EVALUATING TRANSPORT OF CRYPTOSPORIDIUM OOCYSTS THROUGH SOILS USING POLYSTYRENE MICROSPHERE SURROGATES

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Abstract. *Cryptosporidium parvum* causes gastroenteritis in humans and is now recognized as an emerging pathogen. The inactive form, known as an oocyst, is excreted in the feces of infected animals and humans into the environment. Little is known about the transport of *C. parvum* oocysts through soil to reach and contaminate water bodies. A 40x30-m site at the outlet of an 8-ha grazing catchment just above a spring was instrumented to conduct a water budget analysis to evaluate soil-water and particle translocation. A tracer experiment using polystyrene microspheres, as surrogates to the oocysts, showed that microspheres had migrated through unsaturated soil to reach the ground water and spring within 20 days after injection 12-m upstream of the spring.

INTRODUCTION

Cryptosporidium is a small pathogenic parasite 3-5 μm diameter that is found in the intestinal tracts of humans and many animals (e.g., cattle). The species that infects humans and most mammals is referred to as C. *parvum* and the disease it causes is *cryptosporidiosis*. The symptoms in humans include diarrhea, headache, abdominal cramps, nausea, vomiting, and low-grade fever (CDC, 2000), which may lead to loss of weight and dehydration. No safe and effective cure is available for *cryptosporidiosis*.

Dramatic reports of the spread of C. *parvum* through municipal water systems that met all state and federal drinking water standards came from Carrollton, GA, in 1987 (Hayes et al., 1989) and from Milwaukee WS, in 1993 (MacKenzie et al., 1994). An estimated 13,000 people in Carrollton and 400,000 people in Milwaukee became ill and the disease contributed to the death of some AIDS patients in Milwaukee.

The inactive form of *cryptosporidium*, called an oocyst, is excreted in the feces of infected humans and animals. The tough-walled oocysts survive under a wide range of environmental conditions. Cattle, neonatal calves in particular, are considered an important reservoir of C. *parvum* oocysts in the environment (Mawdsley et al, 1995) and can shed billions of oocysts during the period initial infection (Anderson, 1981). As low as 30 doses of oocysts have been shown to induce infection in some healthy human volunteers (DuPont et al., 1995). The mobility of C. *parvum* out of fecal material via water and the hydrologic pathway by which the parasite might be transported to water bodies is unknown.

The overall goals of this research were to investigate transport of *cryptosporidium* in soils using surrogate microspheres and to improve numerical simulation of particle transport in soils.

METHODS

Field research was conducted at a small 8-ha grazing research catchment at the USDA-ARS station in Watkinsville, GA. The site is topographically typical of many catchments in the Southern Piedmont and has Cecil and Pacolet sandy loam and sandy clay loam soils with slopes predominately 2-8%. The catchment is located at the head of a first order stream supplying perennial flow to the stream via a series of springs and seeps.

The procedures included: 1. Intensively instrumenting a 30 by 40-m area upstream of the primary spring and monitoring the site water balance; 2. continuously monitoring the spring flow; 3: conducting tracer experiments within the 30 by 40-m area using fluorescent polystyrene microspheres as surrogates for *cryptosporidium* (Li et al., 1997); and 4: preliminary modeling using HYDRUS 1-D (Šimůnek, 1998) to study conditions under which significant internal drainage, and hence particle transport occurred in the soil profile. A separate laboratory column study looked at transport and retention of microspheres in

Table 1. Field monitoring technique

Parameter	Device	Number
Data recording	Cr10x data logger	1
Rainfall	Tipping bucket gage	1
Evapotranspiration	Bowen ratio	1
Evapotranspiration	Weather station data	1
Surface runoff	H flume	1
Spring flow	H flume	1
Groundwater depth	Piezometer	16
Soil water content	TDR	5
Soil water tension	Tensiometers	10
Pore water samples	Zero-tension lysimeter	6
Spring water sample	SIGMA sampler	1
Spring water sample	Flow-through centrifuge	1
Tracer injector	HPLC pump	1
Soil coring	Giddings probe	1

sand and soil columns under saturated conditions (Kong et al., 2000).

Table 1 presents a list of field monitoring techniques and devices. Rainfall and spring flow were automatically recorded every 5 minutes. Daily evapotranspiration was calculated from standard weather station data automatically recorded every 15 minutes. Ground water depth, soil water content and tension were measured manually two to three times a week.

