Preferential Transport of *Cryptosporidium parvum* Oocysts in Variably Saturated Subsurface Environments

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ABSTRACT: When oocysts of the protozoan *Cryptosporidium parvum* contaminate drinking water supplies, they can cause outbreaks of Cryptosporidiosis, a common waterborne disease. Of the different pathways by which oocysts can wind up in drinking water, one has received little attention to date; that is, because soils are often considered to be perfect filters, the transport of oocysts through the subsoil to groundwater is generally ignored. To evaluate the significance of this pathway, a series of laboratory experiments investigated subsurface transport of oocysts. Experiment 1 was carried out in a vertical 18-cm-long column filled either with glass beads or silica sand, under conditions known to foster fingered flow. Experiment 2 involved undisturbed, macroporous soil columns subjected to macropore flow. Experiment 3 aimed to study the lateral flow on an undisturbed soil block. The columns and soil samples were subjected to artificial rainfall and were allowed to reach steady state. At that point, feces of contaminated calves were applied at the surface along with a known amount of potassium chloride to serve as a tracer, and rainfall was continued at the same rate. The breakthrough of oocysts and chloride, monitored in the effluent, demonstrate the importance of preferential flow on the transport of oocysts. Compared with chloride, peak oocyst concentrations were not appreciably delayed and, in some cases, occurred even before the chloride peak. Recovery rates for oocysts were low, ranging from 0.1 to 10.4% of the oocysts originally applied on the columns. However, the numbers of oocysts present in the effluents were still orders of magnitude higher than 10 oocysts, the infectious dose considered by the U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, to be sufficient to cause Cryptosporidiosis in healthy adults. These results suggest that the transport of oocysts in the subsurface via preferential flow may create a significant risk of groundwater contamination in some situations. *Water Environ. Res.,* **75,** 113 (2003).

KEYWORDS: *Cryptosporidium parvum*, preferential flow, subsurface transport.

Introduction

Pathogenic bacteria, viruses, and protozoa in drinking water are a significant cause of animal and human death in many parts of the world. Chlorination is an efficient treatment against a wide range of pathogens. However, it has no effect on a number of microorganisms, including the protozoan *Cryptosporidium parvum*, which causes Cryptosporidiosis, a common waterborne disease (Smith, 1992). Outside of the lower intestines of humans and domestic and wild animals, where *C. parvum* carries out the active part of its cycle, it is present in the environment in the form of 4- to 6-μm-long ovoid-shaped oocysts, with a double wall that is resistant to most oxidation processes (e.g., ozonation and chlorination) (Atwill et al., 1997; Current, 1986; Fayer and Ungar, 1986; Tzipori, 1983).

During the past two decades, the presence of *C. parvum* in surface waters and groundwaters in the United States and Great Britain (Galbraith et al., 1987; LeChevallier et al., 1991; Rose et al., 1991) has been connected with several major outbreaks of Cryptosporidiosis (Hayes et al., 1989; MacKenzie et al., 1994; Smith et al., 1988). The presence of *C. parvum* oocysts in drinking water supplies results from a number of processes. For example, infected hosts such as cows or deer may defecate in streams and shallow ponds. As a result, feces may end up on the soil via direct release by infected animals or land spreading of manure. Land application of municipal or industrial wastewater sludge may also contribute significant numbers of oocysts at the soil surface. Here, runoff may carry oocysts to nearby waterbodies, or rain infiltration may transport oocysts in the subsoil to groundwater. Among the different pathways for transporting oocysts, rain infiltration was generally considered until recently to be of little significance, in line with the common assumption that soils are effective at filtering a wide range of pathogens (Tim et al., 1988).

Several authors obtained results that seem to support this assumption. For example, Tan et al. (1994) found that movement of bacteria through disturbed, water-saturated soil matrices occurs to any significant extent only under extreme conditions of ionic strength, conditions that are unlikely to be found in agricultural fields. Under normal conditions in saturated and homogeneous media, Brush (1997) found *C. parvum* oocysts to be effectively strained by the soil matrix and to adsorb to soil particles; Harter et al. (2000) also found that the oocysts are subjected to velocity enhancement. Tate et al. (2000), who studied the transport of *C. parvum* oocysts on rangeland watersheds, hypothesized that oocysts can be transported from fecal deposits during rainfall events and are available for transport to waterbodies. During the last decade, laboratory and field experiments have shown that microorganisms can migrate far and fast along with other contaminants through unsaturated, undisturbed soil in both vertical and horizontal directions (Bitton and Harvey, 1992; Tan et al., 1992; van Eslas et al., 1991). This fast transport is due to preferential flow, which encompasses fingering, macropore flow, and funnel flow (Selker et al., 1996). Fingering-flow phenomena resulting from wetting front instabilities occurring at the interfaces of two fluids contributes to transport variability in sand–air–water systems. Hill and Parlange (1972) studied wetting front instability phenomenon and found that the vertical growth in the wetting front instability is driven by gravity, forming ''fingers'' that are stabilized horizontally by capillarity of the surrounding media.

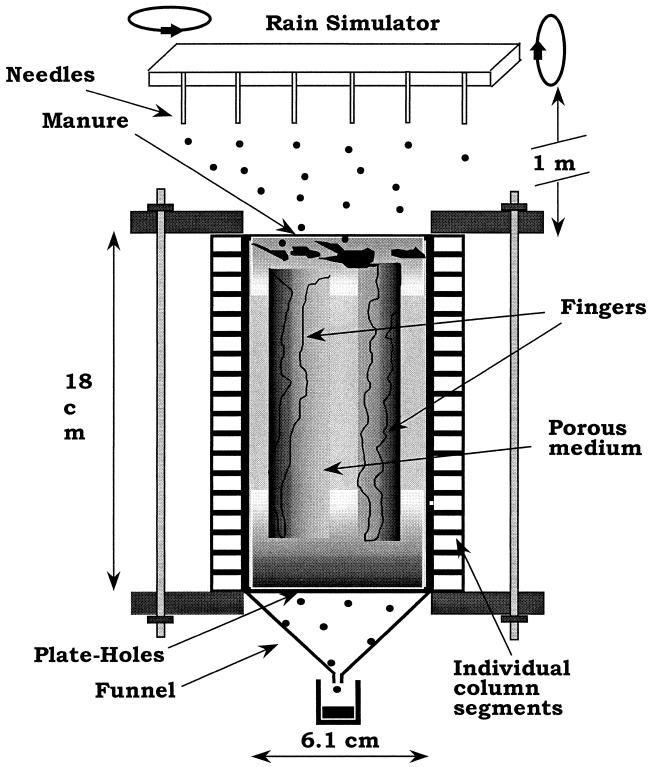

Figure 1—Laboratory experimental setup: column under rainfall simulator.

