Overland and Near-Surface Transport of *Cryptosporidium parvum* from Vegetated and Nonvegetated Surfaces

Jennifer R. Trask, Prasanta K. Kalita,* Mark S. Kuhlenschmidt, Ronald D. Smith, and Ted L. Funk

ABSTRACT

Understanding microbial pathogen transport patterns in overland flow is important for developing best management practices for limiting microbial transport to water resources. Knowledge about the effectiveness of vegetative filter strips (VFS) to reduce pathogen transport from livestock confinement areas is limited. In this study, overland and near-surface transport of *Cryptosporidium parvum* has been investigated. Effects of land slopes, vegetation, and rainfall intensities on oocyst transport were examined using a tilting soil chamber with two compartments, one with bare ground and the other with brome (*Bromus inermis* Leyss.) vegetation. Three slope conditions (1.5, 3.0, and 4.5%) were used in conjunction with two rainfall intensities (25.4 and 63.5 mm/h) for 44 min using a rainfall simulator. The vegetative surface was very effective in reducing *C. parvum* in surface runoff. For the 25.4 mm/h rainfall, the total percent recovery of oocysts in overland flow from the VFS varied from 0.6 to 1.7%, while those from the bare ground condition varied from 4.4 to 14.5%. For the 63.5 mm/h rainfall, the recovery percentages of oocysts varied from 0.8 to 27.2% from the VFS, and 5.3 to 59% from bare-ground conditions. For all slopes and rainfall intensities, the total (combining both surface and near-surface) recovery of *C. parvum* oocysts was considerably less from the vegetated surface than those from the bare-ground conditions. These results indicate that the VFS can be a best management practice for controlling *C. parvum* in runoff from animal production facilities.

Runoff from animal production facilities has been a concern for decades due to the environmental risk of source water contamination. Runoff from these facilities may contain high amounts of nutrients, solids, and microorganisms that have the potential to degrade surface water (Sutton et al., 1976; Young et al., 1980; Dickey and Vanderholm, 1981; Ongerth and Stibbs, 1987; Dillaha et al., 1989; Hansen and Ongerth, 1991; Bukhari et al., 1997). This issue becomes of great concern when there is a substantial risk for disease transmission by water-borne microorganisms, especially pathogens. *Cryptosporidium parvum* is one such pathogen; this protozoan parasite, which is excreted in the form of an oocyst, may be found in large quantities in the feces of diseased mammalian animals. Livestock, both open range and those in concentrated animal facilities, have been associated with high concentrations of *C. parvum* (Garber et al., 1994; DuPont et al., 1995; Fayer et al., 1997, 2000; Olson et al., 1997; Lefay et al., 2000; Sischo et al., 2000; Wade et al., 2000). Infected animals can pass as many as 10 billion oocysts per gram of feces, and a small number of infected animals can produce enough oocysts to potentially contaminate a large water source (Marshall et al., 1997). There have been reports of an increase in the number of infectious outbreaks of water-borne cryptosporidiosis (the disease associated with *C. parvum*) worldwide (Rose, 1997; Roefer et al., 1996; Goldstein et al., 1996; Gallaher et al., 1989). The largest outbreak of cryptosporidiosis occurred in Milwaukee, WI, in 1993, in which 400 000 individuals were infected with *C. parvum*; it has been suspected that the contamination source was either infected livestock or sewage (MacKenzie et al., 1994).

Information on measures for source control of microbial pathogens from animal production facilities is limited. Few studies have investigated the effectiveness of vegetative filter strips (VFS) in improving the quality of runoff, especially from animal feedlots. Williamson et al. (1999) and Keaton et al. (1998) reported that VFS can reduce 18 to 100% of nutrients and bacteria when runoff from feedlots traveled through VFS at three experimental sites containing brome and fescue grasses. Chaubey et al. (1994) found that fescue VFS were effective in removing most nutrients from incoming runoff containing swine manure. They also found that reduction of most parameters was dependant on filter strip length excluding fecal coliform, which was not affected by length and NO_3–N, which increased with filter strip length. Lim et al. (1998) used fescue filter strips to remove nutrients and fecal coliform from cattle manure; their results indicated a significant removal of fecal coliform and all nutrients except nitrate and ammonium. Young et al. (1980) used orchard grass, sorghum–sudangrass, and oat filter strips to evaluate VFS effectiveness in removing pollutants from feedlot runoff; their results indicated an average 83% removal of total N, total P, and PO_4–P, and a 70% removal of fecal coliform.

Little research has been conducted on the effectiveness of filter strips in removing *C. parvum* from feedlot runoff. Several studies have focused on the role of environmental factors affecting oocyst viability such as temperature, effectiveness of chemical disinfectants, soil type, and sedimentation velocity; however, information on transport mechanism of oocysts within a watershed

J.R. Trask, P.K. Kalita, and T.L. Funk, Department of Agricultural and Biological Engineering, University of Illinois, 1304 West Pennsylvania Avenue, Urbana, IL 61801. M.S. Kuhlenschmidt and R.D. Smith, Department of Veterinary Pathobiology, University of Illinois, 2001 South Lincoln, Urbana, IL 61802. Received 4 Mar. 2002. *Corresponding author (pkalita@age.uiuc.edu).

Published in J. Environ. Qual. 33:984–993 (2004).

677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: VFS, vegetative filter strips.

is limiting (Peeters et al., 1989; Robertson et al., 1992; Fayer, 1994; Drozd and Schwartzbrod, 1996; Medema et al., 1998; Olson et al., 1999; Bukhari et al., 2000). Few studies have investigated vertical transport of *C. parvum* with and without vegetation (Brush et al., 1999; Mawdsley et al., 1996a; Harter et al., 2000). Mawdsley et al. (1996b) measured the vertical and horizontal movement of *C. parvum* using three soil-tilting beds with silty clay loam covered with perennial ryegrass at 7.5% slope under simulated rainfall conditions. Analysis of runoff and leachate for oocyst concentrations indicated that movement of the pathogen lasted for at least 21 d (in one case for 70 d). They suggested the need for further studies using different slopes, application distance, and application methods to enhance understanding of oocyst transport in overland flow.

Some management practices have been suggested to control nonpoint sources of *C. parvum* oocysts. These include health management methods for domesticated animals and practices associated with the sediment and nutrient control related to animal waste management. The effectiveness of using sediment and nutrient control practices (such as VFS) for oocyst reduction has not been pursued at length (Walker et al., 1998). The objective of this study was to develop understanding on the relative transport of *C. parvum* in overland flow from bare and vegetated soil surfaces under a variety of slope and simulated rainfall conditions.

MATERIALS AND METHODS

Tilting Soil Chamber Description

A single horizontal tilting soil chamber was constructed to investigate the overland and near-surface transport of *C. parvum*. Figure 1 shows the tilting soil chamber with two compartments and runoff collection system from each compartment. The soil chamber (3.6 m long, 1.5 m wide, 0.3 m deep) was constructed using 10-gauge (3.4-mm thickness) sheet metal. The chamber was supported by a steel frame that contained a hydraulic cylinder located at one end of the frame for controlling slope conditions. The soil chamber was divided into two separate compartments with a steel plate divider placed at the center of the 1.5-m-wide chamber across its 3.6-m length and sealed. Each compartment had twelve 9.5-mm-diameter holes at the bottom of the bed in the last one-third section (i.e., bottom of the slope); this facilitated collection of near-surface or subsurface flow that would percolate through the bottom of each compartment. A steel tray was placed beneath each compartment to collect the subsurface water samples. The overland sample collection system included a separate collection device from each compartment.