Site water balance was determined from:

$$(R+GWu)-(ET+SRO+I+GWo) = +/-\Delta(GWS+SWS)$$

Variables are: rainfall R, evapotranspiration ET, surface runoff SRO, interflow I, groundwater under and out flow GWu and GWo, groundwater storage GWS, and soil water storage SWS. Kriged surfaces from piezometer data were used to get GWS. Water content measurements were used to get SWS. Interflow was calculated by separating base flow from total flow using a recursive digital filter technique of Chapman (1991):

$$IF_t = (3\alpha - 1)(3 - \alpha)^{-1} IF_{t-1} + 2(3 - \alpha)^{-1}(TF_t - \alpha TF_{t-1})$$

where, IF_t is the interflow response at time t; α is a filter parameter that controls attenuation of the signal; and TF is the original spring flow. As α increases toward unity, high frequency responses of base flow are removed and the spring flow is dominated by interflow. There was no runoff from the intensively monitored site.

The tracer experiment consisted of injecting, from a 1 L stock solution, approximately 2.03×10^{10} microspheres at 450 locations, on a 5 by 5-cm grid, 5-cm below the soil surface, within a 152 by 76-cm rectangular area, 12-m upstream of the spring. Injection was done on February 27, 2000. Water samples were collected from four types of locations/samplers following rain events: zero-tension lysimeters installed 30, 60 and 90-cm under the injection area, and immediately downstream; two monitoring wells installed between the injecting area and the spring; and the spring using flow-trough centrifuge and a sampler. Spring water was automatically sampled at half hour intervals and with the flow-though centrifuge run 1 to 2 hours after rain events. Microsphere enumeration was done by filtering water samples through 3 μm polycarbonate track-etch filters, and counting the number of microspheres using an epifluorescence microscope.

RESULTS

Hydrologic System

Near continuous data collection has occurred at the site since July of 1999, providing a large data set for water balance calculations. However rainfall events have been well below normal due to unusually dry conditions. Only 15 rain events with 20 or more mm were recorded between July 1999 and December 2000 inclusive, and only 8 events were recorded with 30-mm or more.

For the limited rainfall events, spring flow showed highly dynamic response to rainfall, especially when the soil was wet. A typical response curve is shown in Figure 1 for the rain event of December 16, 2000. Spring discharge rose sharply to as high as 40 L min^{-1} within an hour after the onset of the first precipitation event. The second, less-intensive event also caused a very rapid increase in spring discharge, which reverted to base flow conditions within a day or more. This behavior was typical at the site, especially when antecedent water content was high prior to the onset of precipitation. Generally, base flow was about 5 L min^{-1} at the beginning of the field monitoring, but eventually reduced to a trickle after May 2000, when volumetric soil water contents in the profile were less than 10%.

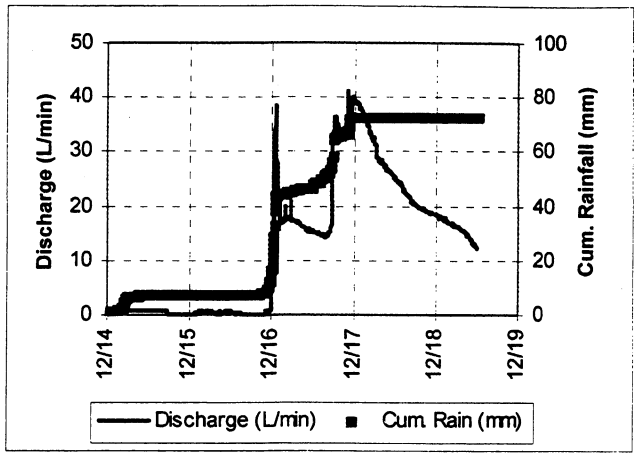

Figure 1. Typical spring discharge response curve after significant rainfall event on December 16, 2000. The thicker line is for cumulative rainfall.