Circumstantial evidence suggests that water movement through macropores accounts for the much faster transport of *C. parvum* observed by Mawdsley et al. (1996a, 1996b) in undisturbed soil columns compared with that in disturbed and repacked columns.

In this context, the key objective of the present research was to determine the subsurface transport of *C. parvum* in columns filled with sand and glass beads and in undisturbed soil columns. To simulate actual field conditions as closely as possible, calves' feces containing *C. parvum* oocysts were applied at the soil surface. All of the experiments were designed to represent a worst-case scenario where defecation occurs during a rainfall event and rain continues uninterrupted for an extended period of time.

To achieve these objectives, three sets of experiments were carried out. Experiment 1 examines the influences of two different porous media on the vertical transport of oocysts by fingered flow. Experiment 2 was designed to analyze the vertical transport of oocysts by macropore flow in soils. Experiment 3 investigates the role of lateral flow in the transport of oocysts.

Materials and Methods

***Cryptosporidium parvum* Oocysts.** Fecal samples were collected from 6- to 21-day-old Holstein calves exhibiting diagnostic signs of Cryptosporidiosis that were housed in hutches at the Cornell University Teaching and Research Center, Hartford, New York. Fecal samples were obtained by palpating the rectum to induce defecation into sample cups and were then sieved through a food strainer with a 2- to 3-mm mesh to remove large solids and mucus. All collected feces were mixed together and stored in a refrigerator at 4 °C until needed. This procedure ensured uniformity of the input concentration. The oocyst concentration in the fecal samples was determined prior to the experiments in 100-μL samples (5 replicates) via immunofluorescence staining and microscopic counting (method described in the following section).

Columns. In experiment 1, the columns consisted of 18 segments of Plexiglas tubing (6.1-cm i.d. × 1-cm high) that were held together by two large rings at the top and bottom connected by nuts and bolts that were tightened so the rings would stay in place (Figure 1). The bottom of the column consisted of a plate with 0.2-mm holes that allowed the effluent to drop into a funnel, which was located immediately underneath. The columns were filled with either industrial quartz sand (Unimin Co., LeSueur, Minnesota) or glass beads, each with two particle sizes: fine (40/50, with 0.36 mm average diameter) and coarse (12/20, with 1.1 mm average diameter). Filling of the columns with these different media was performed under constant shaking to produce packing as uniform as possible and to prevent particles from moving during the experiments.

For experiment 2, an undisturbed soil column was obtained from an orchard at Cornell University in Ithaca, New York. The soil in the orchard belongs to the Hudson soil type and is classified as a fine, illitic, mesic Glossaquic Hapludalf characterized by the presence of numerous macropores and worms. Undisturbed soil blocks (20-cm diameter × 12-cm high) were hand-excavated in the field. A 12-cm-long polyethylene culvert pipe with an inner diameter of 20 cm was placed around the blocks and expandable polyurethane foam (Great Stuff, Dow Chemical Co., Menomonee Falls, Wisconsin) was injected between the soil and the pipe. The foam was allowed to expand and seal for 24 hours and then the bottom of the column was dug out.

In experiment 3, a 35-cm × 30-cm × 41-cm undisturbed column of Hudson soil type was dug in the side of a hill with a 19.3° slope. A three-walled stainless steel lateral lysimeter was placed around three sides of the column with the exposed side downhill of the middle frame of the lysimeter (Mendoza and Steenhuis, 2002). Connectors were placed along the exposed side of the column, and the space between the soil column and the lysimeter was filled with expandable polyurethane foam (Great Stuff, Dow Chemical Co.). After the foam had completely dried, three lateral-flow collectors were installed at depths of 8.5, 20.5, and 33.5 cm from the surface (Figure 2).

Rainfall Simulator. In experiments 1 and 2, artificial rainfall was produced with a laboratory rainfall simulator developed by Andreini and Steenhuis (1990). The simulator has up to six needles installed on a frame with rotating patterns in two directions to randomize raindrop distributions. Each syringe needle has a uniform flowrate, and the number of needles used in a given experiment depends on the targeted flowrate. In each experiment, the rain simulator was calibrated using volumetric gauges to ensure that targeted flowrates were achieved. The overall application rate was 2 cm/h for experiment 1, in which six needles were used. A rate of 1 cm/h in experiment 2 was achieved with four needles. In experiment 3, the field rainfall simulator device used was a cubic reservoir made of polycarbonate sheet with holes on the bottom plate.

Experimental Procedures. In experiment 1, the columns were placed under the rainfall simulator. Feces (25 mL), with enough added potassium chloride to produce a 5.5 g/L chloride concentration in the resulting mix, was applied on the top surface of the column after the column had reached steady-state flow. Sampling frequency varied over the duration (16 hours) of the

experiment. For the first 30 minutes, the effluent was sampled every 30 seconds, then, for the following 90 minutes, every minute. For the next 8 hours, it was sampled every 4 minutes and, for the last 6 hours, every 5 minutes. Each sample was collected in 5-mL plastic tubes either by hand or with an automatic fraction collector. At the end of the experiment, the soil contained in each ring was carefully separated from the rest of the column using a thin aluminum plate and put into 50-mL plastic tubes for analysis of oocyst distribution within the column.