Soil, Vegetation, and Experimental Setup

A moderately well drained silt-loam (Catlin series: fine-silty, mixed, superactive, mesic Oxyaquic Argiudolls) soil was selected for these experiments because this soil type is predominantly found around central Illinois where several concentrated livestock facilities exist. The soil was obtained from an old crop field that had not been farmed for 15 yr. The top 0.3 m of soil (after removing the vegetation from the surface) was collected in two separate layers of 15-cm thickness (0–0.15 and 0.15–0.3 m). Each layer was transported separately to the laboratory. Typically, the top 0.3 m of this soil has average

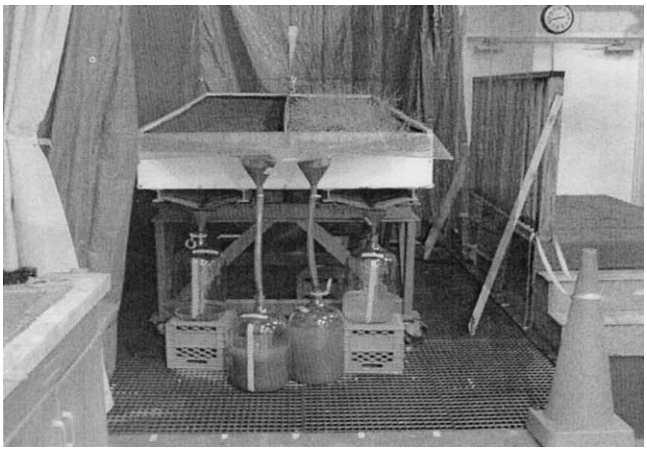

Fig. 1. Laboratory setup of the horizontal tilting soil chamber with surface runoff and near-surface flow collection systems.

clay contents of 18 to 30% and average sand contents of 0 to 8%. Organic matter content is typically between 3 and 4% with bulk densities around 1.4 g/cm^3. As each layer of soil was placed in the chamber, it was compacted using a 25.4- × 25.4-cm steel plate. After compaction, the whole soil bed was saturated and resaturated with water; this allowed natural compaction of the soil in the chamber. The edges of each compartment were compacted tightly to eliminate leakage of surface water along the edges of the bed (as suggested by Mersie and Seybold, 1997).

The vegetation selected for this study was smooth brome. One compartment of the bed was seeded with brome vegetation (at the rate of 16 kg/ha), and the other was left bare. The optimum seeding rate for brome vegetation in central Illinois is about 13.5 to 16.8 kg/ha (Bro-Max brome grass; Growmark, Bloomington, IL). It took almost 90 d after seeding to establish the healthy stand of vegetation (with more than 95% ground cover) during late summer and early fall of 2000. Greenhouse lights were used inside the laboratory to maintain plant growth during the fall and winter. Throughout the experimental period, the soil surfaces were wetted periodically to avoid excessive surface crusting conditions.

In central Illinois, typical VFS slopes are between 1 to 5%. We have used three slope conditions in our experiments: 1.5, 3.0, and 4.5% for this study. These values cover the low, medium, and high slopes for a reliable VFS. To guarantee that the soil chamber was adjusted to the proper slope each time, wooden slope blocks were cut to size for all three slope conditions and placed under both sides of the soil chamber. The soil bed was tilted and then allowed to sit on these blocks during the experiments to ensure uniform slope conditions longitudinally.

Rainfall Simulation

Rainfall was applied to the soil chamber using a microcomputer-controlled laboratory rainfall simulator. The rainfall simulator consisted of two modules, 1.3 m apart, each containing five Spraying Systems (Wheaton, IL) Veejet 80100 nozzles that operate at 41 kPa. The modules are located 10 m from the floor to ensure that most of the drops attain terminal velocity by the time they hit the floor (Hirschi et al., 1990), thus simulating natural rainfall events. The rainfall intensity was controlled by the microcomputer. We decided to use two rainfall intensities to observe the transport characteristics of *C. parvum* under light and heavy rainfall. Before the actual experiments began, a trial rainfall simulation run was con-

ducted to determine the proper intensities and rainfall time duration needed for the experiments. This was also done to select a rainfall duration that would provide sufficient runoff volumes to facilitate sample analysis.

For the preliminary simulation run, we used the low intensity or 25.4 mm/h rainfall. After completing the simulation run, we found that a time of 44 min rainfall duration provided ample volume for sample analysis. Therefore, a duration of 44 min was used for all experimental runs. We have used two rainfall intensities, 25.4 and 63.5 mm/h, with a duration of 44 min for both intensities during this study. These two intensities (with a duration of 44 min) represent 1- and 10-yr storm events, respectively, in east-central Illinois. For both 25.4 and 63.5 mm/h rainfall intensities, three experimental sets were completed; each set consisted of two or three independent rainfall events at one selected slope (1.5, 3.0, or 4.5%). Two rainfall events were conducted on the 1.5% slope while three rainfall events were applied at 3.0 and 4.5%. Before any rainfall experiment started, *C. parvum* was applied on the tilting soil bed as described in the following section.

Application of *Cryptosporidium parvum*

Cryptosporidium parvum was obtained from a neonatal calf by experimental inoculation of 1×10^7 oocysts previously purified by Sheather's sugar flotation and cesium chloride density gradient centrifugation (Fayer et al., 1990, p. 41–42, 44–45). Feces containing oocysts were collected in plastic drop pans maintained over melting ice. To the collection pans we added 100 mL pen/strep/amphotericin (containing 6 g penicillin, 10 g streptomycin, and 25 mg amphotericin per liter of 0.85% sterile saline) and 100 mL nystatin (containing 1.98 g per liter sterile saline) per 1 to 1.5 L of feces. The collection pans were emptied twice daily and oocysts containing antibiotics were stored at 4°C until use.

For application to the tilting soil chamber, oocysts in fecal slurries were washed free of antibiotics and concentrated by centrifugation ($10\,000 \times g$, 30 min) and resuspended in distilled water to a concentration of approximately 3×10^{10} oocysts/250 mL. Just before application on the soil beds, the solution containing the oocysts (250 mL) was mixed thoroughly to ensure a uniform mixture for application. This concentration of oocysts was selected to ensure the detection of oocysts in overland flow and in the range expected from feedlots or dairy runoff. These concentrations also represent similar concentrations that may be shed by a neonatal calf in the environment (Ongerth and Stibbs, 1987; Marshall et al., 1997; Olson et al., 1999; Uga et al., 2000; Nydam et al., 2001). The use of fecal slurries was used rather than purified oocysts because this mimics similar consistencies seen in an acutely shedding cow. Based on our observations, infected cows have this consistency of feces (diarrheal conditions) when infected with *C. parvum*. Therefore, the application suspension was designed to resemble, as closely as possible, the consistency of that being deposited in the field from an infected cow. Initial aliquot samples were taken from each 250-mL solution to analyze for exact concentrations of oocysts applied to both compartments (bare ground and vegetated surface) of the soil chamber. The solution was then applied all at once in a band at the upper end (i.e., the top of the slope) of each compartment to mimic runoff entering the bare ground and vegetated surface (or VFS) at the inlet. Immediately following the application, rainfall was applied to the soil chamber.