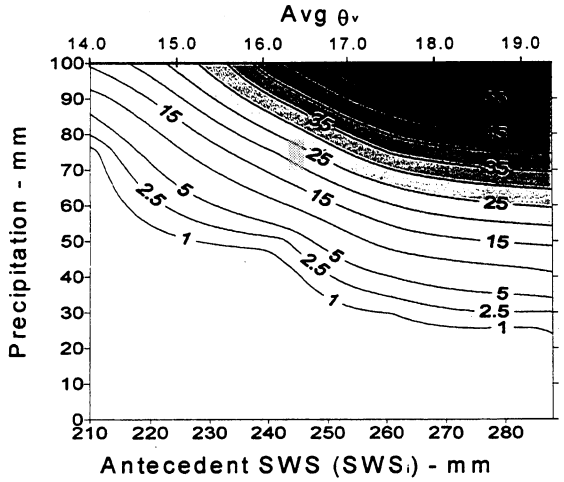

Figure 2. Contour map showing internal soil water drainage in mm after 10 days in 1-D soil profile as functions of rainfall and antecedent water content.

Groundwater levels, and hence volume, also generally declined except for the occasional recharge following larger rainfall events. Most rainfall events only replenished soil water storage and did not significantly recharge ground water or spring flow. These hydrologic observations are very important to understanding the potential for the movement of C. *parvum* oocysts through soils at this and similar locations.

HYDRUS Modeling

Using soil hydraulic properties at the site, and a range of initial water content conditions and precipitation, HYDRUS-1D was used to study the conditions under which significant internal drainage, and hence particle transport, could occur in the profile. Figure 2 shows that for average volumetric water content, θ_v, in the profile of less than ~16%, no internal drainage will occur for precipitation events of up to 50 mm (Young et al., 2000). Water drainage and particle transport through preferential flow pathways is more likely to occur for wetter conditions (e.g., volumetric water content and antecedent SWS (SWS_i) are at or above about 16.5% and 250 mm, respectively).

Microsphere Transport Through Soil

Table 2 presents details of samples from which positive identification of microsphere beads were made prior to December 2000. More than 85% of positive detection of microspheres occurred when SWS exceeded 250 mm. Positive detection in samples from the zero tension lysimeter indicates vertical and lateral transport of beads. Positive detection in monitoring wells indicates that microspheres were transported to, and traveled along the water table. Detection at the spring showed movement both through soil and along water table. Although the absolute number of microspheres detected in samples was very small compared to what was injected into the soil, the finding is significant since infection doses for *cryptosporidiosis* are very low.

Microsphere Bead Transport in Laboratory Columns

Kong et al (2000) reported a strong dependence of microsphere bead transport on soil particle size and pore-water velocity based on their laboratory column study using the same type of beads as used here, and soil material from the research site. They suggest that

Table 2. Microsphere transport up to Dec. 2000

Collection Method [a]	Location	#samples collected	#samples with beads	Percent positive
ZTL	30-cm depth	4(2/2)	3(2/1) [b]	100/66
ZTL	60-cm depth	4(2/2)	1(0,1) [b]	0/50
ZTL	90-cm depth	5(2/3)	4(2/2) [b]	100/66
Well	10-m from spring	44	6	13.6
Well	5-m from spring	53	9	17.0
FLC	At spring	37	8	21.6
SIGMA	At spring	954	21	2.2

a - ZTL- zero tension lysimeter; FLC-flow-through centrifuge; SIGMA- spring water sampler

b – indicates measurement (inside/outside) injection area

in natural soils, the majority of C. *parvum* oocysts were likely to be retained near the soil surface in the absence of preferential flow paths or soil water conditions that facilitated transport mechanisms.

SUMMARY AND CONCLUSIONS

This research looked at potential mechanisms for transport of C. *parvum* oocysts through unsaturated soil to surface and subsurface water sources using microspheres as surrogates. An area 30 by 40-m that was hydrologically connected to a spring was intensively instrumented to monitor hydrologic events and microsphere transport after injecting nearly 10-billion beads near the soil surface about 12-m upstream of the spring.

Internal drainage in soil profile controlled the rapid response of the spring flow following precipitation events. Water drainage and particle transport through preferential flow pathways is more likely when soil water storage is elevated. Matrix-dominated flow for reduced soil water storage will lead to significant filtration of microspheres in upper soil layers. Positive identification of beads in fluid samples in zero tension lysimeters buried up to 90-cm from the surface indicates vertical and lateral transport of microspheres through soil. Positive detection in wells indicates microspheres were transported to, and traveled along the water table. Subsurface transport of *cryptosporidium* would most likely occur during period of high soil water storage and precipitation, typically in late Winter and early Spring in the Southern Piedmont.

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