In experiment 2, 100 mL of feces with enough added potassium chloride to produce 12.5 g/L of chloride concentration in the resulting mix was applied on the soil surface after the column had reached steady-state flow. Each effluent sample was collected in 50-mL plastic tubes during the experiment. At the end of the percolation experiment, the column was cut into three sections from top to bottom: 0 to 2 cm, 2 to 7 cm, and 7 to 12 cm. Each section was individually mixed to homogenize the soil. Roots were removed from the samples by hand. Three soil samples of approximately 7.5 mL were taken from each section to extract oocysts and enumerate them.

In experiment 3, the soil was brought to steady-state flow by a 1-cm rainfall on the column for 3 hours with a field rainfall simulator. Once steady-state flow was achieved, 100 mL of manure mixed with 20 g of potassium chloride was applied to the column. Manure was applied to the upslope two-thirds of the column to prevent vertical flow into the collectors. Samples were collected and recorded from each shelf every 10 minutes for 11 hours. The change in depth of the water in the rainmaker was also recorded every 10 minutes. All effluent and soil samples from the experiments were stored at 4 °C until analysis.

Characterization of Preferential Flow Paths. The characterization of preferential flow paths was performed qualitatively in experiment 1 by adding blue dye (FD&C Blue Dye #1, Warner Jenkinson, St. Louis, Missouri) to the infiltrating water in accordance with Baveye et al. (1998). After the experiment was finished, blue stains were observed in successive horizontal cross sections.

Chemical and Microbiological Analyses. Chloride concentration in the effluent was determined with a digital chloridometer (Buchler Instruments, Lenexa, Kansas) to obtain chloride breakthrough curves. The microbiological analysis of *C. parvum* oocysts consisted of a permeability assay combined with immunofluorescence staining (Anguish and Ghiorse, 1997). Effluent samples were either directly analyzed or concentrated by centrifugation at 1.0×10^4 *g* for 2 and 15 minutes for experiments 1 and 2, respectively. The resulting pellets were resuspended in a fraction of the original liquid and subjected to analysis and counting.

Enumeration of *C. parvum* oocysts in the columns at different depths requires a preliminary extraction using the following procedure. Approximately 15- to 20-cm^3 samples of porous medium containing entrapped feces and *C. parvum* are placed in a 50-mL centrifuge tube, to which 15 mL of an extraction solution are added. This solution consists of 0.1 M of phosphate-buffered saline (PBS; 0.028 M dibasic sodium phosphate [$Na_2HPO_4 \cdot H_2O$], 0.072 M monobasic sodium phosphate [NaH_2PO_4], 0.145 M sodium chloride [NaCl], pH 7.2) and 0.1% by weight of a commercial surfactant, Tween 80 (polyoxyethylene [20] sorbitan monooleate) (Baker Chemical Co., Phillipsburg, New Jersey). The tubes are placed on a horizontal shaker set on low speed (3 strokes/s) for 20 minutes and then on the same shaker at high speed (5 strokes/s) for 10 minutes. Coarse particles are eliminated in each case by siphoning the slurry into a new centrifuge tube. A cold sucrose solution (15 mL, 5 °C, sp gr 1.18) is then injected in the centrifuge tubes with a syringe needle (18G 1 ½″) in such a way that the soil and water slurry is on top of, and not mixed with, the sucrose. After centrifugation at 2700 *g* for 20 minutes (without automatic braking at the end), the bilayer system in each tube evolves into a trilayer one, with most of the organic matter accumulating in a layer of distinct yellow color. Aliquots (10 mL) of this intermediate layer are removed using a syringe equipped with an 18G 1 ½″ hypodermic needle, diluted 4 times with 0.1-M PBS to obtain a total volume of 50 mL, and homogenized by hand. The aliquots are then centrifuged at 2700 *g* for 30 minutes (brake on). The bulk (approximately 49 mL) of the supernatant is discarded and the remaining liquid and pellet are vortexed (Vortex Genie 2, Fisher, Bohemia, New York), transferred to a 1.5-mL Eppendorf tube, and vortexed again. In each Eppendorf tube, a 100-µL sample is taken for the dye permeability assay.

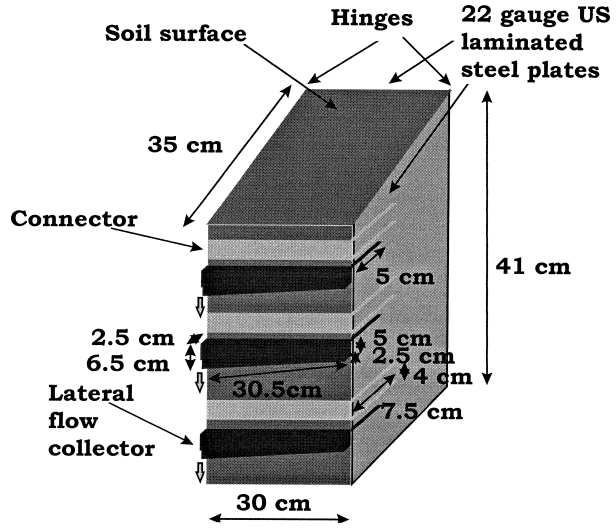

Figure 2—Field experimental setup: lysimeter.

The dye permeability assay is the fluorescent dye exclusion method developed by Anguish and Ghiorse (1997), who improved the earlier method of Campbell et al. (1992) by adding a fluorescent antibody detection step. In this assay, 10 µL each of 4′,6-diamidino-2-phenyllindole (DAPI) (2 mg/mL in high-pressure performance liquid chromatography-grade methanol) and propidium iodide (1 mg/mL in 0.1 M PBS) are added to 100-µL samples. The resulting solution is mixed (Vortex, Bohemia, New York), then incubated in the dark at 37 °C for 90 minutes. Each solution is stained by the immunofluorescence staining procedure (Anguish and Ghiorse, 1997) with an antibody (Hydrofluor Combo, Strategic Diagnostics, Inc., Newark, Delaware). Undiluted primary antibody (10 µL, Hydrofluor) is added to each sample, and the sample is mixed (Vortex) and returned to the incubator in the dark at 37 °C. After the second incubation, 1.0 mL of the PBS solution is added to the sample, the suspension is vortexed and microcentrifuged for 30 seconds at 1.0×10^4 *g*, and the supernatant is discarded. Undiluted secondary reagent (10 µL) is added to each tube, which is then vortexed. The tubes are incubated at room temperature (17 to 20 °C) for 30 minutes in the dark. After incubation, the tubes are again microcentrifuged for