For all experimental sets, *C. parvum* was applied only before the first rainfall event for any given slope. The second rainfall was applied 7 to 10 d after the first rain, while the third rainfall was applied 7 to 10 d following the second rain. The purpose of the second and third rain was to check for residual concentrations of oocysts (left behind by the first rain and runoff event in the soil chamber) that may flush out with runoff from both soil compartments. The time between the rains (7–10 d) was selected to ensure similar soil-moisture conditions before each rainfall event. To ensure these conditions, a small soil sample was collected from each compartment before each rainfall event. The soil-moisture contents of the samples were measured gravimetrically. For all the experimental runs, the soil-moisture contents (before each rainfall event) for both soil compartments were within a range of 22 to 30% on mass basis. After a soil sample was collected from the soil bed, the small hole (from where the sample was collected) was refilled with new soil (we collected more soil for this purpose). During the experiments, few small rills were observed on the bare soil surface after the rains at high surface slope (4.5%). For the vegetated surface, a chlorophyll meter (SPAD 502; Minolta, Tokyo, Japan) was used to determine the chlorophyll content of the vegetation before each rainfall event. This provided a measure of vegetation health throughout the experiments and indicated that the vegetation remained healthy (chlorophyll content between 25 and 33). Toward the end of the experiments, vegetation growth started to slow down.

Runoff Sample Collection

Surface runoff and near-surface flow were collected independently for each rainfall event for each experimental set. For the 25.4 mm/h rainfall conditions, surface runoff was collected in 22.7-L (6-gallon) glass bottles from each compartment for the entire flow period. From each bottle, a 1-L sample was collected, transferred to the laboratory, and analyzed for *C. parvum* oocysts (sample analysis method is described in next section). Since the near-surface (subsurface) flow rate was very slow, these samples were collected for a 24-h period from the start of rainfall application and 1-L samples were extracted and then analyzed for oocyst concentrations.

For the 63.5 mm/h rainfall intensity, two sampling methods were used to collect surface runoff. The first sampling method was performed only for the first rain (that followed immediately after *C. parvum* oocysts application to the soil chamber) to obtain kinetics data for oocyst transport rate with time. Samples were collected in glass bottles for every 5 min from the start of surface runoff until the end of the rainfall event. Since runoff rate decreased after rainfall ended, the excess surface runoff (after the rainfall ended) was collected in one bottle until surface runoff ceased. The second sampling method for the 63.5 mm/h rainfall intensity was the same as that for the 25.4 mm/h rainfall experiments. This method was performed for the second and third rainfall events in each experiments set. In this method, the samples were collected until a 22.7-L bottle was filled and replaced with another one. The time to fill a bottle varied from 2 to 20 min during the rainfall event. Near-surface (subsurface) flow was also collected for a 24-h time period.

Oocyst Detection in Overland and Near-Surface Flow

Concentrations of *C. parvum* oocysts were determined in both surface runoff and near-surface flow from each compartment. Aliquots (40 mL) were taken from each well-stirred, 1-L sample, concentrated by centrifugation ($10\,000 \times g$, 30 min), resuspended in 2 mL water, and analyzed for concentrations of *C. parvum*. Oocysts were enumerated using a *C. parvum*–specific antigen capture enzyme immunoassay (EIA) (Premier *Cryptosporidium* EIA; Meridian Diagnostics, Cin-

cinnati, OH), using modifications of the manufacturer's protocol. This commercial kit, according to the manufacturer's data, has 98.6% specificity (95% confidence interval equal to 96.8–99.5%) and a sensitivity of 100% (95% confidence interval equal to 88.4–100%).

Enzyme immunoassay standard curves were determined using purified oocyst suspensions as the reference standards. The oocyst concentration of the standard was determined by direct counting using a hemocytometer and phase contrast microscopy. Known numbers of oocysts were then assayed using the EIA kit to create a reference standard curve. The EIA assay is linear from 1×10^3 to 1×10^5 oocysts (R^2 = 0.99). For each EIA assay, a standard curve was determined to account for potential variability between assays. This standard curve was then used for the collected water samples for the given assay. The standard curve for the second and third rainfall events at the 3.0% slope can be seen in Fig. 2.

Quantification of oocysts in surface and near-surface water samples collected from the soil chamber was performed as follows. Water samples (50- to 500-mL aliquots) of the total volumes collected (1–22.7 L) were centrifuged at 8000 × *g* for 30 min and the pellets resuspended in 2.5 mL of phosphate buffer saline. Aliquots (5–200 μL) of these concentrates are then assayed directly in the EIA assay and the resultant optical density/μL slope values for each sample are compared to the known oocyst/μL slope determined from the standard curve. Based on this comparison, the total concentration of oocysts in the water samples was calculated. Using the initial concentration of applied oocysts to the soil chamber, a value of the total percent recovery of applied oocysts was determined for all water samples.

Statistical Method

Experiments were conducted using three slopes (1.5, 3.0, and 4.5%), two rainfall intensities (25.4 and 63.5 mm/h), one vegetation type (brome), and one soil type (Catlin) during this study. Statistical analyses were completed for oocyst recovery data in surface and near-surface flow for bare-ground and vegetated conditions using SAS (SAS Institute, 2001). All parameters were evaluated using a 10% (p = 0.10) significance level. Due to the large number of variables involved with this study, it was difficult to view all of the possible main effects and interactions between the variables (ground surface, slope, intensity, and time for surface and near-surface flow). In addition, true repetitions were not possible in this study to estimate a mean square error, which is the best estimate of the common variance. For each trial, two or three rainfall events were performed; however, oocysts were only applied during one of those events, and therefore, a true repetition was not performed. An analysis of variance (ANOVA) was performed to evaluate the interaction between variables to determine which interactions were negligible (small sums of squares). Another ANOVA was conducted using a mixed linear model with a general Satterthwaite approximation for the denominator degrees of freedom. This was done to compare the main effects of land conditions (bare-ground or vegetated surface), slope (1.5, 3.0, and 4.5%), rainfall intensity (25.4 and 63.5 mm/h), and time as well as the interactions between these variables that seemed to be of the most importance.

RESULTS

Cryptosporidium parvum oocysts were detected in both surface runoff and near-surface flow for both bare-ground and vegetated conditions for each slope (1.5, 3.0, and 4.5%) under low rainfall intensity (25.4 mm/h) conditions following the initial application of the pathogen. For the high-intensity rainfall (63.5 mm/h), *C. parvum* oocysts were detected in surface runoff from both bare-ground and vegetated conditions. However, *C. parvum* oocysts were only detected from the vegetated conditions in near-surface flow. There was no near-surface flow volume collected from the bare-ground conditions during any of the rainfall events under high-intensity rainfall conditions. The volumes of surface runoff obtained from the bare-ground conditions were consistently higher than those from vegetated conditions

