



## Pathogen and chemical transport in the karst limestone of the Biscayne aquifer:

### 3. Use of microspheres to estimate the transport potential of *Cryptosporidium parvum* oocysts

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[1] The vulnerability of a municipal well in the Northwest well field in southeastern Florida to potential contamination by *Cryptosporidium parvum* oocysts was assessed in a large-scale, forced-gradient (convergent) injection and recovery test. The field study involved a simultaneous pulse introduction of a nonreactive tracer (SF<sub>6</sub>, an inert gas) and oocyst-sized (1.6, 2.9, and 4.9 μm diameter) carboxylated polystyrene microspheres into karst limestone of the Biscayne aquifer characterized by a complex triple (matrix, touching-vug, and conduit) porosity. Fractional recoveries 97 m down gradient were inversely related to diameter and ranged from 2.9% for the 4.9 μm microspheres to 5.8% for 1.6 μm microspheres. Their centers of mass arrived at the pumping well approximately threefold earlier than that of the nonreactive tracer SF<sub>6</sub> (gas), underscoring the need for use of colloid tracers and field-scale tracer tests for these kinds of evaluations. In a modified triaxial cell using near in situ chemical conditions, 2.9 and 4.9 μm microspheres underestimated by fourfold to sixfold the attachment potential of the less electronegative 2.9–4.1 μm oocysts in the matrix porosity of limestone core samples. The field and laboratory results collectively suggested that it may take 200–300 m of transport to ensure even a 1-log unit removal of oocysts, even though the limestone surfaces exhibited a substantive capability for their sorptive removal. The study further demonstrated the utility of microspheres as oocyst surrogates in field-scale assessments of well vulnerability in limestone, provided that differences in attachment behaviors between oocysts and microspheres are taken into account.

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#### 1. Introduction

[2] Karst aquifers are important sources of drinking water in many parts of the world. However, these aquifers are highly vulnerable to contamination by pathogens, because they can include solution-enlarged conduits and fracture systems that can result in rapid transport along preferential flow paths. Contamination of karst aquifers by *Cryptosporidium parvum*, a waterborne pathogenic protist that causes severe intestinal infection, is a concern in areas where shallow karst systems are the sources of drinking water. This concern is exacerbated by the fact that oocysts (the encysted, environmental form of this parasite) typically exhibit low infective doses (ID<sub>50</sub> values as low as one to nine oocysts) [Okhuysen *et al.*, 1999], long (weeks to

months) survival in aquatic environments [Pokorny *et al.*, 2002], and high resistance to chlorination [Carpenter *et al.*, 1999], the most common method of chemical disinfection in the United States. Although the standards for treated groundwater and surface water are the same, groundwater often requires fewer treatment operations to attain those standards than surface water. Consequently, occurrence of oocysts in aquifers characterized by high fluxes and velocities can create serious public health concerns for water supply companies, municipal water districts, and users of individual wells. Karst aquifers are capable of transporting a wide spectrum of particle types and sizes [Atteia and Kozel, 1997; Mahler *et al.*, 2000]. Much has been learned about the transport of virus-sized particles in karst aquifers [Hötzl *et al.*, 1991; Paul *et al.*, 1995; Rossi *et al.*, 1998], but very little is known about the fate and transport of oocysts in such systems.

[3] *C. parvum* oocysts are ubiquitous in surface waters throughout much of North America [e.g., LeChevallier *et al.*, 1991]. Consequently, shallow, karst limestone aquifers hydraulically connected to surface waters, such as the Biscayne aquifer in southeastern Florida, may be particularly

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vulnerable to contamination by oocysts. The proposed expansion of extractive limestone-mining activities in the immediate vicinity of the Northwest well field (NWWF) has attracted widespread attention [Renken *et al.*, 2005] because the resulting borrow pit lakes bring potentially contaminated surface waters within close proximity of Florida's largest municipal well field, in some cases within a few hundred meters. Many of the existing borrow pit mines have been excavated to depths of 12–18 m, an interval approximately equivalent to the depth of the well field's primary production zone. As of 2006, Miami-Dade County water treatment was limited to lime softening, sand/anthracite filtration, and subsequent chlorination; however, the potential for transport of *C. parvum* oocysts through the Biscayne aquifer remains a significant public health concern because of their high resistance to chlorine disinfection. Also, it has been documented that sand filtration sometimes fails to effectively remove these pathogens resulting in outbreaks of cryptosporidiosis [e.g., Mac Kenzie *et al.*, 1994; Rose, 1997].

[4] The overall objective of our study was to assess the vulnerability of the municipal supply wells within the NWWF to contamination by *C. parvum* oocysts from existing and newly proposed borrow pit lakes. This paper focuses on the outcome and interpretation of an in situ (injection-and-recovery) colloidal transport tracer test conducted on 5 February 2004 and supporting laboratory studies. The field study involved a pulse release (“instantaneous”) injection of three sizes of carboxylated polystyrene microspheres within the NWWF [Renken *et al.*, 2008] at a distance of 97 m from municipal well S-3164. The sizes of the inert, nontoxic microspheres (1.6 to 4.9  $\mu\text{m}$  diameters) were chosen to bracket the size ranges of most oocysts. Additional information sought in the studies described herein included the efficacy of Biscayne aquifer karst limestone for removing oocyst-sized particles on an environmentally relevant field scale and, under more controlled laboratory conditions, the extent by which oocysts and oocyst-sized microspheres become immobilized in the matrix porosity of limestone core samples taken from the field site.

## 2. Methods and Materials

### 2.1. Microspheres and Oocyst

[5] For safety and permitting reasons, oocysts were not used in the NWWF field study, whereas both microspheres and oocysts were used in the lab. The field study employed a polydispersed mixture that included  $1.6 \pm 0.1$ ,  $2.9 \pm 0.1$ , and  $4.9 \pm 0.2$   $\mu\text{m}$  carboxylated, polystyrene microspheres (Polysciences, Warrington, Pennsylvania; Molecular Probes, Eugene, Oregon) that bracketed the size ranges of oocysts used in our study (2.9–4.1  $\mu\text{m}$ ). To facilitate differentiation of each size class, the 2.9  $\mu\text{m}$  microspheres were labeled with brilliant blue fluorescence (type BB, 365 nm excitation and 445 nm emission), whereas the 1.6 and 4.9  $\mu\text{m}$  microspheres, which could be easily differentiated on the basis of size, were both labeled with yellow-green fluorescence (type YG, 434 nm excitation and 521 nm emission). Laboratory columns employed initial concentrations of  $10^6$ – $10^8$  microspheres/mL. For the field test, an initial concentration  $5.38 \pm 0.22 \times 10^8$ /mL of 1.6  $\mu\text{m}$  microspheres was added to the aquifer, whereas the more expensive 2.9 and

4.9  $\mu\text{m}$  microspheres were added at  $6.57 \pm 0.06 \times 10^5$ /mL and  $2.59 \pm 0.02 \times 10^5$ /mL, respectively.

[6] Formalin-inactivated *C. parvum* oocysts were obtained from Sterling Parasitology Laboratory (SPL), University of Arizona, Tucson. Oocysts were harvested from a calf infected with the “Iowa” isolate of *C. parvum* (Harvey Moon, National Animal Disease Center, Ames, Iowa), purified at SPL by centrifugation using discontinuous sucrose/cesium chloride gradients [Brush *et al.*, 1998], resuspended in a solution containing formaldehyde (5%), Tween 20 (0.01%), NaCl (0.85%), and three antibiotics (111 U/mL penicillin, 111 U/mL streptomycin, 56  $\mu\text{g}$ /mL gentamicin), and stored at 4°C [Abudalo *et al.*, 2005]. Oocysts were pelleted from the formalin solution by centrifugation (3500 g, 5°–10°C, 30 min), stained with the DNA-specific fluorescent dye DAPI (4,6-diamidino-2-phenylindole) at 0.1 mg/L (final concentration) for 15 min, and resuspended in artificial groundwater (AGW) at final concentrations of  $10^4$  to  $10^6$  oocyst/mL. Optimal excitation and emission wavelengths were 359 and 461 nm, respectively.