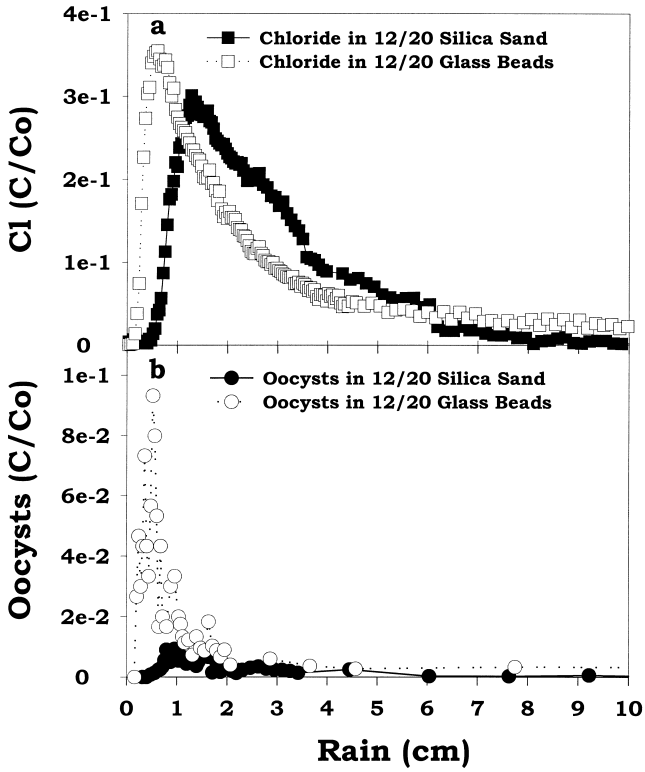

Figure 3—Breakthrough curves of chloride (3a) and *Cryptosporidium parvum* oocysts (3b) in 12/20 silica sand and in 12/20 glass beads plotted as a function of cumulative rain. In this and all subsequent figures, Co refers to the concentration (mg/L for Cl^-, oocysts/L for *Cryptosporidium parvum*) in the manure.

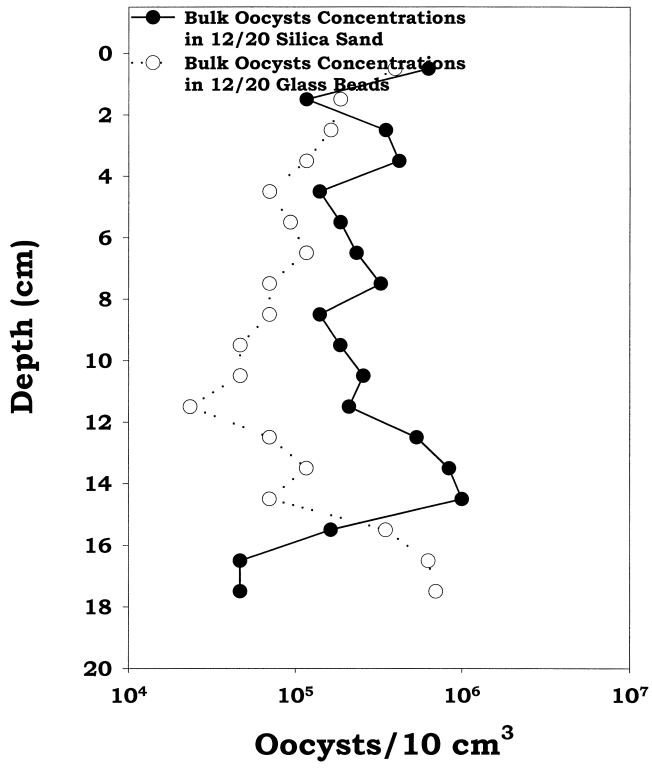

Figure 4—Spatial distribution of *Cryptosporidium parvum* oocysts in 12/20 silica sand and 12/20 glass beads.

30 seconds at 1.0×10^4 *g*, and the pellet material is washed once with 1 mL of 0.3 M 1,4-diazabicyclo[2.2.2]octane in 0.1 M PBS. The samples are stored in the dark at 4 °C and are examined within 72 hours. A 10-µL portion of each sample is placed on an air-dried agar-coated slide and mounted under a 22-mm^2 cover slip. Mounted slides are put aside for 1 minute before examination to allow water in the sample to diffuse into the dried-agar layer, creating a hydrated agar matrix around the sample. The agar-coated slides are prepared by air drying an even film of molten 1% purified agar produced by spreading 1 mL of 1% agar solution in distilled water on an alcohol-cleaned glass slide (Anguish and Ghiorse, 1997).

Samples were examined by microscrope (model LSM-210, Zeiss, Thornwood, New York) and observed with a 100× (NA, 1.3) oil immersion objective lens (Neofluar, Zeiss) under both conventional differential-interference contrast and epifluorescence mode with a triple excitation-emission filter (Chroma Technology Corp., Brattleboro, Vermont). For optimal imaging, the top element of the 1.4-NA condenser lens was also immersed in oil. *Cryptosporidium parvum* can be classified in three categories: nonviable, viable, and intermediate. Nonviable oocysts are permeable to DAPI and propidium iodide. Viable oocysts are impermeable to DAPI and propidium iodide. Intermediate oocysts are impermeable to propidium iodide and permeable to DAPI (Campbell et al., 1992).

Additional visualization of oocysts was obtained through an attached color charge-coupled device (CCD) video camera (model VI-470, Optronics, Golita, California), and the video images were displayed on a second color video monitor (model PV 1343MD, Sony Trinitron, Ichinomiya, Japan) and then printed using a color video printer (Mavigraph model, Sony).