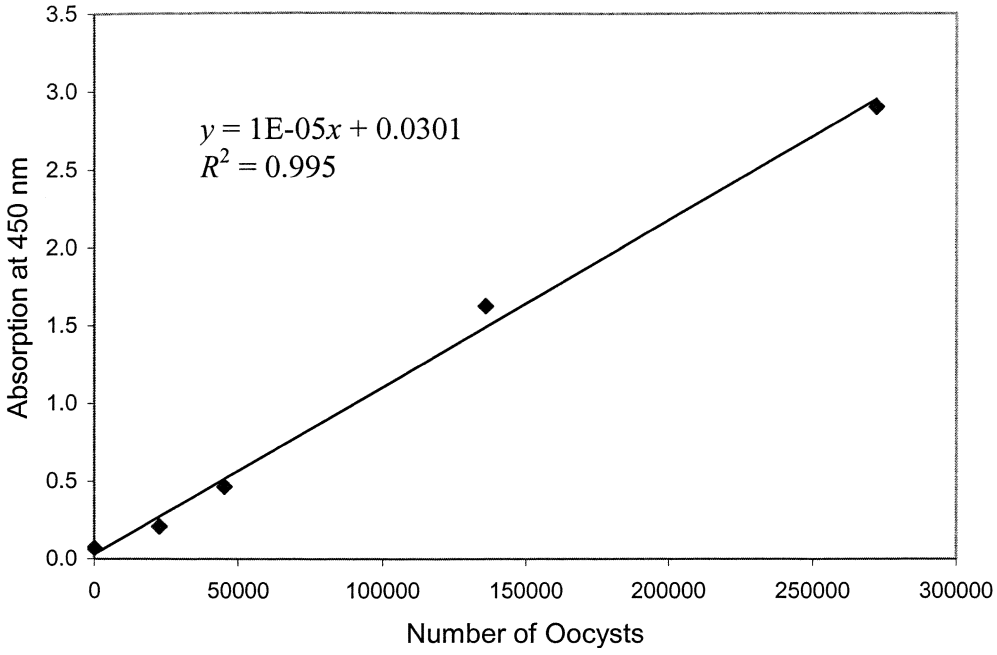

Fig. 2. Standard oocyst curve used for quantification of oocysts in water samples.

Table 1. Average runoff volumes for all slopes and rainfall conditions.

	Volume					
	25.4 mm/h rainfall			63.5 mm/h rainfall		
	Slope (%)					
Surface condition	1.5	3.0	4.5	1.5	3.0	4.5
	L					
Bare-ground surface runoff	28.6	34.1	31.7	111.5	109.5	104.9
Vegetated surface runoff	16.3	23.6	20.8	59.6	64.0	76.7
Bare-ground near-surface flow	3.4	1.4	0.6	0	0	0
Vegetated near-surface flow	9.7	8.3	4.7	8.6	14.1	13.8

(Table 1). This was observed for all slope conditions and for both rainfall intensities. Total oocyst recovery was related to total volume of water collected. For any slope and rainfall intensity, the first rainfall event produced higher recovery of *C. parvum* as compared with those from the second and third rainfall events.

Statistics of Results

Statistical analyses were evaluated using a significance level of p equal to 0.10. The results of the statistical analysis can be seen in Table 2. Statistical results indicate that the rainfall intensity ($p = 0.47$) and slope ($p = 0.21$) did not play a considerable role in reducing the transport of *C. parvum*. However, the main source of variation was due to surface conditions ($p = 0.09$) indicating that oocyst transport in overland flow was considerably less from the vegetated surface conditions. Time ($p = 0.05$) and the interaction between slope and intensity ($p = 0.05$) had also played important roles in oocyst transport. Although true replications were not possible in this study, this general statistical analysis clearly indicates that time and ground surface conditions are significant factors in the overland transport of *C. parvum*.

Cryptosporidium parvum Recovery from the 25.4 mm/h Rainfall and Three Slope Conditions

Table 3 shows all the oocyst recovery data from all of the experimental runs. Total percent recovery of oocysts was based on the initial concentration of oocysts applied to the soil chamber and the total concentration of oocysts calculated in the water samples. For the 25.4 mm/h rainfall conditions, the total percent recoveries of applied oocysts in surface runoff for the 1.5, 3.0, and 4.5% slopes (as seen in Table 3) show that for each slope,

Table 2. Statistical analyses for surface runoff and near-surface flow under bare-ground and vegetated conditions.

Variable	*P* value
Surface condition	0.09
Slope	0.21
Surface condition × slope	0.77
Intensity	0.47
Slope × intensity	0.04
Surface condition × intensity	0.82
Surface condition × slope × intensity	0.36
Time	0.05
Surface condition × time	0.17
Slope × time	0.27
Time × intensity	0.37

Table 3. Total percent recovery of applied oocysts for surface runoff and near-surface flow for bare-ground and vegetated conditions.

		Recovery					
		25.4 mm/h rainfall			63.5 mm/h rainfall		
		Slope (%)					
Surface condition	Rainfall event	1.5	3.0	4.5	1.5	3.0	4.5
		%					
Bare ground	1	13.77	9.53	3.35	2.53	5.32	52.45
	2	0.71	4.61	0.89	2.72	2.90	3.89
	3	–	3.84	0.13	–	1.05	2.67
	total	14.48	17.98	4.37	5.25	9.27	59.01
Bare-ground near-surface	1	0.04	0.02	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.03	0.00	0.00	0.00
	3	–	0.00	0.00	–	0.00	0.00
	total	0.04	0.02	0.03	0.00	0.00	0.00
Bare-ground total		14.52	18.00	4.40	5.25	9.27	59.01
Vegetated surface	1	1.65	0.84	0.34	0.80	0.39	20.34
	2	0.00	0.00	0.25	0.65	0.18	4.61
	3	–	0.00	0.00	–	0.19	2.20
	total	1.65	0.84	0.59	1.45	0.76	27.15
Vegetated near-surface	1	1.25	0.04	0.01	0.08	0.18	1.62
	2	0.00	0.00	0.01	0.11	0.00	2.16
	3	–	0.00	0.00	–	0.00	1.38
	total	1.25	0.04	0.02	0.19	0.18	5.16
Vegetated total		2.90	0.88	0.61	1.64	0.94	32.31

vegetated surface condition had much lower percent recovery rates compared to those from the bare-ground conditions. For this rainfall condition, the second and third rainfall events applied to the chamber resulted in reduced total recovery rates in surface runoff from both bare and vegetated conditions. The overall percent recovery of oocysts in surface runoff from the bare-ground conditions varied for all slopes (Table 3). It was observed that the total percent recovery usually declined as the slope increased from 1.5 to 4.5% for the first rainfall events. For the second rainfall, however, the recovery rate increased when the slope changed from 1.5 to 3.0% and then again decreased for 4.5% slope. For the vegetated conditions, the overall percent recoveries of applied oocysts were considerably lower than those from the bare-ground conditions. The total percent recoveries for the vegetated conditions show that almost no oocysts were recovered from second and third rainfalls for all the three slopes under 25.4 mm/h rainfall.

The total percent recovery rates for near-surface flow (Table 3) indicated that some oocysts were percolating through the 0.3-m soil profile for both the bare-ground and vegetated conditions under 25.4 mm/h rainfall condition. For each slope, the total recovery of oocysts in near-surface flow declined with second or third rainfall applications; it resulted in 0% recovery at the end of the final rainfall application (second rain for 1.5% slope and third rain for both 3.0 and 4.5% slopes). Although near-surface flow had very small recoveries of oocysts compared to that in surface flow, overall percent recovery of oocysts in near-surface flow was generally higher from the vegetated conditions than that in the bare-ground conditions (Table 3).