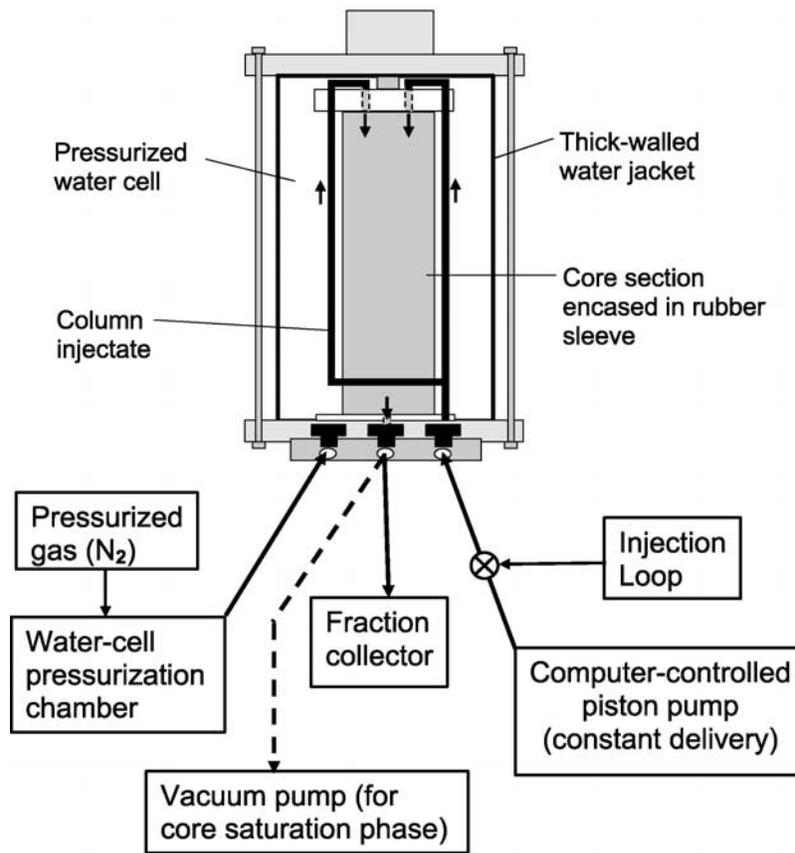
### 2.2. Enumerations

[7] Two methods (epifluorescence direct counting and flow cytometry) were used to quantify differentially the 1.6, 2.9, and 4.9  $\mu\text{m}$  microspheres in municipal well water samples and oocysts in the limestone core studies. On-site enumerations were performed at 500–1260 total magnifications using an Optiphot-2 model epifluorescence microscope (Nikon Corporation) equipped with a 100 W Hg lamp, FITC/AO and DAPI filter cubes [Harvey *et al.*, 1989]. Groundwater containing oocysts and (or) microspheres were filtered (0.34 bar) onto black, 1.0  $\mu\text{m}$  pore size polycarbonate membranes (Osmonics). On average, 100–300 microspheres or oocysts were counted using the epifluorescent microscope during examinations of each membrane filter.

[8] Flow cytometry was employed to enumerate microspheres in the column studies and as a check for the epifluorescence enumerations performed at the field site. The flow cytometer (Biorad, Hercules, California, model HS Bryte) utilizes 3 colors and Apogee 1.3 software (Apogee, London, UK). For each sample of column eluent and groundwater collected from the production well, a 120  $\mu\text{L}$  subsample was analyzed at a flow rate of 1–5  $\mu\text{L}/\text{min}$  and a pressure of 2.8 kPa. The carrier fluid was filtered (0.1  $\mu\text{m}$  pore size) Milli-Q water. A mercury lamp (OSRAM, HBO 103 W/2) was used to excite the DAPI and fluorescein stains and a combination FITC-520-DAPI filter cube (395 and 496 nm maximum peak excitations, respectively) was used to separate the emission spectra. Emission spectra specific to the microspheres were filtered with Biorad HS Bryte fluorescence channel cubes (OR1 and GR2). Fluorescent particle emissions were captured with three down-beam fluorescence channel photomultiplier tubes.

### 2.3. Physicochemical Measurements

[9] Physicochemical characteristics were compared to better evaluate the utility of carboxylated polystyrene microspheres as surrogates for oocysts in the field environment. Parameters included aspect ratio (length/width), diameter, buoyant density, and zeta potential ( $\zeta$ ) at pH 7.0 and at pH 7.8, which bracket the pH range (7.1–7.8) reported for the Biscayne aquifer [Radell and Katz, 1991].



**Figure 1.** Modified triaxial cell design for comparing transport behaviors of *C. parvum* oocysts and similar-sized carboxylated polystyrene microspheres in intact karst limestone cores.

[10] Aspect ratios (length/width) were determined at 788–1260 magnification using epifluorescence microscopy/computer-coupled image analysis and checked by flow cytometry. This involved a Nikon Optiphot II (Nikon, Buffalo, New York) epifluorescence microscope fitted with a Dage SIT66 black and white camera connected to an ITC (Image Technology Corporation, Deer Park, New York) image processor.

[11] Buoyant density determinations were performed using the method of Wolff [1975]. This method involved creating density gradients within transparent, 50 mL tubes (Nalgene) by using a 1.131 g/mL colloidal silica suspension (Percoll I solution, Sigma Chemical Company). After the suspensions were diluted with 0.15 M NaCl, the tubes were spun for 30 min at 15000 g in a refrigerated centrifuge (Dupont, Sorvall RC-5B). Brightly colored density marker beads (Sigma Chemical Company) were used to indicate specific buoyant density values along the longitudinal axis of the tubes. Measured (2.5 mL) suspensions of microsphere or oocyst were carefully layered on the top of the preformed gradients, spun at 15000 g for 1 h, and their buoyant densities estimated by the position of their bands relative to those of the marker beads.

[12] Electrophoretic mobility of oocysts and microspheres were assessed by laser Doppler microelectrophoresis (Particle Sizing Systems, 380 ZLS) at pH 7.0 and 7.8 in artificial groundwater (AGW) with an ionic strength of  $10^{-2}$  M, an alkalinity of 308 mg/L (as  $\text{CaCO}_3$ ), and chloride, sodium, calcium, and magnesium concentrations of 2.2, 3.2,

1.8, and 0.35 mM, respectively [Osborn, 2004], in order to reasonably approximate the groundwater chemistry around the NWWF. However, a slightly higher pH (8.0 versus 7.5) was chosen to facilitate buffering the pH. Electrophoretic mobilities were converted to zeta ( $\zeta$ ) potential using the Smoluchowski approximation [Abudalo et al., 2005]. Zeta potential refers to the electrostatic potential generated by accumulation of ions at the surface of a colloidal particle. The relevance of zeta potential to transport of microspheres or oocysts through Biscayne aquifer limestone is that the higher the absolute zeta potential (the greater the net positive or negative charge), the greater the Coulombic attraction to areas of oppositely charged surface on the limestone.

#### 2.4. Limestone Column Studies

[13] Flow-through column studies incorporating a triaxial cell (Figure 1) were conducted in order to assess degrees of removal of oocyst-sized colloids that might occur within the matrix porosity of the limestone, which could not be measured in the field test, and to compare the relative affinities of oocysts and microspheres for limestone surfaces. Two intact 10 cm (diameter) cylindrical samples of limestone core (Figure 2) were obtained from a test core hole adjacent to production well S-3168 at the NWWF and cut to 16.5 cm lengths. One core sample (sample 30) was obtained from a depth interval between 13.7–13.9 m below land surface (bls) from a limestone classified as a sandy skeletal wackestone and packstone [Dunham, 1962] dominated by fossil moldic pores. The other core sample (sample 18) was obtained from a shallower depth (6.0–6.3 m bls) charac-



**Figure 2.** Photographs of limestone core samples (a) 18 and (b) 30. Both limestone cores are examples of matrix porosity from a test core hole adjacent to production well S-3168 at the Northwest well field [see Renken *et al.*, 2008, Figure 1]. Core sample 18 was taken from interval depth of 6.0–6.3 m. Core sample 30 was taken from interval depth of 13.7–13.9 m. Note the greater visible (secondary) porosity in core 18 versus core 30.

terized by a more porous and permeable limestone (Table 1) classified as a pelecypod floatstone with a matrix of skeletal wackestone and packstone [Embry and Klovan, 1971]. Fossil molds are the principal pore type of the floatstone.