Cryptosporidium parvum oocysts were enumerated within smears of 10-µL samples spread over glass slides. The *Cryptosporidium* concentration, C (oocysts/L) is given by the following formula:

$$C = \frac{N}{F}\frac{S}{S'}\cdot\frac{1}{V} \qquad (1)$$

Where

N = number of oocysts counted in F fields;
S = total area covered by the smear (22 mm^2 for the cover slide);
S' = area of one field (3.14×0.115 mm^2); and
V = volume of the smear (10 µL).

Results

Fingered-Flow Experiments. Experiment 1 was designed to study the transport of *C. parvum* oocysts through uniform porous media consisting of silica sand and glass beads. Hill and Parlange (1972) showed that unstable fingered flow occurs in these media.

The breakthrough curves obtained in the 12/20 sand and glass-bead columns under a rainfall rate of 2 cm/h were similar (Figures 3a and 3b). In general, the relative concentrations of oocysts in the effluent from the glass-bead column are higher (at the same

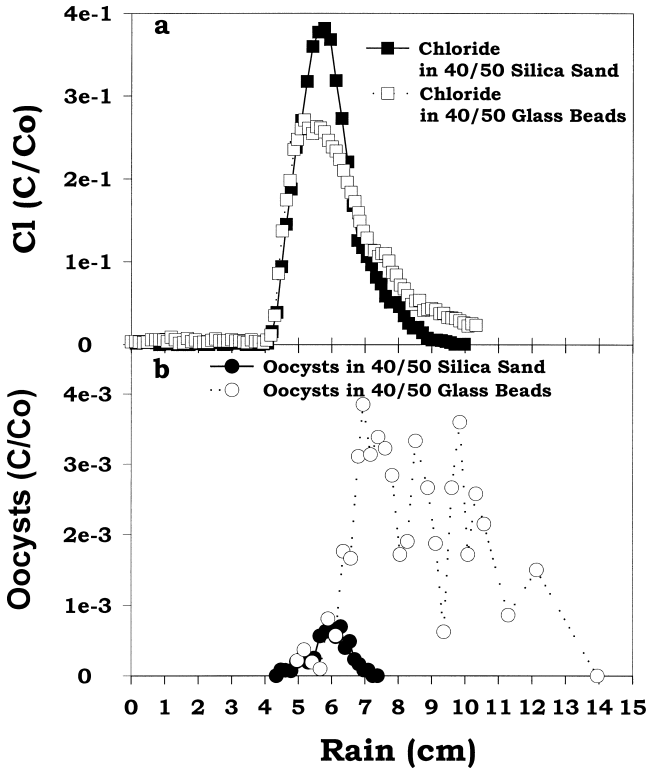

Figure 5—Breakthrough curves of chloride (a) and *Cryptosporidium parvum* oocysts (b) in 40/50 silica sand and 40/50 glass beads.

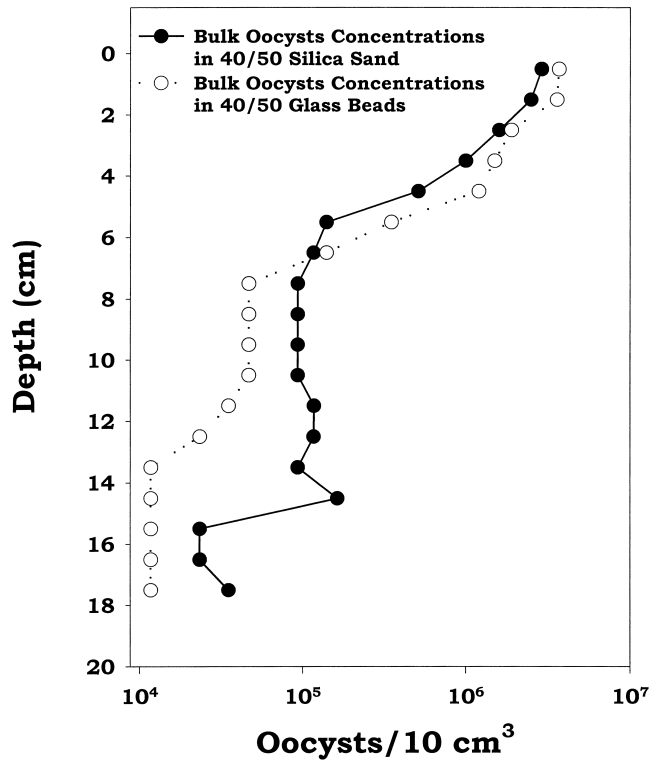

Figure 6—Spatial distribution of *Cryptosporidium parvum* oocysts in 40/50 silica sand.

cumulative rain) than in the effluent from the sand column. In both columns, the chloride breakthrough curves exhibit a sharp increase followed by a long tail. After 4 cm of rain has been applied, most of the chloride has passed through the sand and glass beads. In the glass beads, the oocysts breakthrough peak occurred slightly before the chloride peak, but the relative concentration of the oocysts at the peak is 4 times lower than that for chloride. In the sand column, the oocysts peak is not clearly identifiable. Although the highest relative concentration occurs after 0.9 cm of applied rain, it corresponds only to less than one thirtieth of the peak relative chloride concentration (after 1.2 cm of rain). Evidence that preferential (fingered) flow occurred during the breakthrough experiments was ascertained by visual observation of the distribution of blue dye in horizontal cross sections after dismantlement of the columns. In both columns, several fully developed fingers were clearly present. In the sand column, five fingers of approximately 2 cm in diameter were present at a depth of 5 cm, merging into three larger fingers (3 cm in diameter) in lower sections.

The spatial distribution of the oocysts inside the columns exhibited some similarities in the sand and glass-bead columns as well as a number of differences (Figure 4). Overall, more oocysts were retained in the sand than in the glass beads, which was in agreement with the results of Figure 3b. In both sand and glass beads, a zone from 0 to 3 cm in depth is characterized by locally high oocyst concentrations. Below this layer, oocyst concentrations decrease progressively with depth in both columns until a depth of approximately 12 cm, where oocyst concentrations increase sharply. Visual observation (during the experiments) and analysis of dye distribution indicate that this depth corresponds to the top of the capillary fringe in both columns. In the saturated zone of the columns, oocyst concentrations decrease dramatically in the sand (by more than an order of magnitude), whereas they keep increasing in the glass beads.