Cryptosporidium parvum Recovery from the 63.5 mm/h Rainfall and Three Slope Conditions

The results for the high-intensity rainfall (63.5 mm/h) included total percent recovery of applied oocysts from

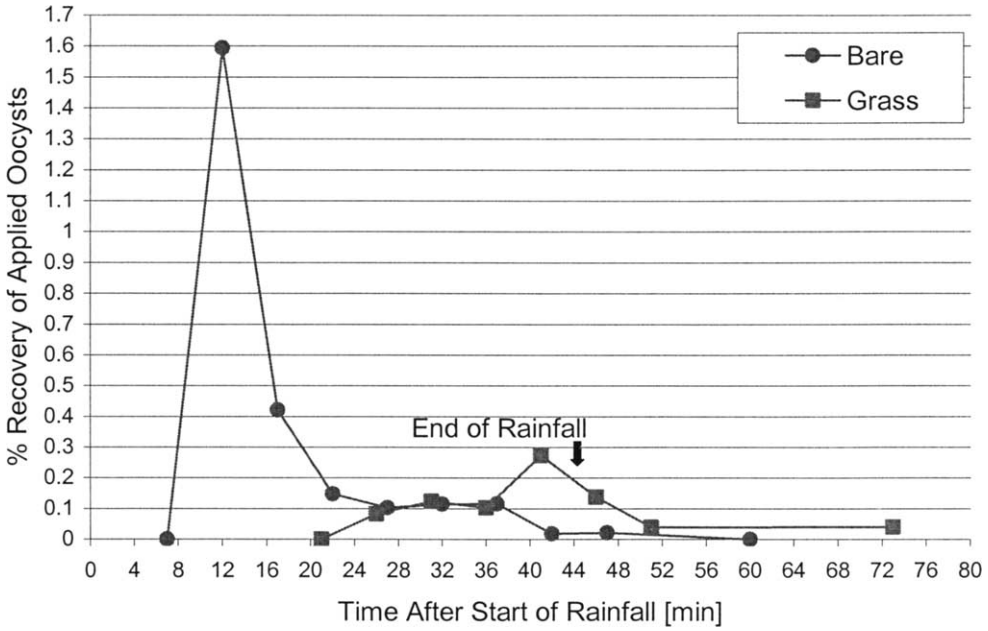

Fig. 3. Kinetics of *Cryptosporidium parvum* recovery in surface runoff from bare-ground and vegetated conditions for 1.5% slope.

surface runoff and near-surface flow (Table 3) as well as kinetics data for *C. parvum* at each slope (Fig. 3, 4, and 5). Table 4 shows times to peak flow rate in oocyst transport during the runoff events. For the second and third rainfall events, samples were not collected in 5-min intervals, but had sampling time durations of 5 to 20 min depending on time to fill the 22.7-L glass bottles. Near-surface flow was collected for 24 h as was done for the 25.4 mm/h rainfall conditions.

Table 3 shows that there was a lower total percent recovery of applied oocysts from the 63.5 mm/h rainfall trials at the 1.5 and 3.0% slopes as compared to the 25.4 mm/h intensity rainfall trials for the corresponding slopes. With the 63.5 mm/h rainfall, there was more sediment loss with runoff that made it more difficult to analyze the water samples for oocyst concentrations. The mircowells for oocyst analysis were heavily packed with sediment, which reduced the detection of *C. parvum*. As a result, fewer oocysts were detected than may have been present in surface runoff for the 63.5 mm/h rainfall trials. The volumes of surface runoff were also much greater (Table 1) than those obtained from the 25.4 mm/h rainfall. Bare-ground conditions showed much higher oocyst recovery than that from the vegetated surface for the 63.5 mm/h rains as was observed for the low-intensity rainfall conditions. The 4.5% slope had the greatest percent recovery of oocysts from both vegetated and bare-ground conditions.

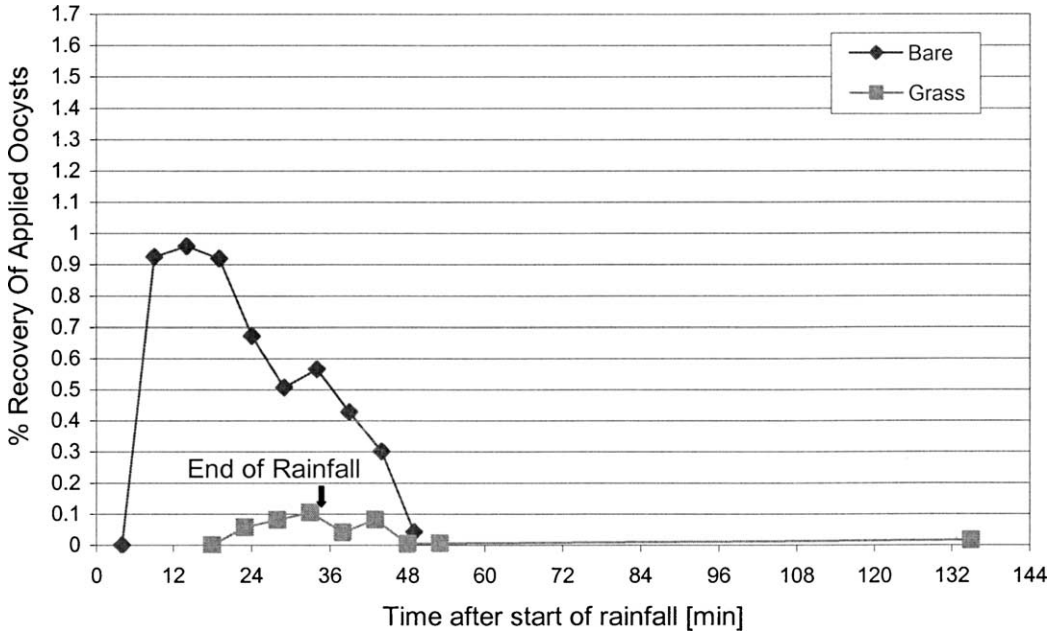

Fig. 4. Kinetics of *Cryptosporidium parvum* recovery in surface runoff from bare-ground and vegetated conditions for 3.0% slope.

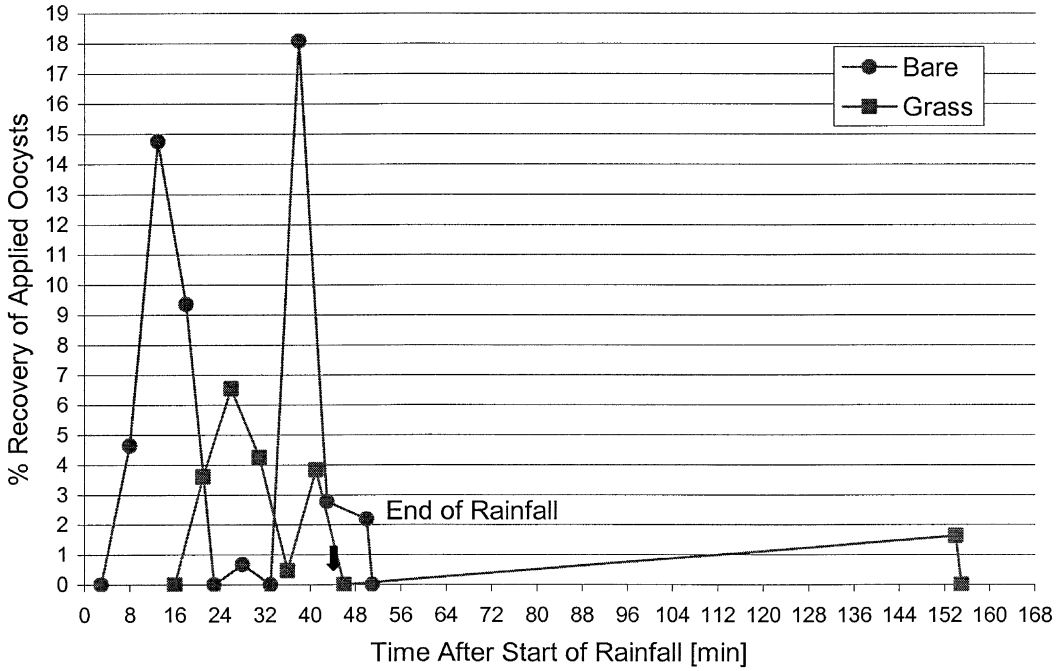

Fig. 5. Kinetics of *Cryptosporidium parvum* recovery in surface runoff from bare-ground and vegetated conditions for 4.5% slope.