[14] The triaxial cell housing the intact core samples was run in an upflow mode. Dry limestone core samples (10 cm diameter) were encased within three concentric, 0.63 mm thick latex sleeves (3D Polymers, Gardena, California) that conformed to the irregular porous limestone core surface in order to preclude short-circuiting along the exterior core boundaries. To saturate the limestone core, a negative pressure ( $\sim 81$  kPa) was applied for 1 h in order to evacuate air from the interconnected pore spaces. AGW was then introduced through the inlet valve for 1 h under the same negative pressure until the flow rates exiting and entering the core were equal. A water pressure cell and compressed nitrogen gas were used to apply an external pressure to the exterior of the core that was 34 kPa greater than the internal core pressure in order to ensure that the rubber sleeves tightly conformed to the somewhat irregular outside surfaces of the core samples. An injection loop, which was agitated to minimize settling of particles, provided a “step injection” delivery of injectate (nitrate, a nonreactive tracer, and microspheres and/or *C. parvum* oocysts or both). Flow of AGW through the column (20 mL/min,  $\sim 1.6$  cm/min) was provided with two high-precision piston pumps (ISCO model LC5000) controlled to operate sequentially. Eluent was continuously collected using a fraction collector.

## 2.5. Biscayne Aquifer Field Test

[15] Approximately 164 L of groundwater (formation water collected from the injection zone) containing the microspheres and  $\sim 172$  L of groundwater containing the nonreactive tracer SF<sub>6</sub> (gas) were pumped into an interval of injection well G-3817 (see Figure 3) isolated by a downwell packer device built specifically for this experiment. A detailed description of the injection apparatus is given by Shapiro *et al.* [2008]. The isolated interval included a 0.8 m thick, high-permeability groundwater flow zone [Renken *et al.*, 2005]. The microspheres and conservative tracer (dissolved SF<sub>6</sub>) were pumped from separate tanks simultaneously downhole using a system of valves and pumps [Shapiro *et al.*, 2008]. Total numbers of 1.6, 2.9, and 4.9  $\mu\text{m}$  diameter microspheres added to the borehole during a

**Table 1.** Comparison of Physical/Hydrologic Properties of the Two Core Samples Employed in the Flow-Through Column Studies

Parameter	Physical Characteristics	
	Core Sample 18	Core Sample 30
Limestone rock type <sup>a</sup>	Pelecypod floatstone with a limestone matrix of skeletal wackestone and packstone	Sandy skeletal wackestone and packstone
Porosity	Matrix pore system composed of mainly fossil molds	Matrix pore system composed of mainly fossil molds
Core sample diameter, cm	10	10
Core sample length, cm	16.5	16.5
Depth of core sample, m	6.0–6.3	13.7–13.9
Vertical hydraulic conductivity, <sup>b</sup> cm/s	$2.6 \times 10^{-2}$	$0.5 \times 10^{-2}$
Helium porosity, <sup>c</sup> %	37	25
Measured dry mass, g	2100	2580

<sup>a</sup>Dunham [1962] and Embry and Klovan [1971].

<sup>b</sup>Porosity measurements made by Core Laboratories (Midland, Texas).

<sup>c</sup>Calculated from air permeability values measured by Core Laboratories (Midland, Texas) using a factor of 1 millidarcy equal to 0.0835 cm/d.



**Figure 3.** Digital image of the borehole wall that spans the injection interval in injection well G-3816 [see *Renken et al.*, 2008, Figure 1]. Note the major groundwater flow zone near the base of the injection interval with a pore system characterized by touching-vug porosity and overlying limestone with a pore system mainly characterized by matrix porosity [Cunningham *et al.*, 2006a, 2006b]. Injection well G-3816 is a horizontal distance of 1 m from injection well G-3817.

19.5 min interval were  $9.5 \times 10^{13}$ ,  $1.2 \times 10^{11}$ , and  $4.5 \times 10^{10}$ , respectively. This was followed by addition of a “chaser” volume ( $\sim 380$  L of formation water containing no microspheres or conservative tracers) that helped ensure complete displacement of the injectate from the borehole into the limestone aquifer.

[16] Following the injection of microspheres and SF<sub>6</sub>, groundwater samples were collected periodically from production well S-3164, located 97 m down gradient from the injection well G-3817 and analyzed for each size class of microspheres. The pumping rate of the production well was

initially 476 L/s and remained relatively steady during the first 50 h of the test. In addition to the groundwater samples collected in acid-washed polypropylene bottles for epifluorescence microscopy enumerations, 3 L volumes of groundwater were concentrated onto 47 mm diameter, 1  $\mu$ m pore size cellulose acetate filters (Gelman), and resuspended in deionized water (Milli Q, 18 M $\Omega$  resistivity) for subsequent microsphere analysis by flow cytometry. Samples were collected every 15 min during the first 12 h, every 30 min during the next 12 h, and every hour thereafter. Only data obtained during the first 26 h following injection were used because subsequent microsphere concentrations were too low to enumerate accurately. Separate samples were collected for the purpose of assessing concentrations of conservative tracers. Breakthrough curves (concentration histories) were determined for each size class of microspheres. Total numbers of microspheres transported to well S-3164 were estimated for each size class using numerical integrations of the respective breakthrough curves and the pumping rates corresponding to the time the samples were taken.

### 3. Results

#### 3.1. Laboratory Comparisons of Oocysts and Microspheres

[17] A comparison of physicochemical characteristics that influence the degrees of settling (buoyant densities, aspect ratios, and average diameters) and attachment (zeta potentials at pH 7.5) is detailed in Table 2 for oocysts and 4.9  $\mu$ m microspheres. Aspect ratios of oocysts were, on average, slightly greater than those of the 1.6, 2.9, and 4.9  $\mu$ m diameter microspheres, although both were nearly spherical. The *C. parvum* oocysts used in these evaluations exhibited a range of buoyant densities (1.03–1.07 g/cm<sup>3</sup>) that bracketed that of the microspheres (1.05 g/cm<sup>3</sup>). However, under ionic strength (10<sup>-2</sup> M) and near-neutral to slightly alkaline conditions typical of the Biscayne aquifer [Radell and Katz, 1991], carboxylated microspheres exhibited substantially more negative zeta potentials than those measured for the oocysts (Table 2). In a preliminary study involving static minicolumns, AGW, and fragments of crushed limestone core, both oocysts and 4.9  $\mu$ m microspheres exhibited a tendency for increasing sorption to limestone surfaces in response to increasing dissolved calcium from 0–2 mM [Osborn, 2004].

#### 3.2. Transport Through Limestone Core Samples

[18] Substantial core-to-core differences in microsphere transport behavior were observed for two limestone core samples described in detail in Table 1 and representative of matrix porosity [Cunningham *et al.*, 2006a, 2006b]. Transport of two sizes (2.9 and 4.9  $\mu$ m) of microspheres occurred through 16.5 cm of core sample 30 containing 25% helium porosity. Peak abundances of the two sizes of microspheres were coincident with that of the conservative tracer (nitrate) (Figure 4), although fractional recoveries for the 2.9 and 4.9  $\mu$ m microspheres (Table 3) were only 3.6 and 2.1%, respectively, compared with a fractional recovery of 91% observed for the nitrate. Most (>73%) of the nitrate recovered from the column eluted within the first collected pore volume and only a modest degree of “tailing” was observed.

**Table 2.** Comparison of Properties for *Cryptosporidium Parvum* Oocysts and 2, 3, and 5  $\mu\text{m}$  Size Classes of Carboxylated Polystyrene Microspheres Used in the Northwest Well Field Study

Property	<i>C. parvum</i> Oocysts	Carboxylated Polystyrene Microspheres		
Source of colloid	Parasitology Lab, University of Arizona at Tucson	Polysciences	molecular probes	Polysciences
Size class, $\mu\text{m}$	3–5	2	3	5
Average diameter, $\mu\text{m}$	$3.6 \pm 0.3$	$1.6 \pm 0.1$	$2.9 \pm 0.1$	$4.9 \pm 0.2$
Aspect ratio	1.06	1.03	1.03	1.03
Buoyant density, $\text{g}/\text{cm}^3$	1.03–1.07	1.05	1.05	1.05
Zeta potential at pH 7.0, mv	$-3.6^a$	ND <sup>b</sup>	ND	$-18.7^a$
Zeta potential at pH 7.8, mv	$-6.7^a$	ND	ND	$-21.0^a$