In the case of the 40/50 sand and glass-bead columns, under a 2-cm/h artificial rainfall, the observed breakthrough patterns are again similar in both columns (Figure 5). As in the 12/20 sand and glass-bead columns, effluent concentrations for both chloride and oocysts exhibit a sharp increase and significant tailing. Compared with the 12/20 glass-bead column, the relative concentration of oocysts in the 40/50 glass-bead column remains high for some period of time after breakthrough, and the peak concentration is not clearly marked. Unlike the earlier case, however, it takes a full 4 cm of rain for any breakthrough to occur in either column, and the chloride concentration peaks now appear earlier than that of the oocysts. Another difference is that the peak relative oocyst concentration is now between 100 and 500 times lower than the peak relative chloride concentration.

Once again, dye patterns indicated the presence of fingers downward in the columns. Because of the fine texture of the materials, causing the presence of a thick capillary fringe, the fingers were only approximately 7 cm long.

More oocysts are retained in the sand column than in the glass-bead column (Figure 6). The highest concentrations are found in the first 2 cm near the surface. Visual information after dismantlement of the columns suggests that these high numbers are due to a slight penetration and filtration of the manure. Underneath this top zone, oocyst concentrations decrease sharply with depth in both columns until the top of the capillary fringe is

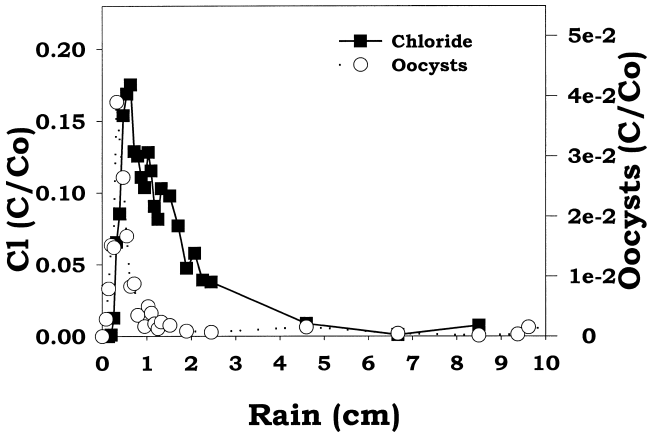

Figure 7—Breakthrough curves of chloride and *Cryptosporidium parvum* oocysts in 12-cm Hudson soil.

reached (at a depth of approximately 7 cm). In the capillary fringe, oocyst concentrations (per unit volume of soil) remain relatively stable. At depths of approximately 11 and 15 cm for the glass beads and sand, respectively, the porous media become visibly saturated with water and, at that point, oocyst numbers decrease appreciably.

Macropore Flow Experiments. In the 12-cm Hudson soil column (Figure 7), the peak concentration of *C. parvum* oocysts occurred shortly before the chloride peak. However, the decrease in oocysts concentration was much more precipitous than that for chloride. Although oocyst concentrations in the effluent, even at the peak, amount to only a few percents of the applied concentration, C_0, they are still several orders of magnitude (10^6 times) higher than the concentration generally considered infectious (10 oocysts or less per healthy human) assessed by the Center for Food Safety and Applied Nutrition of the U.S. Food and Drug Administration (U.S. FDA) (1992). Numerical integration of the oocysts breakthrough curves indicates that 10% of the applied oocysts ended up in the effluent (Table 1).

Sampling in the top 2 cm of the soil showed that most oocysts were filtered in this part of the soil column and were not able to move downward with the percolating water.

Lateral Transport. In the lateral transport experiments, oocysts appeared rapidly in the collector located 8.5 cm below the surface (Figure 8), and their concentration in the effluent had decreased to virtually zero by the time a sharp chloride peak occurred. The initial oocysts and chloride peaks had almost identical shapes, except that oocyst concentrations were substantially lower than the corresponding chloride concentrations.

Discussion

Different physical parameters and possibly one chemical feature of artificial reconstituted porous media control the transport of *C. parvum* oocysts. Percent recovery data in the sand and glass-bead columns (Table 1) clearly show that the texture of the medium has a strong influence on the number of oocysts that end up in the effluent. In the sand columns, 18 times more oocysts were found in the effluent from the coarse-textured column (12/20) than from the fine-textured one (40/50). The trend is similar for the glass-bead columns, although the ratio is only approximately 7 in this case. These observations are consistent with earlier results related to the

Table 1—Percentages of *Cryptosporidium parvum* oocysts recovered in the effluents after percolation through 12/20 and 40/50 silica sand and glass beads.

	Silica sand	Glass beads
12/20	1.8%	10.4%
40/50	0.1%	1.5%

movement of bacteria and viruses in sand and soil columns (Bashan and Levanony, 1988; Tan et al., 1991).

Texture alone is not able to account for the experimental evidence, however. Indeed, the breakthrough curves of Figures 3b and 5b and the summary in Table 1 indicate that the sand and glass beads, although of identical granulometry, produce drastically different oocyst breakthrough patterns. Because particle sizes are statistically similar in both cases, and one can expect pore diameters and pore neck sizes to be comparably large, straining or filtering of oocysts cannot account for the observed difference in breakthrough.

Another explanation may be a slight preference of oocysts to adsorb on sand particles rather than glass beads. This adsorption may, in turn, lead to significantly enhanced filtration in the sand following a collaborative or autocatalytic mechanism suggested by Gerba et al. (1975) to account for the retention of bacteria in soil columns. Surface properties of the sand particles that may result in increased adsorption by oocysts include the small yet generally nonzero negative surface charge of the sand particles as well as the possible presence of a thin organic coating frequently found on natural sands, but less likely on synthetic glass beads.