There was no near-surface flow collected from the bare-ground conditions under this high rainfall intensity, yielding no oocyst recovery. However, *C. parvum* oocysts were detected in near-surface flow from the vegetated conditions. The total percent recovery of oocysts increased when slope increased from 1.5 to 3.0%, and the highest recovery was at the 4.5% slope. Even though oocysts were recovered in near-surface flow from the vegetated conditions, these concentrations were much smaller than those in surface runoff from the vegetated condition.

The kinetics data of oocyst recovery were collected for each slope only for the first rainfall under the 63.5 mm/h rainfall intensity. Figures 3, 4, and 5 show these data for 1.5, 3.0, and 4.5% slopes, respectively. Table 4 shows that the bare-ground conditions produced similar times to peak for the 1.5, 3.0, and 4.5% slopes; peak times occurred between 8 and 15 min and more than one peak value was observed. The peak times for vegetated conditions were much delayed in comparison to those from bare-ground conditions, indicating the resistance to flow provided by the vegetation. Usually, two peaks occurred during the rainfall event with the first peak between 23 and 33 min and the second one between 36 and 43 min. Only one peak took place at the 1.5% slope, which corresponded to the second peak of the 3.0 and 4.5% slopes. Even though the vegetated conditions delayed the times to peak, the percent recovery during the peak was much smaller than those from the bare-ground conditions.

Table 4. Time to peak for surface runoff at 63.5 mm/h rainfall for bare-ground and vegetated conditions.

	Time to peak		
	Peak number		
Slope	1	2	3
%	min		
	Bare ground		
1.5	12–15	–	–
3.0	9–14	33–36	–
4.5	8–13	23–28	33–38
	Vegetated		
1.5	36–41		
3.0	28–33	38–43	–
4.5	21–26	36–41	–

DISCUSSION

Table 3 shows that in general, there was a greater recovery of oocysts from the first rainfall event as compared with the second and third rainfall for all slopes, rainfall, and surface cover conditions. This was expected because a limited number of oocysts were applied to the soil chamber and the opportunity for oocyst settlement existed as time progressed after the first runoff event. Giddens and Barnett (1980) and Coyne et al. (1998) found similar results with fecal bacteria concentrations following manure-amended soils.

Surface runoff volumes from the bare-ground conditions (Table 1) were greater than those from the vegetated conditions. These findings were expected because reduced runoff volumes from the vegetated surface were associated with more resistance to overland flow and more infiltration opportunity time. Infiltration has been considered one of the primary mechanisms for decreasing surface runoff volume from VFS in other studies (Mersie and Seybold, 1997; Dickey and Vanderholm, 1981; Srivastava et al., 1998).

As seen in Table 3, the total percent recovery of applied oocysts decreased in surface runoff from both bare and vegetated surfaces as bed slope increased at 25.4 mm/h rainfall. A decrease in oocyst concentration may be associated with an increase in surface runoff

volumes (Table 1) that resulted from increasing slopes. Chaubey et al. (1994) found that decrease in concentrations of various parameters could be attributed to filtration mechanisms as well as dilution. Additionally, we observed that the amount of sediment coming with runoff volume increased with increasing slope. This might have reduced concentrations and mass of *C. parvum* oocysts in runoff water, since more oocysts might have adsorbed to sediments. Studies involving various soil types and microorganisms have shown that adsorption, sedimentation, and filtration are mechanisms that influence microorganism transport. However, the dominating mechanism depends on microorganism characteristics (Burge and Enkiri, 1978; Mawdsley et al., 1996a). It has also been shown that the major soil components controlling microbial adsorption are organic matter, silt, clay, and minerals (Bitton and Harvey, 1992; Burge and Enkiri, 1978). The interaction of soil particles with pathogens, in particular *C. parvum*, has not been investigated in this study. This interaction may play a major role in determining the actual *C. parvum* concentrations in surface runoff. For the vegetated surface, however, the difference between oocyst recovery from the 1.5% slope and the 3.0 and 4.5% slopes was not as high as in the bare-ground conditions (Table 3). This may be due to less sediment loss from the vegetated compartment as compared to the bare-ground conditions. In addition, only the first or second rainfall event recovered *C. parvum* oocysts. This may suggest that overall oocyst entrapment is greater at a higher slope on vegetated soils under low rainfall intensity.

Near-surface flow consistently produced lower oocyst recovery than surface runoff for all rainfall and slope conditions. Oocyst adhesion to the steel collection plate was not measured, but it was assumed to retain small numbers of oocysts, if any. For all rainfall events, the near-surface flow from the vegetated chamber contained higher oocyst recovery than the bare-ground conditions (Table 3). Macropores or preferential flow paths in the vegetated soil increased infiltration and might have influenced oocyst migration through the soil profile. Madson and Alexander (1982) and Coyne et al. (1998) found similar results. The percent recovery of applied oocysts in near-surface flow was also greater at the 1.5% slope than the 3.0 and 4.5% slopes, which might be related to increase in infiltration with lower slopes.

With the 63.5 mm/h rainfall, there was considerable sediment loss from the bare-ground surface that made it more difficult to analyze the runoff samples for oocyst concentrations. The mircowells for oocyst analysis were heavily packed with sediment, which reduced the detection of *C. parvum*. As a result, fewer oocysts might have been detected than actually were present in surface runoff. The 4.5% slope produced the greatest total percent recovery of applied oocysts during the initial rainfall event. At this slope with higher rainfall intensity, the time for oocyst settling was perhaps minimum. Biskie et al. (1988) found that with rapid water flows, the possibility of fecal bacteria settlement from surface runoff is less. The high velocity of surface runoff and sediment loss may have prevented the oocysts from adhering to the soil particles and the chance for oocyst settlement. These conditions may account for the maximum transport of *C. parvum* under such circumstances.

The results in Table 3 indicate that although the vegetated surface considerably reduced the oocyst transport in surface runoff compared to bare-ground conditions, the effect of the vegetation in controlling oocyst transport at 4.5% slope is reduced at high rainfall intensity. The infiltration opportunity time and adhesion to soil and vegetation were perhaps greatly reduced at this slope, and erosion and sedimentation increased; therefore, concentrations of oocysts in surface runoff considerably increased at 4.5% slope compared with those at 1.5 and 3% slopes. However, even under the maximum slope and rainfall conditions, oocyst recovery from VFS was considerably lower than that from bare-ground conditions.