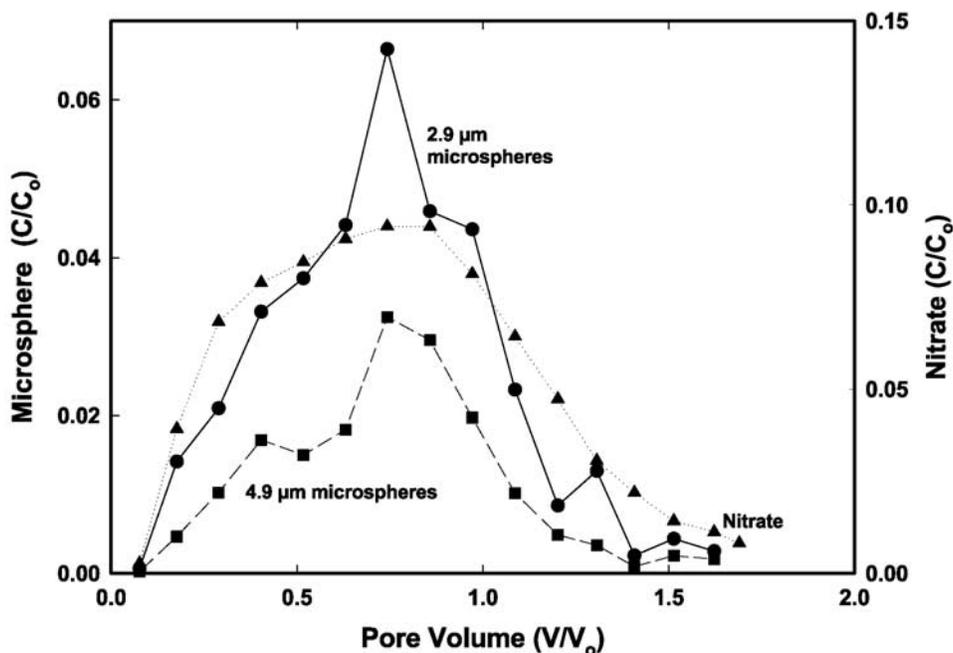
<sup>a</sup>Measurements made at  $10^{-2}$  ionic strength using artificial groundwater.

<sup>b</sup>ND means not determined.

[19] In contrast to the transport study in core sample 30, peak abundances for the two size classes of microspheres eluting through 16.5 cm of the more porous (37% helium porosity) core sample 18, which was taken at a shallower depth, preceded that of the nitrate by about 0.3 pore volumes and the center of mass for the microsphere breakthrough curves preceded that for the nitrate by about 0.4 pore volumes (Figure 5). Most of the microspheres were also retained within the column, similar to the study involving core sample 30. However, fractional recoveries for the 2.9 and 4.9  $\mu\text{m}$  microspheres (4.8 and 3.3%, respectively) were  $\sim 1.5$  times higher than that for sample 30. The oocysts were subject to about fourfold and sixfold less immobilization than the 2.9 and 4.9  $\mu\text{m}$  carboxylated microspheres, respectively (Table 3). In contrast to the earlier study involving core sample 30, substantial tailing of nitrate was observed.

### 3.3. Transport Through the Biscayne Aquifer

[20] Transport parameters involving breakthrough of the 1.6, 2.9, and 4.9  $\mu\text{m}$  microspheres at the pumping well (S-3164) after 97 m of transport through the karst limestone of the Biscayne aquifer are summarized in Table 4. First detection of the 1.6  $\mu\text{m}$  microspheres at well S-3164 was observed 4.7 h after they were introduced to the injection borehole 97 m up gradient. First detection of the 2.9 and 4.9  $\mu\text{m}$  microspheres, which were introduced at substantially lower concentrations, did not occur until 5.5 and 5.7 h, respectively, after their introduction to the aquifer. The appearance of the peak concentrations of microspheres at well S-3164 occurred within 2 h of first detection for all three sizes. However, the elapsed time between injection and detection of the peak abundance varied from 6.3 h for the 2.9  $\mu\text{m}$  microspheres to 7.3 h for the 4.9  $\mu\text{m}$  microspheres.



**Figure 4.** Dimensionless concentration histories for nitrate (conservative tracer) and 2.9 and 4.9  $\mu\text{m}$  fluorescent microspheres after transport through core sample 30 that is representative of pore class II [Cunningham *et al.*, 2006a] taken from 13.7–13.9 m below land surface at the Northwest well field.  $C_0$  is the influent concentration,  $C$  is the effluent concentration,  $V_0$  is the measured void volume, and  $V$  is the cumulative volume of eluent collected.

**Table 3.** Fractional Recoveries of *Cryptosporidium Parvum* Oocysts, Carboxylated Microspheres, and Nitrate (a Conservative Solute) in Flow-Through Columns of Karst Limestone Taken From the Northwest Well Field, Miami-Dade County, Florida

Tracer	Fractional Recoveries (Percent of Amount Injected)	
	Core Sample 18 <sup>a</sup>	Core Sample 30 <sup>b</sup>
2.9 $\mu\text{m}$ microspheres	4.8	3.6
4.9 $\mu\text{m}$ microspheres	3.3	2.1
3–4 $\mu\text{m}$ <i>C. parvum</i> oocysts	19	ND <sup>c</sup>
Nitrate	64	92

<sup>a</sup>Core 18 taken at the 6.0–6.3 m depth. Total helium porosity is 37%.

<sup>b</sup>Core 30 taken at the 13.7–13.9 m depth. Total helium porosity is 25%.

<sup>c</sup>Not determined because *Cryptosporidium parvum* oocysts were not injected into this core.

[21] Patterns of breakthrough at well S-3164 were similar among the three sizes of microspheres, although overall removal was clearly size-dependent (Figure 6). Fractional recoveries were inversely related to microsphere diameter and ranged from 2.9% for the largest (4.9  $\mu\text{m}$ ) size to 5.8% for the smallest (1.6  $\mu\text{m}$ ) size (Table 4). In general, microspheres that moved through the 97 m of Biscayne aquifer limestone did so much more rapidly than did SF<sub>6</sub>. For microspheres and SF<sub>6</sub> recovered at well S-3164, the center of mass of the SF<sub>6</sub> was retarded relative to that of the microspheres by a factor of about 3.

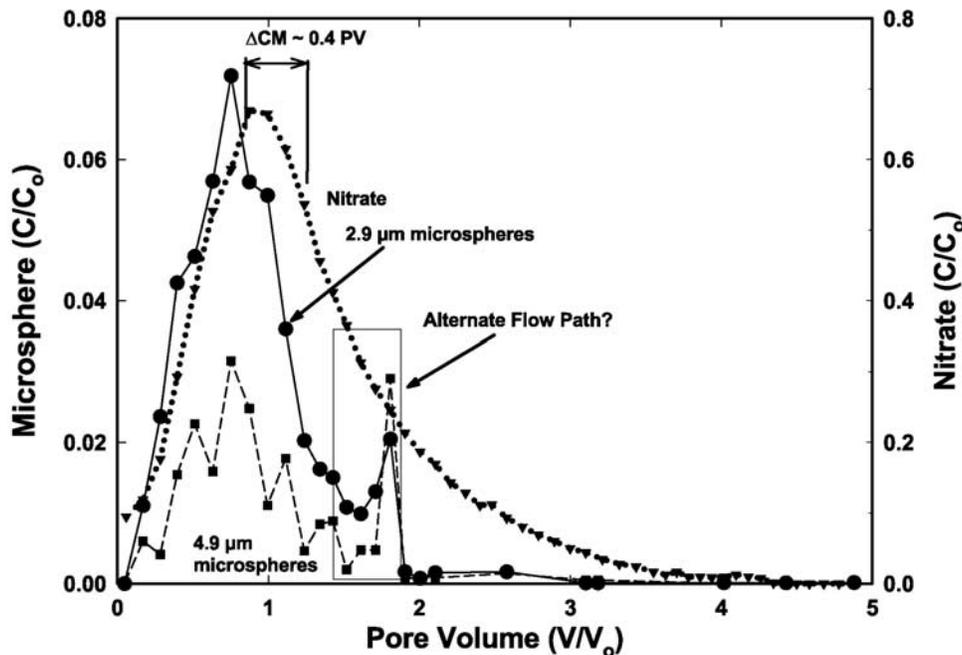
[22] The concentration history for the 1.6  $\mu\text{m}$  microspheres at well S-3164 was characterized by a sharp initial peak followed by smaller secondary peaks (Figure 7). The two methods used to enumerate the microspheres (epifluorescence microscopy and flow cytometry) resulted in similar-

shaped breakthrough curves. There was a strong correlation ( $r^2 = 0.94$  at  $p < 0.0001$ ) between counts made by the two methods (Figure 7 inset). However, microsphere abundances determined by flow cytometry were consistently a bit lower, most likely because of a systematic incomplete recovery of microspheres from the filter used to concentrate them from well water.

