180-min event on 10° slope) to $10^{4.5}$ oocysts over a distance of 1 m on unvegetated loam soil (55-mm h^{-1}, 30-min event on 10° slope) (Table 4). The results from the study of Atwill et al. (7) suggested that vegetated buffers constructed with sandy loam or soils with higher bulk densities were less effective at removing oocysts (1- to 2-$\log_{10}$ reduction m^{-1}) than buffers constructed with silty clay, loam, or soils with lower bulk densities (2- to 3-$\log_{10}$ reduction m^{-1}). These workers also suggested that on slopes of >20%, a length of >3 m should function to remove >99.9% of *C. parvum* oocysts from agricultural runoff generated during events involving mild to moderate precipitation (15 to 40 mm h^{-1} for 4 h).

Tate et al. (46) examined the transport of *C. parvum* oocysts over a 1-m distance in overland flows from fecal deposits under natural rainfall and rangeland conditions. Paired plots were located on 10, 20, and 30% slope sites. In addition to the slope being a significant factor in oocyst transport, there was an apparent flushing effect of oocysts across four subsequent storm events, with the majority being transported by the first two storm events (although the effect was not statistically significant). A pilot rainfall simulation experiment also revealed a flushing phenomenon from pats during individual rainfall events. Hence, there is a need to focus in the future on 1- to 20-m travel distances with successive rain events and measurements of soil saturation to investigate the potential travel times

for mobile or remobilized oocysts from land to waterways. Nonetheless, data presented here are sufficient to facilitate the prediction of oocyst export to surface water, of the water quality benefits of improved riparian buffer management, and of the importance of providing design criteria for setback distances to waterways. Above all, it provides evidence of the importance of maintaining riparian buffers, in particular the importance of vegetative cover on those buffers for managing *Cryptosporidium* transport within watersheds. An understanding of the influence of stock densities, management practices, vegetation cover, distance to water, slope, drainage, soil, and climate on pollutant export from land units provides a basis for setting watershed management priorities. The same understanding can be used for predicting the pollution reduction benefits available through rehabilitation and improved management practice programs.