The kinetics for oocyst transport under the 63.5 mm/h rainfall were similar at the 1.5 and 3.0% slopes for both the bare-ground and vegetated conditions, while those at the 4.5% slope were different (Fig. 3, 4, and 5). With the higher slopes (3.0 and 4.5%) and high-intensity rainfall, there was a great deal of soil erosion obstructing the surface runoff collection system from the bare-ground conditions. This caused water to build up at the end of the soil chamber. The soil had to be removed manually to provide continuous flow conditions. This design error was not anticipated in the initial chamber design. This minor error probably caused a shift in the total percent recovery of applied oocysts. The peak times may have occurred later than actually took place because surface runoff had a little time to attenuate at the end of the soil chamber. In general, the 1.5 and 3.0% produced similar peak times (Table 4). At 4.5% slope, the second peak may have been due to the build up of water on the soil chamber, and the third peak (which was slightly greater than the first peak) may indicate possible settlement and resuspension of oocysts in surface runoff. For the vegetated surface, only one or two peak times were observed. The delayed peak times clearly show the effects of vegetation on flow resistance and corresponding oocyst transport. The vegetation acted as an effective barrier and filter to overland flow and transport of oocysts. The peaks might be indications of threshold periods for oocyst entrapment and adsorption to vegetation. This may suggest that the oocysts would adsorb or attach to the vegetation until the flow velocity is large enough to dislodge the oocysts back into surface runoff. Coyne et al. (1998) explained that increases in fecal coliform from filter strips may be attributed to subsequent releases of fecal bacteria from sediment by the mechanical activity of rainfall and lateral surface flow with time.

Among all the experimental runs, the greatest percent recovery of *C. parvum* oocysts was 52.5% from the bare ground and 20.3% from the vegetated surface at 4.5% slope under 63.5 mm/h rainfall intensity. Recovery of all applied *C. parvum* oocysts (3×10^{10} oocysts/250 mL applied) was not achieved in entirety from any slope condition or rainfall intensity. Therefore, a portion of the applied oocysts resided in the soil chamber. The

distribution of nonrecovered oocysts within the soil chamber needs to be addressed in future studies to determine the risks associated with using VFS as cattle feed as well as to understand the transport characteristics of *C. parvum* in greater detail.

CONCLUSIONS

Vegetated surfaces, and hence the VFS, are very effective in reducing the overall transport of *C. parvum* in overland flow. Bare-ground conditions continuously produced higher concentrations of oocysts than that from the vegetated conditions for all experimental conditions. The vegetation acted as an effective barrier allowing for possible oocyst entrapment within the vegetation, adsorption to the plant material, and infiltration through the soil profile. Even at high slope and rainfall intensities, vegetation proved to reduce oocyst transport in surface runoff considerably. While the vegetation root system may provide a preferential transport mechanism for oocysts to the near-surface zone, the total combined percent recovery of applied oocysts from surface runoff and near-surface flow for the vegetation was substantially less than that from the bare-ground conditions. For controlling the transport of *C. parvum* in overland flow, VFS at 1.5 and 3.0% slopes can be more effective for both low and high rainfall intensities. Due to the fact that certain environmental conditions cannot be controlled, the major design criterion for VFS to achieve maximum percent reduction in oocyst transport is the slope factor. Vegetative filter strips when combined with other agricultural practices can be a best management practice to control *Cryptosporidium parvum* transport in overland flow.

ACKNOWLEDGMENTS

This study was partially funded by the Illinois Council on Food and Agricultural Research (C-FAR; Project 011-050-2).

REFERENCES

Biskie, H.A., B.M. Sherer, J.A. Moore, J.R. Miner, and J.C. Buckhouse. 1988. Fate of organisms from manure point loading into rangeland stream. Paper 88-2081. *In* ASAE Int. Summer Meeting, Rapid City, SD. 26–29 June 1988. Am. Soc. Agric. Eng., St. Joseph, MI.

Bitton, G., and R.W. Harvey. 1992. Transport of pathogens through soils and aquifers. p. 103–124. *In* R. Mitchell (ed.) Environmental microbiology. Wiley-Liss, New York.

Brush, C.F., W.C. Ghiorse, L.J. Anguish, J. Parlange, and H.G. Grimes. 1999. Transport of *Cryptosporidium parvum* oocysts through saturated columns. J. Environ. Qual. 28:809–815.

Bukhari, Z., M.M. Marshall, D.G. Korich, C.R. Fricker, H.V. Smith, J. Rosen, and J.L. Clancy. 2000. Comparison of *Cryptosporidium parvum* viability and infectivity assays following ozone treatment of oocysts. Appl. Environ. Microbiol. 66:2972–2980.

Bukhari, Z., H.V. Smith, N. Sykes, S.W. Humphreys, C.A. Paton, R.W.A. Girdwood, and C.R. Fricker. 1997. Occurrence of *Cryptosporidium* spp oocysts and *Giardia* spp cysts in sewage influents and effluents from treatment plants in England. Water Sci. Technol. 35(11):385–390.

Burge, W.D., and N.K. Enkiri. 1978. Virus adsorption by five soils. J. Environ. Qual. 7:73–76.

Chaubey, I., D.R. Edwards, T.C. Daniel, P.A. Moore, Jr., and D.J. Nichols. 1994. Effectiveness of vegetative filter strips in retaining surface-applied swine manure constituents. Trans. ASAE 37:845–850.

Coyne, M.S., A.Z. Vilalba, and R.A.Z. Gilfillen. 1998. Fecal bacteria trapping by grass filter strips during simulated rain. J. Soil Water Conserv. 53(2):140–145.

Dickey, E.C., and D.H. Vanderholm. 1981. Vegetative filter treatment of livestock feedlot runoff. J. Environ. Qual. 10:279–284.

Dillaha, T.A., R.B. Reneau, S. Mostaghimi, and D. Lee. 1989. Vegetative filter strips for agricultural nonpoint source pollution control. Trans. ASAE 32:513–519.

Drozd, C., and J. Schwartzbrod. 1996. Hydrophobic and electrostatic cell surface properties of *Cryptosporidium parvum*. Appl. Environ. Microbiol. 62:1227–1232.

DuPont, H.L., C.L. Chappell, C.R. Sterling, P.C. Okhuysen, J.B. Rose, and W. Jakubowski. 1995. The infectivity of Cryptosporidium parvum in healthy volunteers. N. Engl. J. Med. 332(13):855–859.

Fayer, R. 1994. Effect of high temperature on infectivity of Cryptosporidium parvum oocysts in water. Appl. Environ. Microbiol. 60: 2732–2735.

Fayer, R., C.A. Speer, and J.P. Dubye. 1990. Cryptosporidiosis of man and animals. CRC Press, Boca Raton.

Fayer, R., C.A. Speer, and J.P. Dubey. 1997. The general biology of *Cryptosporidium*. p. 1–42. *In* R. Fayer (ed.) Cryptosporidium and cryptosporidiosis. CRC Press, Boca Raton, FL.

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(2): 103–112.

Gallaher, M.M., J.L. Herndon, L.J. Nims, C.R. Sterling, D.J. Grabowski, and H.F. Hull. 1989. Cryptosporidiosis and surface water. Am. J. Public Health 79:39–42.

Garber, L.P., M.D. Salman, H.S. Hurd, T. Keefe, and J.L. Schlater. 1994. Potential risk factors for Cryptosporidium infection in dairy calves. J. Am. Vet. Med. Assoc. 205:86–91.