## 4. Discussion

### 4.1. Microspheres as Surrogates

[23] At the time of the field test, the utility of 2–5  $\mu\text{m}$  diameter polystyrene microspheres as surrogates of *C. parvum* oocysts in a limestone aquifer had not been previously evaluated. They were used as an abiotic surrogate at the NWWF because they are nonhazardous in tracer applications [Behrens *et al.*, 2001], chemically inert, negatively charged, easy to detect, available in a wide variety of size classes, and their aspect ratios and buoyant densities are reasonably similar to those of *C. parvum* oocysts (Table 2). Recent tracer tests involving karst aquifers in Germany, Slovenia, and Switzerland have employed bacteria-sized (1  $\mu\text{m}$ ) diameter microspheres [Auckenthaler *et al.*, 2002; Goldscheider *et al.*, 2003; Göppert *et al.*, 2005; Göppert and Goldscheider, 2007]. Although 1  $\mu\text{m}$  carboxylated microspheres were determined to be a poor analog for simulating the subsurface transport behaviors of bacteria in a well-sorted, sandy glacial outwash aquifer in Cape Cod, Massachusetts [Harvey and Garabedian, 1991], larger (2 and 3  $\mu\text{m}$ ) carboxylated microspheres reasonably captured the transport behavior of the 2–3  $\mu\text{m}$  groundwater protist, *Spumella guttula* Kent [Harvey *et al.*, 1995]. Consequently, it



**Figure 5.** Dimensionless concentration histories for nitrate (conservative tracer) and 2.9 and 4.9  $\mu\text{m}$  fluorescent microspheres in the effluent column of core sample 18 taken from the 6.0–6.3 m depth of Northwest well field at well S-3168.  $C_0$  is the influent concentration,  $C$  is the effluent concentration,  $V_0$  is the measured void volume, and  $V$  is the cumulative volume of eluent collected.  $\Delta\text{CM}$  refers to the difference in the centers of mass for the 2.9  $\mu\text{m}$  microsphere and the nitrate breakthrough curves.

**Table 4.** Fractional Recoveries of Carboxylated Polystyrene Microspheres After 97 m of Transport Through the Biscayne Aquifer at the Northwest Well Field, Miami-Dade County, Florida

Parameter	Microsphere Size		
	1.6 $\mu\text{m}$	2.9 $\mu\text{m}$	4.9 $\mu\text{m}$
Time of first detection, h	4.75	5.5	5.75
Time of peak concentration, h	6.75	6.25	7.25
Fractional recovery, %	5.8	3.9	2.9

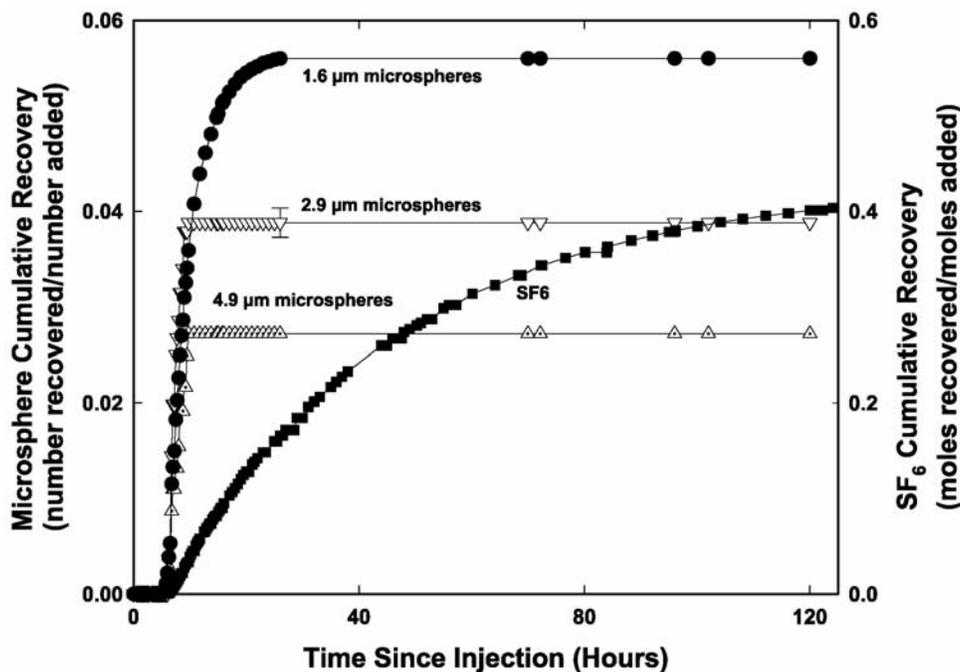
was suggested that microspheres may be useful as abiotic analogs for *C. parvum* oocysts in subsequent field studies.

[24] It was expected that the sorptive behavior of the microspheres in the presence of the karst limestone might differ from that of the oocysts of similar size because of the difference in the surface electrical properties [Dai and Hozalski, 2003]. There is considerable disagreement among recent studies that compared attachment and (or) transport behaviors of *C. parvum* oocysts and oocyst-sized carboxylated microspheres. Although some studies involving granular media suggest that transport behaviors of inactivated oocysts and 4–5  $\mu\text{m}$  carboxylated microspheres are similar [Emelko et al., 2003; Tufenkji et al., 2004], other studies suggest that they can be quite different [e.g., Bradford et al., 2005]. In our study, *C. parvum* oocysts at pH 8 and  $10^{-2}$  M ionic strength were transported, respectively,  $\sim 4$  and  $\sim 6$  times more readily through intact limestone core samples characterized largely by matrix porosity than 2.9 and 4.9  $\mu\text{m}$  carboxylated microspheres (Table 3).

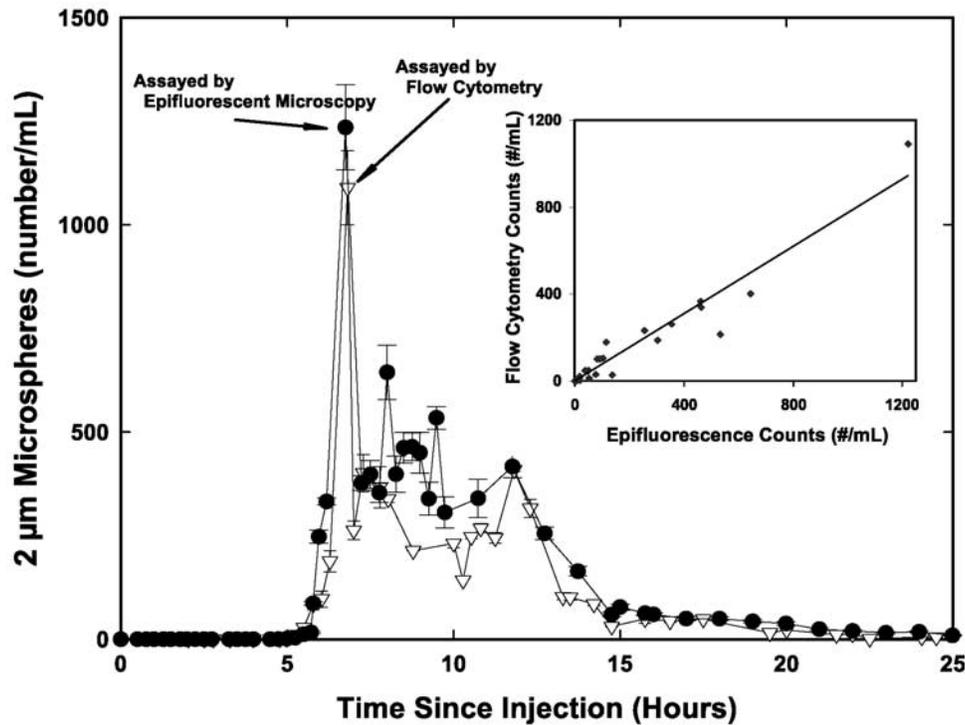
[25] Observed differences in the attachment behaviors of *C. parvum* oocysts and oocyst-sized microspheres have been attributed to variability in electrical properties of the

oocyst surface. Although several reports suggest a very weak surface charge at neutral pH [e.g., Brush et al., 1998], others suggest that oocysts at  $10^{-2}$  and  $10^{-3}$  M ionic strength carry a more substantive negative charge [e.g., Hsu and Huang, 2002]. The apparent contradictions in reported oocyst  $\zeta$  potentials where similar conditions and methods are employed are likely due to sensitivity of surface electrical properties to the source of the oocysts [Butkus et al., 2003], age, exposure to antibiotics, and method of purification [Brush et al., 1998]. In our study, the  $\zeta$  potentials of the oocysts were more negative than those of the carboxylated microspheres under neutral to slightly alkaline conditions. Because mineral surfaces in aquifers are typically characterized by patches of positive charge due to presence of iron and aluminum oxides, oocysts should experience less attachment and more transport than microspheres having a more negative surface charge.