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REFERENCES

1. **American Public Health Association.** 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
2. **Asare, S. N., R. P. Rudra, W. T. Dickinson, and A. Fenster.** 2001. Soil macroporosity distribution and trends in a no-till plot using a volume computer tomography scanner. J. Agric. Eng. Res. **78:**437–447.
3. **Ashbolt, N. J., and D. Roser.** 2003. Interpretation and management implications of event and baseflow pathogen data. *In* P. E. Black (ed.), Proceedings of the 2003 International Congress on Watershed Management: Watershed Management for Water Supply Systems. American Water Resources Association, Middleburg, Va.
4. **Atherholt, T. B., M. W. LeChevallier, W. D. Norton, and J. S. Rosen.** 1998. Effect of rainfall on *Giardia* and *Cryptosporidium.* J. Am. Water Works Assoc. **90:**66–80.
5. **Atwill, E. R.** 1996. Assessing the link between rangeland cattle and waterborne *Cryptosporidium parvum* infection in humans. Rangelands **18:**48–51.
6. **Atwill, E. R., B. Hoar, M. das Gracas Cabral Pereira, K. W. Tate, F. Rulofson, and G. Nader.** 2003. Improved quantitative estimates of low environmental loading and sporadic periparturient shedding of *Cryptosporidium parvum* in adult beef cattle. Appl. Environ. Microbiol. **69:**4604–4610.
7. **Atwill, E. R., L. Hou, B. M. Karle, T. Harter, K. W. Tate, and R. A. Dahlgren.** 2002. Transport of *Cryptosporidium parvum* oocysts through vegetated buffer strips and estimated filtration efficiency. Appl. Environ. Microbiol. **68:**5517–5527.
8. **Bradford, S. A., and J. Schijven.** 2002. Release of *Cryptosporidium* and *Giardia* from dairy calf manure: impact of solution salinity. Environ. Sci. Technol. **36:**3916–3923.
9. **Brouwer, J., and R. W. Fitzpatrick.** 2002. Restricting layers, flow paths, and correlation between duration of soil saturation and soil morphological features along a hillslope with an altered soil water regime in western Victoria. Aust. J. Soil Res. **40:**927–946.
10. **Chalmers, R. M., A. P. Sturdee, S. A. Bull, A. Miller, and S. E. Wright.** 1997. The prevalence of *Cryptosporidium parvum* and *C. muris* in *Mus domesticus*, *Apodemus sylvaticus* and *Clethrionomys glareolus* in an agricultural system. Parasitol. Res. **83:**478–482.
11. **Chaubey, I., D. R. Edwards, T. C. Daniel, P. A. Moore, and D. J. Nichols.** 1994. Effectiveness of vegetative filter strips in retaining surface-applied swine manure constituents. Trans. ASAE **37:**845–850.
12. **Clough, H. E., D. Clancy, P. D. O'Neill, and N. P. French.** 2003. Bayesian methods for estimating pathogen prevalence within groups of animals from faecal-pat sampling. Prev. Vet. Med. **58:**145–169.
13. **Considine, R. F., D. R. Dixon, and C. J. Drummond.** 2002. Oocysts of *Cryptosporidium parvum* and model sand surfaces in aqueous solutions: an atomic force microscope (AFM) study. Water Res. **36:**3421–3428.
14. **Davies, C. M., C. Kaucner, D. A. Deere, and N. J. Ashbolt.** 2003. Recovery and enumeration of *Cryptosporidium parvum* from animal fecal matrices. Appl. Environ. Microbiol. **69:**2842–2847.
15. **Drozd, C., and J. Schwartzbrod.** 1996. Hydrophobic and electrostatic cell surface properties of *Cryptosporidium parvum.* Appl. Environ. Microbiol. **62:**1227–1232.
16. **Fawcett, R. G., and N. Collis-George.** 1967. A filter-paper method for determining the moisture characteristics of soil. Aust. J. Exp. Agric. Anim. Husbandry **7:**162–167.
17. **Fayer, R., J. M. Trout, T. K. Graczyk, and E. J. Lewis.** 2000. Prevalence of *Cryptosporidium*, *Giardia* and *Eimeria* infections in post-weaned and adult cattle on three Maryland farms. Vet. Parasitol. **93:**103–112.
18. **Ferguson, C. M., N. Altavilla, N. J. Ashbolt, and D. A. Deere.** 2003. Prioritizing watershed pathogen research. J. Am. Water Works Assoc. **95:**92–102.
19. **Ferguson, C. M., B. G. Coote, N. J. Ashbolt, and I. M. Stevenson.** 1996. Relationships between indicators, pathogens and water quality in an estuarine system. Water Res. **30:**2045–2054.
20. **Ferguson, C. M., A. M. de Roda Husman, N. Altavilla, D. Deere, and N. Ashbolt.** 2003. Fate and transport of surface water pathogens in watersheds. Crit. Rev. Environ. Sci. Technol. **33:**299–361.
21. **Fleming, R., D. Hocking, H. Fraser, and D. Alves.** 1999. Extent and magnitude of agricultural sources of *Cryptosporidium* in surface water. Final report project #40. Agricultural Adaptation Council, West Guelph, Ontario, Canada.
22. **Gary, H. L., S. R. Johnson, and S. L. Ponce.** 1983. Cattle grazing impact on surface water quality in a Colorado front range stream. J. Soil Water Conserv. **38:**124–128.
23. **Geering, H. R.** 1995. Field soil physical measurements, p. 187–215. *In* P. A. Hazelton and A. J. Koppi (ed.), Applied soil science: a course of lectures. Australian Society of Soil Science Inc., Sydney, Australia.
24. **Graczyk, T. K., B. M. Evans, C. J. Shiff, H. J. Karreman, and J. A. Patz.** 2000. Environmental and geographical factors contributing to watershed contamination with *Cryptosporidium parvum* oocysts. Environ. Res. **82:**263–271.
25. **Hoar, B. R., E. R. Atwill, C. Elmi, W. W. Utterback, and A. J. Edmondson.** 1999. Comparison of fecal samples collected per rectum and off the ground for estimation of environmental contamination attributable to beef cattle. Am. J. Vet. Res. **60:**1352–1356.
26. **Hudson, N. W.** 1993. Rainfall simulators. *In* Field measurement of soil erosion and runoff. Food and Agriculture Organization of the United Nations, Rome, Italy. [Online.] http://www.fao.org/docrep/T0848E/t0848e-11.htm#P1556_157119.
27. **Hunter, P. R., M. Waite, and E. Ronchi.** 2002. Drinking water and infectious disease: establishing the links. IWA Publishing, London, United Kingdom.
28. **Jellison, K. L., H. F. Hemond, and D. B. Schauer.** 2002. Sources and species of *Cryptosporidium* oocysts in the Wachusett Reservoir watershed. Appl. Environ. Microbiol. **68:**569–575.
29. **Kistemann, T., T. Claβen, C. Koch, F. Dangendorf, R. Fischeder, J. Gebel, V. Vacata, and M. Exner.** 2002. Microbial load of drinking water reservoir tributaries during extreme rainfall and runoff. Appl. Environ. Microbiol. **68:**2188–2197.
30. **Kress, M., and G. F. Gifford.** 1984. Fecal coliform release from cattle fecal deposits. Water Resources Bull. **20:**61–66.
31. **Laegdsmand, M., K. G. Villholth, M. Ullum, and K. H. Jensen.** 1999. Processes of colloid mobilization and transport in macroporous soil monoliths. Geoderma **93:**33–59.
32. **Larsen, R. E., J. C. Miner, J. C. Buckhouse, and J. A. Moore.** 1994. Water-quality benefits of having cattle manure deposited away from streams. Bioresource Technol. **48:**113–118.
33. **LeChevallier, M. W., and R. H. Moser.** 1995. Occurrence of *Giardia* and *Cryptosporidium* in raw and finished drinking water. J. Am. Water Works Assoc. **87:**54–68.
34. **Loch, R. J., B. G. Robotham, L. Zeller, N. Masterman, D. N. Orange, B. J. Bridge, G. Sheridan, and J. J. Bourke.** 2001. A multi-purpose rainfall simulator for field infiltration and erosion studies. Aust. J. Soil Res. **39:**599–610.
35. **Mawdsley, J. L., A. E. Brooks, and R. J. Merry.** 1996. Movement of the protozoan pathogen *Cryptosporidium parvum* through three contrasting soil types. Biol. Fert. Soils **21:**30–36.
36. **Mawdsley, J. L., A. E. Brooks, R. J. Merry, and B. F. Pain.** 1996. Use of a novel soil tilting table apparatus to demonstrate the horizontal and vertical movement of the protozoan pathogen *Cryptosporidium parvum* in soil. Biol. Fert. Soils **23:**215–220.
37. **McGechan, M. B.** 2002. Transport of particulate and colloid-sorbed contaminants through soil. Part 2. Trapping processes and soil pore geometry. Biosystems Eng. **83:**387–395.
38. **McMurry, S. W., M. S. Coyne, and E. Perfect.** 1998. Fecal coliform transport through intact soil blocks amended with poultry manure. J. Environ. Qual. **27:**86–92.
39. **Medema, G. J., H. A. M. Ketelaars, W. Hoogenboezem, G. B. J. Rijs, and J. F. Schijven.** 2001. *Cryptosporidium* and *Giardia*: occurrence in sewage,