Both filtration and adsorption might also account for the depth profile of oocyst concentrations found in the columns (Figures 4 and 6). In the fine-textured (40/50) columns, significant retention of the oocysts occurs in the surface layer. In fact, the overwhelming majority of oocysts are strained and adsorbed in the top 4 cm regardless of the type of medium (sand or glass). Similar observations have been made by other authors. Wollum and Cassel (1978) monitored the movement of bacteria and *Streptomycete conidia* in a sandy soil and showed that the majority of microorganisms were retained near the soil surface. In the coarse-

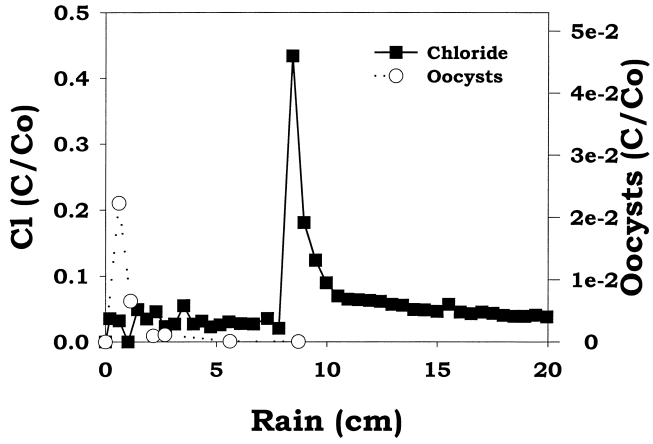

Figure 8—Breakthrough curves of chloride and *Cryptosporidium parvum* oocysts in lateral flow occurring in Hudson soil.

textured (12/20) columns (Figure 4), straining is less pronounced in the surface layer. In the sand column at least, significant oocyst accumulation seems associated with the capillary fringe, perhaps because of preferential adsorption at the air–water interfaces, but most likely because of the higher water content in that part of the column. Indeed, oocyst concentrations in the liquid phase (obtained by dividing oocyst concentration per volume of soil by the volumetric water content) shows no significant increase.

In spite of significant retention inside the columns, oocyst concentrations in the effluents (Figures 3 and 5) consistently exceed, by several orders of magnitude, the minimum infectious doses (10 oocysts or less per healthy human) assessed by the Center for Food Safety and Applied Nutrition of the U.S. FDA (1992). Therefore, an 18-cm thick layer of sand or glass beads is not able to filter oocysts effectively, at least under conditions where preferential fingered flow occurs. The situation is considerably worse even in the soil columns (Figure 7), where macropore flow is able to produce peak oocyst concentrations in the effluent that are 4 to 5 orders of magnitude higher than the minimum infectious doses.

One aspect of the transport of oocysts that the results obtained to date cannot elucidate is whether oocysts move alone as ''biocolloids'' (i.e., microorganisms of the size of colloids) or in association with soil constituents such as clay-sized (<2 μm) particles. The association of oocysts with clay minerals, oxides and hydroxides, or organic colloids may change the surface and adsorptive properties of the oocysts to such an extent that it may explain the increased transport in soil columns (Figure 7) compared with that in sand or glass-bead columns. An alternative and simpler explanation is that water flow is significantly faster in wormholes, cracks, and decayed root channels than in fingers, and is, therefore, able to carry oocysts farther and faster. Whichever explanation is correct, the results depicted in Figure 7 for undisturbed soil columns suggest that risks of groundwater contamination resulting from the downward transport of *C. parvum* oocysts through soils may have been vastly underestimated.

Conclusion

The results of laboratory breakthrough experiments suggest that significant numbers of *C. parvum* oocysts are able to percolate through sand, glass beads, and soil columns under conditions where preferential flow occurs. In all cases, oocyst concentrations in the effluent were orders of magnitude higher than what regulators consider sufficient to cause Cryptosporidiosis in healthy humans. Therefore, under field conditions and the worst-case scenario where a contaminated animal defecates on a soil when it rains, the transport of oocysts in soil via preferential flow may create a significant risk of groundwater contamination. Until now, this risk has been ignored. Although the risk may be minor in situations of deep aquifers, further research is needed to determine its significance in cases (such as riverbank filtration operations) where water is pumped out of aquifers at shallow depths.

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References

Andreini, M. S.; Steenhuis, T. S. (1990) Preferential Paths of Flow under Conventional and Conservational Tillage. *Geoderma,* **46,** 85.

Anguish, L. J.; Ghiorse, W. C. (1997) Computer-Assisted Laser Scanning and Video Microscopy for Analysis of *Cryptosporidium parvum* Oocysts in Soil, Sediment and Feces. *Appl. Environ. Microbiol.,* **63,** 724.

Atwill, E. R.; Sweitzer, R. A.; Pereira, M. D. C.; Gardner, I. A.; Van Vuren, D.; Boyce, W. M. (1997) Prevalence of and Associated Risk Factors for Shedding *Cryptosporidium parvum* Oocysts and *Giardia* Cysts within Fecal Pig Populations in California. *Appl. Environ. Microbiol.,* **63,** 3946.

Bashan, Y.; Levanony, H. (1988) Adsorption of the Rhizosphere Bacterium *Azospirillum brasilense* Cd to Soil, Sand and Peat Particles. *J. Gen. Microbiol.,* **134,** 1811.

Baveye, P.; Boast, C. W.; Ogawa, S.; Parlange, J.-Y.; Steenhuis, T. (1998) Influence of Image Resolution and Thresholding on the Apparent Mass Fractal Characteristics of Preferential Flow Patterns in Field Soils. *Water Resour. Res.,* **34,** 2783.

Bitton, G.; Harvey, R. W. (1992) Transport of Pathogens through Soils and Aquifers. In *Environmental Microbiology*; Mitchell, R., Ed.; Wiley-Liss: New York; pp 103–124.

Brush, C. F. (1997) Surface and Transport Properties of *Cryptosporidium parvum* Oocysts. Ph.D. Dissertation, Cornell University, Ithaca, New York.

Campbell, A. T.; Haggart, R.; Robertson, L. J.; Smith, H. V. (1992) Viability of *Cryptosporidium parvum* Oocysts: Correlation of In Vitro Excystation with Inclusion or Exclusion of Fluorogenic Dyes. *Appl. Environ. Microbiol.,* **58,** 3488.