[26] Unfortunately, there is little information about surface properties for oocysts found in natural waters, because of the difficulties in concentrating a large enough number to perform surface measurements. However, the considerable range of surface properties reported for *C. parvum* oocysts suggests that it may be incorrect to assume that *C. parvum* oocysts in aquifers will always be highly charged. For vulnerability assessments involving drinking water aquifers, a more cautious approach should be taken that assumes some oocysts in natural waters may be characterized by low charge, at least until there is more definitive evidence to the contrary. Although carboxylated microspheres proved useful surrogates in the NWWF test, it is recommended that a mixture of different types of microspheres [see Harvey et al., 1989] representing a wider variety of surface charge having carboxylated, neutral, and carbonyl surfaces be



**Figure 6.** Cumulative breakthrough of  $\text{SF}_6$  and the 1.6, 2.9, and 4.9  $\mu\text{m}$  microspheres at well S-3164 in Northwest well field (Miami-Dade County, Florida) after 97 m of transport through karst limestone of the Biscayne aquifer.



**Figure 7.** Concentration history of 1.6  $\mu\text{m}$  microspheres appearing at Northwest well field well S-3164 after 97 m of transport through karst limestone of the Biscayne aquifer (Miami-Dade County, Florida). Circles, counts made using epifluorescence microscopy; triangles, counts made using flow cytometry for microspheres resuspended from a filter. Inset depicts linear regression between microscopy and flow-cytometry counts ( $y = 0.780x - 7.780$ ;  $r^2 = 0.94$  at  $p < 0.0001$ ).

employed in future well field vulnerability assessments regarding this pathogen.

## 4.2. Oocyst Transport Potential in Biscayne Aquifer

### 4.2.1. Time-of-Travel Considerations

[27] Because little was known about the efficiency of karst limestone of the Biscayne aquifer to remove pathogens, time-of-travel well field protection zones were established in 1981 for the NWWF [Metropolitan Dade County, 1980, 1981]. The zones were based upon a numerical groundwater flow model [e.g., Prickett and Lonquist, 1971], existing knowledge of bacterial transport and viral die-off rates, and permitted water withdrawal rates (587,740  $\text{m}^3/\text{day}$ ). As noted by Renken *et al.* [2005], a major uncertainty involved the application of an equivalent continuum model, which oversimplifies aquifer heterogeneities and may poorly represent field conditions [Bear, 1993] in the area of the NWWF. Indeed, in the preliminary 2003 NWWF tracer test and in the current study, the conservative tracer traveled between the injection borehole and production well S3164 one to two orders of magnitude faster than predicted. Depending on whether one assumes that the tracer moved through one or more touching vug flow zones, transport (effective) porosity could range between 0.4 to 0.04 [Renken *et al.*, 2008].

[28] Peak concentrations of microorganisms or microspheres or both have been observed to travel faster through karst limestone aquifers than would be predicted from an in situ transport test employing a nonreactive tracer. Auckenthaler *et al.* [2002] showed that peak breakthrough for the marine bacteriophage H4 traveled almost 50% faster

through 1250 m of a shallow karst aquifer in northern Switzerland than uranine, a conservative tracer. In a tracer test involving a shallow karst aquifer in Germany, the peak concentration of 1  $\mu\text{m}$  diameter microspheres traveled about 10% faster than that of the fluorescent solute tracer, sulforhodamine [Göppert *et al.*, 2005], although Göppert and Goldsheider [2007] have more recently shown that such differences can depend upon the fluid velocity. In the present study, the centers of mass for the three sizes of microspheres appearing at production well S-3164 after 97 m of transport traveled  $\sim 3$  times faster than that of the conservative tracer  $\text{SF}_6$ , suggesting that preferred flow path structure is particularly important for pathogen transport through the Biscayne aquifer near the NWWF. This is likely due, at least in part, to matrix exclusion of the colloids [Lawrence and Hendry, 1996] and underscores the need for employing microbial-sized particles in well vulnerability assessments.

[29] In contrast to the microspheres, breakthrough of  $\text{SF}_6$  displayed a peak concentration followed by a monotonically decreasing concentration that extended over 6 days following the injection of the tracer solutions. Shapiro *et al.* [2008] showed that chemical diffusion into and out of smaller pore openings in the Biscayne limestone affected the  $\text{SF}_6$  breakthrough curve and interpreted the elongated tail as being an artifact of multiple pathways with different mean velocities varying over several orders of magnitude. Although the touching-vug flow zones [Cunningham *et al.*, 2006a, 2006b] are the principal pathways through which most fluids, solutes and particulate tracers migrate in the Biscayne

aquifer, preferential pathways within the touching-vug porosity (porosity consisting of vugs that are interconnected by direct contact) are likely to have evolved *Shapiro et al.* [2008]. That microspheres were not detected after one day following their introduction, whereas substantive breakthrough of SF<sub>6</sub> continued for several days thereafter, suggests particulate tracers may only migrate significant distances through the highest-velocity flow paths in the karst. In the slower velocity flow paths, microspheres should be rapidly attenuated because of settling and filtration.

#### 4.2.2. Removal Efficiency Considerations

[30] Fractional recoveries per unit distance traveled for the microspheres (Table 3) were considerably greater than was observed in earlier injection and recovery tests involving sandy [*Harvey et al.*, 1995] and fractured-rock [*Becker et al.*, 2003] aquifers. Furthermore, microsphere recoveries likely underestimate the transport potential of oocysts within the NWWF for the following reasons. (1) The quantity of microspheres immobilized within the bore hole could not be accurately quantified and, consequently, was not accounted for. (2) Concentrations of microspheres appearing at well S-3164 after the first 26 h were too low to quantify. (3) Our intact column studies suggest that oocysts have less propensity for attachment to the limestone surfaces than the microspheres and, consequently, should be transported through the Biscayne aquifer more readily. Given that the injectate in the borehole was recirculated to preclude settling of microspheres before release, it is estimated that, collectively, the first two sources of error are minor, i.e., <10% of the total microspheres pumped out of the injectate tank. However, differences in propensity for attachment between the microspheres and oocysts would cause a substantial underestimation of oocyst transport potential.

[31] For *C. parvum* oocysts, which are capable of causing disease in very low numbers, it is desirable to have 4–5 log(10) units of removal between contaminant source and well. However, it would be overly optimistic to assume more than one factor of ten removal of oocysts—sized particles per 100 m of travel through the karst limestone underlying the NWWF. Assuming our laboratory observation that oocysts have lower propensities for attachment to limestone than microspheres is applicable to the field, it may take 200–300 m of travel to safely reduce by one log unit the concentration of 3 to 5  $\mu\text{m}$  *C. parvum* oocysts from a hypothetical introduction into the aquifer, depending upon local-scale heterogeneity. Relative to transmissive granular systems, it appears that groundwater extracted by the NWWF may be particularly vulnerable to contamination by protozoan pathogens because of the higher potential for transport. In contrast to the considerable distance required to remove by one-log unit 2–3  $\mu\text{m}$  sized microspheres in the present study, a one-log unit removal occurred in less than a meter for 2–3  $\mu\text{m}$  microspheres being advected through undisturbed, highly transmissive siliciclastic sand and gravel of the Cape Cod, Massachusetts, aquifer [*Harvey et al.*, 1995].

[32] Rapid flow rates and the presence of solution-enhanced porosity have been implicated in the enhanced transport potential of pathogens in karst limestone aquifers. However, additional characteristics of the Biscayne aquifer are likely to facilitate microbial transport. These include

slightly alkaline conditions (which should result in more electrostatic repulsion between oocysts and limestone surfaces), a dearth of iron oxide coatings on surfaces of pores within the limestone of the NWWF, which have been shown to be highly efficient in sorbing microorganisms in granular media [e.g., *Ryan et al.*, 1999, 2002], and high levels (13–18 mg/L) of dissolved organic carbon (DOC). Oocyst transport through porous media appears to be particularly sensitive to the presence of natural organic matter (NOM). For example, the presence of only 5 mg/L of NOM was reported to decrease by more than threefold the removal efficiency for *C. parvum* oocysts being advected through 30 cm of glass beads [*Dai and Hozalski*, 2003]. It was also reported that indicator bacteria appearing at a karst spring was correlated with organic carbon [*Pronk et al.*, 2006] and that NOM was correlated with the transport of *Bacillus subtilis* spores through an alluvial aquifer [*Pang et al.*, 2005].