manure and surface water. Association of River Waterworks (RIWA), Utrecht, The Netherlands.
40. **Power, M. L., S. R. Shanker, N. C. Sangster, and D. A. Veal.** 2003. Evaluation of a combined immunomagnetic separation/flow cytometry technique for epidemiological investigations of *Cryptosporidium* in domestic and Australian native animals. Vet. Parasitol. **112:**21–31.
41. **Prosser, I., and L. Karssies.** 2001. Designing filter strips to trap sediment and attached nutrient. Riparian land management technical guideline, update 1. Land and Water Australia, Canberra, Australia.
42. **Rose, J. B., D. E. Huffman, K. Riley, S. M. Farrah, J. O. Lukasik, and C. L. Hamann.** 2002. Reduction of enteric microorganisms at the upper occoquan sewage authority water reclamation plant. Water Environ. Res. **73:**711–720.
43. **Simmons, O. D., M. D. Sobsey, C. D. Heaney, F. W. Schaefer, and D. S. Francy.** 2001. Concentration and detection of *Cryptosporidium* oocysts in surface water samples by method 1622 using ultrafiltration and capsule filtration. Appl. Environ. Microbiol. **67:**1123–1127.
44. **Smith, H. V., A. M. Grimason, C. Benton, and J. F. W. Parker.** 1991. The occurrence of *Cryptosporidium* spp. oocysts in Scottish waters, and the development of a fluorogenic viability assay for individual *Cryptosporidium* spp. oocysts. Water Sci. Technol. **24:**169–172.
45. **Smith, M. S., G. W. Thomas, R. E. White, and D. Ritonga.** 1985. Transport of *Escherichia coli* through intact and disturbed soil columns. J. Environ. Qual. **14:**87–91.
46. **Tate, K. W., E. R. Atwill, M. R. George, M. K. McDougald, and R. E. Larsen.** 2000. *Cryptosporidium parvum* transport from cattle fecal deposits on California rangelands. J. Range Management **53:**295–299.
47. **Thelin, R., and G. F. Gifford.** 1983. Fecal coliform release patterns from fecal material of cattle. J. Environ. Qual. **12:**57–63.
48. **Tyrrel, S. F., and J. N. Quinton.** 2003. Overland flow transport of pathogens from agricultural land receiving faecal wastes. J. Appl. Microbiol. **94:**87S–93S.
49. **Upton, S. J.** 1997. In vitro cultivation, p. 181–207. *In* R. Fayer (ed.), *Cryptosporidium* and cryptosporidiosis. CRC Press, Washington, D.C.
50. **Xiao, L., A. Singh, J. Limor, T. K. Graczyk, S. Gradus, and A. Lal.** 2001. Molecular characterization of *Cryptosporidium* oocysts in samples of raw surface water and wastewater. Appl. Environ. Microbiol. **67:**1097–1101.
51. **Xiao, L. H., K. Alderisio, J. Limor, M. Royer, and A. A. Lal.** 2000. Identification of species and sources of *Cryptosporidium* oocysts in waters with a small-subunit rRNA-based diagnostic and genotyping tool. Appl. Environ. Microbiol. **66:**5492–5498.