Giddens, J., and A.P. Barnett. 1980. Soil loss and microbiological quality of runoff from land treated with poultry litter. J. Environ. Qual. 9:518–520.

Goldstein, S., D. Juranek, O. Ravenholt, A. Hightower, D. Martin, J. Mesnik, S. Griffiths, A. Bryant, R. Reich, and B. Herwaldt. 1996. Cryptosporidiosis: An outbreak association with drinking water despite state-of-the-art water treatment. Ann. Intern. Med. 124: 459–468.

Hansen, J.S., and J.E. Ongerth. 1991. Effects of time and watershed characteristics on the concentration of *Cryptosporidium* oocysts in river water. Appl. Environ. Microbiol. 57:2790–2795.

Harter, T., S. Wagner, and E.R. Atwill. 2000. Colloid transport and filtration of Cryptosporidium parvum in sandy soils and aquifer sediments. Environ. Sci. Technol. 34:62–70.

Hirschi, M.C., J.K. Mitchell, D.R. Feezor, and B.J. Lesikar. 1990. Microcomputer-controlled laboratory rainfall simulator. Trans. ASAE 33:1950–1953.

Keaton, M.R., P.K. Kalita, J.P. Harnes, and S.K. Starrelt. 1998. Efficiency of vegetative filter strips in reducing nitrogen and phosphorus from surface runoff. Paper 98-2014. *In* ASAE Annual Int. Meeting, Orlando, FL. Am. Soc. Agric. Eng., St. Joseph, MI.

Lefay, D., M. Naciri, P. Poirier, and R. Chermette. 2000. Prevalence of Cryptosporidium infection in calves in France. Vet. Parasitol. 89:1–9.

Lim, T.T., D.R. Edwards, S.R. Workman, B.T. Larson, and L. Dunn. 1998. Vegetated filter strip removal of cattle manure constituents in runoff. Trans. ASAE 41:1375–1381.

MacKenzie, W.R., N.J. Hoxie, M.E. Proctor, M.S. Gradus, K.A. Blair, D.E. Peterson, J.J. Kazmierczak, D.G. Addiss, K.R. Fox, J.B. Rose, and J.P. Davis. 1994. A massive outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply. N. Engl. J. Med. 331(3):161–167.

Madson, F.L., and M. Alexander. 1982. Transport of *Rhizobium* and *Pseudomonas* through soil. J. Environ. Qual. 46:557–560.

Marshall, M.M., D. Naumovitz, Y. Ortega, and C.R. Sterling. 1997. Waterborne protozoan pathogens. Clin. Microbiol. Rev. 10:67–85.

Mawdsley, J.L., A.E. Brooks, and R.J. Merry. 1996a. Movement of the protozoan pathogen *Cryptosporidium parvum* through three contrasting soil types. Biol. Fertil. Soils 21:30–36.

Mawdsley, J.L., A.E. Brooks, R.J. Merry, and B.F. Pain. 1996b. Use

of a novel soil tilting table apparatus to demonstrate the horizontal and vertical movement of the protozoan pathogen *Cryptosporidium parvum* in soil. Biol. Fertil. Soils 23:215–220.

Medema, G.J., F.M. Schets, P.F.M. Teunis, and A.H. Havelaar. 1998. Sedimentation of free and attached *Cryptosporidium* oocysts and *Giardia* cysts in water. Appl. Environ. Microbiol. 64:4460–4466.

Mersie, W., and C.A. Seybold. 1997. Design, construction, and operation of tilted beds to simulate agricultural runoff in vegetative filter strips. Weed Technol. 11:618–622.

Nydam, D.V., S.E. Wade, S.L. Schaaf, and H.O. Mohammed. 2001. Number of Cryptosporidium parvum oocysts or Giardia spp cysts shed by dairy calves after natural infection. Am. J. Vet. Res. 62(10): 1612–1615.

Olson, M.E., J. Goh, M. Phillips, N. Guselle, and T.A. McAllister. 1999. *Giardia* cyst and *Cryptosporidium* oocyst survival in water, soil, and cattle feces. J. Environ. Qual. 28:1991–1996.

Olson, M.E., C.L. Thorlakson, L. Deselliers, D.W. Morck, and T.A. McAllister. 1997. *Giardia* and *Cryptosporidium* in Canadian farm animals. Vet. Parasitol. 68:375–381.

Ongerth, J.E., and H.H. Stibbs. 1987. Identification of cryptosporidium oocysts in river water. Appl. Environ. Microbiol. 53:672–676.

Peeters, J.E., E.A. Mazas, W.J. Masschelein, I.V.M. de Maturana, and E. Debacker. 1989. Effect of disinfection of drinking water with ozone or chlorine dioxide on survival of Cryptosporidium parvum oocysts. Appl. Environ. Microbiol. 55:1519–1522.

Robertson, L.J., A.T. Campbell, and H.V. Smith. 1992. Survival of Cryptosporidium parvum oocysts under various environmental pressure. Appl. Environ. Microbiol. 58:3494–3500.

Roefer, P.A., J.T. Monscvitz, and D.J. Rexing. 1996. The Las Vegas cryptosporidiosis outbreak. J. Am. Water Works Assoc. 88(9):95–106.

Rose, J.B. 1997. Environmental ecology of *Cryptosporidium* and public health implications. Annu. Rev. Public Health 18:135–161.

SAS Institute. 2001. SAS Version 8.2. SAS Inst., Cary, NC.

Sischo, W.M., E.R. Atwill, L.E. Lanyon, and J. George. 2000. Cryptosporidia on dairy farms and the role these farms may have in contaminating surface water supplies in the northeastern United States. Prev. Vet. Med. 43:253–267.

Srivastava, P., T.A. Costello, D.R. Edwards, and J.A. Ferguson. 1998. Validating a vegetative filter strip performance model. Trans. ASAE 41:89–95.

Sutton, A.L., D.D. Jones, and M.C. Brumm. 1976. A low cost settling basin and infiltration channel for controlling runoff from an open swine feedlot. Am. Soc. Agric. Eng. Paper 76-4516. ASAE, St. Joseph, MI.

Uga, S., J. Matsuo, E. Kono, K. Kimura, M. Inoue, S.K. Rai, and K. Ono. 2000. Prevalence of Cryptosporidium parvum infection and pattern of oocysts shedding in calves in Japan. Vet. Parasitol. 94: 27–32.

Wade, S.E., H.O. Mohammed, and S.L. Schaaf. 2000. Prevalence of *Giardia* sp., *Cryptosporidium parvum*, and *Cryptosporidium muris* (*C. andersoni*) in 109 dairy herds in five counties of southeastern New York. Vet. Parasitol. 93:1–11.

Walker, M.J., C.D. Montemagno, and M.B. Jenkins. 1998. Source water assessment and nonpoint sources of acutely toxic contaminants: A review of research related to survival and transport of Cryptosporidium parvum. Water Resour. Res. 34:3383–3392.

Williamson, T.S., P.K. Kalita, J.P. Harner, G.L. Marchin, and C.W. Rice. 1999. Coliform reduction by filter strips in Kansas watersheds. Paper 99-2076. *In* ASAE Annual Int. Meeting, Toronto. 18–21 July 1999. Am. Soc. Agric. Eng., St. Joseph, MI.

Young, R.A., T. Huntrods, and W. Anderson. 1980. Effectiveness of vegetated buffer strips in controlling pollution from feedlot runoff. J. Environ. Qual. 9:483–487.