#### 4.2.3. Mechanisms for Removal

[33] The observation that fractional retention for the microspheres was directly related to diameter suggests that removal by settling and (or) trapping may be significant. Although settling may be important, it does not appear to be the sole mechanism for microsphere removal. This is because the differences in fractional retentions among the three different size classes (1.6, 2.9, and 4.9  $\mu\text{m}$ ) were considerably less than the respective differences in their predicted settling velocities, based upon modifications of Stokes' law for groundwater applications [e.g., *Wan et al.*, 1995]. This suggests that other mechanisms may be operative in this field test. Straining within the matrix porosity of the karst limestone, physical interception, and diffusion are possibilities that still need to be evaluated. Because delineating subsurface structure is scale-dependent, separating the aforementioned effects may not be possible at the field scale.

[34] For biocolloid injection and recovery tests involving granular aquifers, elevated rates of immobilization often occur near their point of introduction, but the rate of loss down gradient is much reduced [e.g., *Dong et al.*, 2006; *Pieper et al.*, 1997]. Consequently, tests involving microbial transport over longer travel distances typically exhibit average per unit distance removal efficiencies that are considerably lower than tests involving the same injection point, but shorter travel distances. Several explanations have been given for these observations, including variations in surface characteristics within the same population of microorganisms [*Albinger et al.*, 1994] and problems related to the filtration model itself [*Li et al.*, 2004]. Neither of these explanations, however, is directly transferable to the problem of microspheres being advected through the Biscayne aquifer. This is because existing colloid filtration theory, which is based upon granular media containing spherical collectors, is not adequate for describing colloidal transport in karst limestone and because microspheres within a manufacturer's lot number should, in theory, have reasonably similar surface characteristics from one microsphere to the next. It is more likely that in the Biscayne aquifer, where colloidal transport might be expected to occur primarily in preferential flow zones characterized by touching-vug or conduit porosity or both, longer travel distances increase the likelihood that significant portions

of the travel will involve these “faster” zones of high macroporosity and correspondingly lower removals.

#### 4.2.4. Summary

[35] The outcome of the February 2004 field test suggests that all three sizes of carboxylated microspheres can be transported rapidly through 97 m of the Biscayne aquifer near NWWF well S-3164. The fast transport of oocyst-sized microspheres, the low removal efficiency relative to other types of aquifers, and the extensive nature of horizontal flow zones of high macroporosity [Cunningham *et al.*, 2006a, 2006b] collectively suggest that additional attention needs to be focused on ameliorating the threats posed by potential future introductions of *Cryptosporidium* into the Biscayne aquifer. Although our laboratory experiments indicate that matrix porosity of the limestone can be effective in removing oocysts, the very early appearance of the centers of mass for the three sizes of microspheres relative to that of the nonreactive tracer in the field study suggests the importance of preferential flow paths for transporting oocyst-sized colloids. The field observations also demonstrate the inadequacy of using total porosity for determining time-of-travel protection zones around the NWWF and underscore the need for employing oocyst-sized colloidal particles in assessments of well vulnerability for aquifers characterized by complex porosity. Use of microspheres in the field test and the supporting laboratory comparisons of oocysts and microsphere attachment yielded valuable information about the potential for transport of *C. parvum* oocysts in the Biscayne aquifer that conservative tracers alone could not provide.

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## References

- Abudalo, R. A., Y. G. Bogats, J. N. Ryan, R. W. Harvey, D. W. Metge, and M. Elimelech (2005), Effect of ferric oxyhydroxide grain coatings on the transport of bacteriophage PRD1 and *Cryptosporidium parvum* oocysts in saturated porous media, *Environ. Sci. Technol.*, *39*, 6412–6419.
- Albinger, O., B. K. Biesemeyer, R. G. Arnold, and B. E. Logan (1994), Effect of bacterial heterogeneity on adhesion to uniform collectors by monoclonal populations, *FEMS Microbiol. Lett.*, *124*, 321.
- Attea, O., and R. Kozel (1997), Particle size distributions in waters from a karst aquifer: From particles to colloids, *J. Hydrol.*, *201*, 102–119.
- Auckenthaler, A., G. Raso, and P. Huggenberger (2002), Particle transport in a karst aquifer: Natural and artificial tracer experiments with bacteria, bacteriophages and microspheres, *Water Sci. Technol.*, *46*, 131–138.
- Bear, J. (1993), Modeling flow and contaminant transport in fractured rocks, in *Flow and Contaminant Transport in Fractured Rock*, edited by J. Bear *et al.*, pp. 1–37, Academic, San Diego, Calif.
- Becker, M. W., D. W. Metge, S. A. Collins, A. M. Shapiro, and R. W. Harvey (2003), Bacterial transport experiments in fractured crystalline bedrock, *Ground Water*, *41*, 682–689.
- Behrens, H., *et al.* (2001), Toxicological and ecotoxicological assessment of water tracers, *Hydrogeol. J.*, *9*, 321–325.
- Bradford, S. A., J. Simunek, M. Bettahar, Y. F. Tadassa, M. T. van Genuchten, and S. R. Yates (2005), Straining of colloids at textural interfaces, *Water Resour. Res.*, *41*, W10404, doi:10.1029/2004WR003675.
- Brush, C. F., M. F. Walter, L. J. Anguish, and W. C. Ghiorse (1998), Influence of pretreatment and experimental conditions on electrophoretic mobility and hydrophobicity of *Cryptosporidium parvum* oocysts, *Appl. Environ. Microbiol.*, *64*, 4439–4445.
- Butkus, M. A., J. T. Bays, and M. P. Labare (2003), Influence of surface characteristics on the stability of *Cryptosporidium parvum* oocysts, *Appl. Environ. Microbiol.*, *69*, 3819–3825.
- Carpenter, C., R. Fayer, J. Trout, and M. J. Beach (1999), Chlorine disinfection of recreational water for *Cryptosporidium parvum*, *Emerg. Infect. Dis.*, *5*, 579–584.
- Cunningham, K. J., M. A. Wacker, E. Robinson, J. F. Dixon, and G. L. Wingard (2006a), A cyclostratigraphic and borehole geophysical approach to development of a three-dimensional conceptual hydrogeologic model of the karst Biscayne aquifer, southeastern Florida., *U.S. Geol. Surv. Sci. Invest. Rep.*, 2005–5235, 69 pp.
- Cunningham, K. J., R. A. Renken, M. A. Wacker, M. R. Zygner, E. Robinson, A. M. Shapiro, and G. L. Wingard (2006b), Application of carbonate cyclostratigraphy and borehole geophysics to delineate porosity and preferential flow in the karst limestone of the Biscayne aquifer, SE Florida, in *Perspectives on Karst Geomorphology, Hydrology, and Geochemistry—A Tribute Volume to Derek C. Ford and William B. White*, edited by R. S. Harmon and C. M. Wicks, *Spec. Pap. Geol. Soc. Am.*, *404*, 191–208.
- Dai, X., and R. M. Hozalski (2003), Evaluation of microspheres as surrogates for *Cryptosporidium parvum* oocysts in filtration experiments, *Environ. Sci. Technol.*, *37*, 1037–1042.
- Dong, H., T. D. Scheibe, W. P. Johnson, C. M. Monkman, and M. E. Fuller (2006), Change of collision efficiency with distance in bacterial transport experiments, *Ground Water*, *44*, 415–429.
- Dunham, R. J. (1962), Classification of carbonate rocks according to depositional textures, in *Classification of Carbonate Rocks*, edited by W. E. Ham, *AAPG Mem.*, *1*, 108–121.
- Embry, A. F., and J. E. Klován (1971), A Late Devonian reef tract on northeastern Banks Island, N.W.T., *Bull. Can. Pet. Geol.*, *19*, 730–781.
- Emelko, M. B., P. M. Huck, and I. P. Douglas (2003), *Cryptosporidium* and microsphere removal during late in-cycle filtration, *J. Am. Water Works Assoc.*, *95*, 173–182.
- Goldscheider, N., H. Hötzl, W. Käss, and W. Ufrect (2003), Combined tracer tests in the karst aquifer of the artesian mineral springs of Stuttgart, Germany, *Environ. Geol.*, *43*, 922–929.
- Göppert, N., and N. Goldscheider (2007), Solute and colloid transport in karst conduits under low and high flow conditions, *Ground Water*, *46*, 61–68, doi:10.1111/j.1745-6584.2007.00373.
- Göppert, N., N. Goldscheider, and H. Hötzl (2005), Transport of colloidal and solute tracers in three different types of alpine karst aquifers—Examples from southern Germany and Slovenia, paper presented at Sinkholes and the Engineering and Environmental Impacts of Karst: 10th Multidisciplinary Conference, Am. Soc. Civ. Eng., San Antonio, Tex., 24–28 Sept.
- Harvey, R. W., and S. P. Garabedian (1991), Use of colloid filtration theory in modeling movement of bacteria through a contaminated sandy aquifer, *Environ. Sci. Technol.*, *25*, 178–185.
- Harvey, R. W., L. H. George, R. L. Smith, and D. R. LeBlanc (1989), Transport of microspheres and indigenous bacteria through a sandy aquifer—Results of natural-gradient and forced-gradient tracer experiments, *Environ. Sci. Technol.*, *23*, 51–56.
- Harvey, R. W., N. E. Kinner, A. Bunn, D. Macdonald, and D. Metge (1995), Transport behavior of groundwater protozoa and protozoan-sized microspheres in sandy aquifer sediments, *Appl. Environ. Microbiol.*, *61*, 209–217.
- Hötzl, H., W. Käss, and B. Reichert (1991), Application of microbial tracers in groundwater studies, *Water Sci. Technol.*, *24*, 295–300.
- Hsu, B. M., and C. P. Huang (2002), Influence of ionic strength and pH on hydrophobicity and zeta potential of giardia and *Cryptosporidium*, *Colloids Surf. A Physicochem. Eng. Asp.*, *201*, 201–206.
- Lawrence, J. R., and M. J. Hendry (1996), Transport of bacteria through geologic media, *Can. J. Microbiol.*, *42*, 410–422.
- LeChevallier, M. W., W. D. Norton, and R. G. Lee (1991), Occurrence of giardia and *Cryptosporidium* spp. in surface water supplies, *Appl. Environ. Microbiol.*, *57*, 2610–2616.
- Li, X. Q., T. D. Scheibe, and W. P. Johnson (2004), Apparent decreases in colloid deposition rate coefficients with distance of transport under unfavorable deposition conditions: A general phenomenon, *Environ. Sci. Technol.*, *38*, 5616–5625.