Current, W. L. (1986) *Cryptosporidium*: Its Biology and Potential for Environmental Transmission. *CRC Crit. Rev. Environ. Control.,* **17,** 21.

Fayer, R.; Ungar, B. L. P. (1986) *Cryptosporidium* spp. and Cryptosporidiosis. *Microbiol. Rev.,* **50,** 458.

Galbraith, N. S.; Barrett, N. J.; Stanwell-Smith, R. (1987) Water and Disease after Croydon: A Review of Water-Associated Disease in the U.K. 1937–1986. *J. Inst. Water Environ. Manage.,* **1,** 7.

Gerba, C. P.; Wallis, C.; Melnick, J. L. (1975) Fate and Wastewater Bacteria and Viruses in Soil. *J. Irrig. Drain. Div. Am. Soc. Civ. Eng.,* **101,** 157.

Harter, T.; Wagner, S.; Atwill, E. R. (2000) Colloid Transport and Filtration of *Cryptosporidium parvum* in Sandy Soils and Aquifer Sediments. *Environ. Sci. Technol.,* **34,** 62.

Hayes, E. B.; Matte, T. D.; O'Brian, T. R.; McKinley, T. W.; Logsdon, G. S.; Rose, J. B.; Ungar, B. L. P.; Word, D. M.; Pinsky, P. F.; Cummings, M. L.; Wilson, M. A.; Long, E. G.; Hurwitz, E. S.; Juranek, D. D. (1989) Contamination of a Conventionally Treated Filtered Public Water Supply by *Cryptosporidium* Associated with a Large Community Outbreak of Cryptosporidiosis. *New England J. Med.,* **320,** 1372.

Hill, D. E.; Parlange, J.-Y. (1972) Wetting Front Instability in Layered Soils. *Soil Sci. Soc. Am. Proc.,* **36,** 115.

LeChevallier, M. W.; Norton, W. D.; Lee, R. G. (1991) Occurrence of *Giardia* and *Cryptosporidium* spp. in Surface Water Supplies. *Appl. Environ. Microbiol.,* **57,** 2610.

MacKenzie, W. R.; Hoxie, N. J.; Procter, M. E.; Gradus, M. S.; Blair, K. A.; Peterson, D. E.; Kazmierczak, J. J.; Addiss, D. G.; Fox, K. R.; Rose, J. B.; Davis, J. P. (1994) A Massive Outbreak in Milwaukee of *Cryptsoporidium* Infection Transmitted through the Public Water Supply. *New England J. Med.,* **331,** 161.

Mawdsley, J. L.; Brooks, A. E.; Merry, R. J. (1996a) Movement of the Protozoan Pathogen *Cryptosporidium parvum* in Three Contrasting Soil Types. *Biol. Fertility Soils,* **21,** 30.

Mawdsley, J. L.; Brooks, A. E.; Merry, R. J.; Pain, B. F. (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. Fertility Soils,* **23,** 215.

Mendoza, G.; Steenhuis, T. S. (2002) Determination of Hydraulic Behavior of Hillsides with a Hillslope Infiltrometer. *Soil Sci. Soc. Am. J.,* **66,** 1501.

Rose, J. B.; Gerba, C. P.; Jakubowski, W. (1991) Survey of Potable Water Supplies for *Cryptosporidium* and *Giardia. Environ. Sci. Technol.,* **25,** 1393.

Selker, J. S.; Steenhuis, T. S.; Parlange, J.-Y. (1996) An Engineering Approach to Fingered Vadose Pollutant Transport. *Geoderma,* **70,** 197.

Smith, H. V.; Girdwood, R. W. A.; Patterson, W. J.; Hardie, R.; Green, L. A.; Benton, C.; Tulloch, W.; Sharp, J. C. M.; Forbes, G. I. (1988) Waterborne Outbreak of Cryptosporidiosis. *Lancet,* **2,** 8626.

Smith, H. V. (1992) *Cryptosporidium* and Water: A Review. *J. Inst. Water Environ. Manage.,* **6,** 443.

Tan, Y.; Bond, W. J.; Griffin, D. M. (1992) Transport of Bacteria during Unsteady Unsaturated Soil Water Flow. *Soil Sci. Soc. Am. J.,* **56,** 1331.

Tan, Y.; Bond, W. J.; Rovira, A. D.; Brisbane, P. G.; Griffin, D. M. (1991) Movement through Soil of the Biological Control Agent, *Pseudomonas fluorescens*. *Soil Biol. Biochem.,* **23,** 821.

Tan, Y.; Gannon, J. T.; Baveye, P.; Alexander, M. (1994) Transport of Bacteria in an Aquifer Sand: Experiments and Model Simulations. *Water Resour. Res.,* **30,** 3234.

Tate, K. W.; Atwill, E. R.; George, M. R.; McDougald, M. K.; Larsen, R. E. (2000) *Cryptosporidium parvum* Transport from Cattle Fecal Deposits on California Rangelands. *J. Range Manage.,* **53,** 295.

Tim, U. S.; Mostaghimi, S.; Dillaha, T. A. (1988) Modeling the Movement and Persistence of Bacteria and Viruses in Porous Media. AZO Paper No. 88-2627; Kodak Professional: St. Joseph, Michigan.

Tzipori, S. (1983) Cryptosporidiosis in Animals and Humans. *Microbiol. Rev.,* **47,** 84.

U.S. Food and Drug Administration (1992) *Foodborne Pathogenic Microorganisms and Natural Toxins Handbook;* Center for Food Safety and Applied Nutrition: College Park, Maryland.

van Eslas, J. D.; Trevors, J. T.; van Overbeek, L. S. (1991) Influence of Soil Properties on the Vertical Movement of Genetically-Marked *Pseudomonas fluorescens* through Large Soil Microcosms. *Biol. Fertility Soils,* **10,** 249.

Wollum, A. G.; Cassel, D. K. (1978) Transport of Microorganisms in Sand Columns. *Soil Sci. Soc. Am. J.,* **42,** 72.