- Mac Kenzie, 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, and J. B. Rose (1994), A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply, *N. Engl. J. Med.*, *331*, 161–167.
- Mahler, B. J., J. C. Personne, G. F. Lods, and C. Drogue (2000), Transport of free and particulate-associated bacteria in karst, *J. Hydrol.*, *238*, 179–193.
- Metropolitan Dade County (1980), Protection of potable water supply wells program, *Dep. of Environ. Resour. Manage. Tech. Rep. 80-4*, Miami, Fla., 176 pp.
- Metropolitan Dade County (1981), *Adopting the northwest well field time-of-travel protection zones, Resolution R-81-23*, Board of County Comm, Miami, Fla.
- Okhuysen, P. C., C. L. Chappell, J. H. Crabb, C. R. Sterling, and H. L. DuPont (1999), Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults, *J. Infect. Dis.*, *180*, 1275–1281.
- Osborn, C. (2004), The effect of changes in solution chemistry on the attachment of *Cryptosporidium parvum* oocysts and microspheres to limestone and the transport of *Cryptosporidium parvum* oocysts and microspheres in intact limestone cores, M.S. thesis, 105 pp., Univ. of Colo., Boulder.
- Pang, L., M. Close, M. Goltz, M. Noonan, and L. Sinton (2005), Filtration and transport of bacillus subtilis spores and the F-RNA phage MS2 in a coarse alluvial gravel aquifer: Implications in the estimation of setback distances, *J. Contam. Hydrol.*, *77*, 165–194.
- Paul, J. H., J. B. Rose, S. Jiang, C. Kellogg, and E. A. Shinn (1995), Occurrence of fecal indicator bacteria in surface waters and the subsurface aquifer in Key Largo, Florida, *Appl. Environ. Microbiol.*, *61*, 2235–2241.
- Pieper, A. P., J. N. Ryan, R. W. Harvey, G. L. Amy, T. H. Illangasekare, and D. W. Metge (1997), Transport and recovery of bacteriophage PRD1 in a sand and gravel aquifer: Effect of sewage-derived organic matter, *Environ. Sci. Technol.*, *31*, 1163–1170.
- Pokorny, N. J., S. C. Weir, R. A. Carreno, J. T. Trevors, and H. Lee (2002), Influence of temperature on *Cryptosporidium parvum* oocyst infectivity in river water samples as detected by tissue culture assay, *J. Parasitol.*, *88*, 641–643.
- Prickett, T. A., and C. C. Lonquist (1971), *Selected digital computer techniques for groundwater flow modeling*, ISWS Bull. 55, 62 pp., Ill. State Water Surv, Champaign, Ill.
- Pronk, M., N. Goldscheider, and J. Zopfi (2006), Dynamics and interaction of organic carbon, turbidity, and bacteria in a karst aquifer system, *Hydrogeol. J.*, *14*, 473–484.
- Radell, M. J., and B. G. Katz (1991), Major-ion and selected trace-metal chemistry of the Biscayne aquifer, southeast Florida, *U.S. Geol. Surv. Water Resour. Invest. Rep.*, *91-4009*, 18 pp.
- Renken, R. A., A. M. Shapiro, K. J. Cunningham, R. W. Harvey, D. W. Metge, M. R. Zygnerski, M. A. Wacker, C. L. Osborn, and J. N. Ryan (2005), Assessing the vulnerability of a municipal well field to contamination in a karst aquifer, *Environ. Eng. Geosci. J.*, *11*(4), 341–354.
- Renken, R. A., K. J. Cunningham, A. M. Shapiro, R. W. Harvey, M. R. Zygnerski, D. W. Metge, and M. A. Wacker (2008), Pathogen and chemical transport in the karst limestone of the Biscayne aquifer: 1. Revised conceptualization of groundwater flow, *Water Resour. Res.*, *44*, W08430, doi:10.1029/2007WR006058.
- Rose, J. B. (1997), Environmental ecology of *Cryptosporidium* and public health implications, *Annu. Rev. Public Health*, *18*, 135–161.
- Rossi, P., N. Dorfliger, K. Kennedy, I. Müller, and M. Aragno (1998), Bacteriophages as surface and ground water tracers, *Hydrol. Earth Syst. Sci.*, *2*, 101–110.
- Ryan, J. N., M. Elimelech, R. A. Ard, R. W. Harvey, and P. R. Johnson (1999), Bacteriophage PRD1 and silica colloid transport and recovery in an iron oxide-coated sand aquifer, *Environ. Sci. Technol.*, *33*, 63–73.
- Ryan, J. N., R. W. Harvey, D. Metge, M. Elimelech, T. Navigato, and A. P. Pieper (2002), Field and laboratory investigations of inactivation of viruses (PRD1 and MS2) attached to iron oxide-coated quartz sand, *Environ. Sci. Technol.*, *36*, 2403–2413.
- Shapiro, A. M., R. A. Renken, R. W. Harvey, M. Zygnerski, and D. W. Metge (2008), Pathogen and chemical transport in the karst limestone of the Biscayne aquifer: 2. Chemical retention from diffusion and slow advection, *Water Resour. Res.*, *44*, W08430, doi:10.1029/2007WR006059.
- Tufenkji, N., G. F. Miller, J. N. Ryan, R. W. Harvey, and M. Elimelech (2004), Transport of *Cryptosporidium* oocysts in porous media: Role of straining and physicochemical filtration, *Environ. Sci. Technol.*, *38*, 5932–5938.
- Wan, J., T. K. Tokunaga, and C. F. Tsang (1995), Bacterial sedimentation through a porous medium, *Water Resour. Res.*, *31*, 1627–1636.
- Wolff, D. A. (1975), The separation of cells and subcellular particles by colloidal silica density gradient centrifugation, in *Methods in Cell Biology*, edited by D. M. Prescott, pp. 85–104, Academic, New York.

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