APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 2006, p. 1810–1816 Vol. 72, No. 3
0099-2240/06/$08.00+0 doi:10.1128/AEM.72.3.1810–1816.2006

Deposition of *Cryptosporidium* Oocysts in Streambeds

Kristin E. Searcy,[1]* Aaron I. Packman,[1] Edward R. Atwill,[2] and Thomas Harter[3]

Department of Civil and Environmental Engineering, Northwestern University, Evanston, Illinois 60208[1]; Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California—Davis, Tulare, California 93274[2]; and Department of Land, Air, and Water Resources, University of California—Davis, Davis, California 95616[3]

Received 17 June 2005/Accepted 15 December 2005

The transfer of *Cryptosporidium* oocysts from the surface water to the sediment beds of streams and rivers influences their migration in surface waters. We used controlled laboratory flume experiments to investigate the deposition of suspended *Cryptosporidium parvum* oocysts in streambeds. The experimental results demonstrate that hydrodynamic interactions between an overlying flow and a sediment bed cause oocysts to accumulate in the sediments and reduce their concentrations in the surface water. The association of *C. parvum* with other suspended sediments increased both the oocysts' effective settling velocity and the rate at which oocysts were transferred to the sediment bed. A model for the stream-subsurface exchange of colloidal particles, including physical transport and physicochemical interactions with sediment grains, accurately represented the deposition of both free *C. parvum* oocysts and oocysts that were attached to suspended sediments. We believe that these pathogen-sediment interactions play an important role in regulating the concentrations of *Cryptosporidium* in streams and rivers and should be taken into consideration when predicting the fate of pathogens in the environment.

Cryptosporidium parvum is a priority pathogen responsible for many water-borne disease outbreaks within the United States (8, 13, 18, 29, 33). Outside of its host, *C. parvum* exists as a nonreproductive oocyst that can persist for long periods of time in the environment because of its high degree of resistance to chemical and physical stresses (6, 17, 23, 28). Oocysts typically enter surface water systems from the waste of infected hosts, and major sources include municipal wastewater treatment facilities and runoff from agricultural and wildlife populations (3, 11, 15, 39). The potential for humans to be infected with *C. parvum* depends on the transport of viable oocysts from their source to public water supplies. Therefore, a clear understanding of the mechanisms that control the movement and fate of *Cryptosporidium* oocysts in surface waters is essential for the protection of water quality and public health.

Due to their small size (~5 μm) and low specific gravity (~1.05), *C. parvum* oocysts are often considered to move conservatively downstream and to have little interaction with sediments. However, stream-subsurface interactions provide a mechanism for delivery of oocysts to sediment beds. The coupling of surface and pore water flow has been shown to transfer a wide variety of dissolved and suspended substances across the sediment-water interface (4, 9, 10, 36). This process has been called hyporheic exchange and can lead to high rates of suspended particle deposition in sediment beds, even when the suspended particles are very small and have no appreciable settling velocity (25). Hyporheic exchange should also be expected to impact the fate of *Cryptosporidium* oocysts in aquatic systems, but current *C. parvum* transport models do not consider potential oocyst-sediment bed interactions (22, 38). Because of the persistence of *C. parvum* under typical environmental conditions, accumulation of oocysts can cause streambeds to be a source of pathogens during events that cause sediment resuspension. Previous studies have shown elevated concentrations of *C. parvum* in surface waters following periods of heavy rainfall or during high-flow events (2, 11, 16, 30). Although it is typically assumed that this increase is due to runoff from agricultural areas or sewer overflows, oocyst release from streambed sediments could also be important during high-flow events.

Hyporheic exchange can carry suspended particles into the sediment bed, and filtration and settling mechanisms can cause the retention of particles in the subsurface (20, 21, 25). Models that take into account pore water flow, particle filtration, and gravitational settling have been used to predict the hyporheic exchange of solutes and colloidal particles in stream systems (20, 21, 25–27). While these models have successfully predicted the stream-subsurface exchange of dissolved ions and deposition of inorganic colloids, it has yet to be determined if they can accurately simulate the deposition of biocolloids such as *C. parvum* oocysts.

This paper presents the results of laboratory flume experiments designed to investigate the stream-subsurface exchange of *C. parvum* oocysts. The transport of oocysts was observed in both the presence and absence of background particles. Attachment of oocysts to background particles has been shown to increase the effective settling velocity of oocysts, and this process is expected to increase the rate of oocyst deposition (19, 31, 40). Experimental results are compared with those from simulations of a stream-subsurface exchange model to clarify the processes that control the transport and fate of *Cryptosporidium* oocysts in the environment.

* Corresponding author. Mailing address: Department of Civil and Environmental Engineering, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208. Phone: (847) 467-4980. Fax: (847) 491-4011. E-mail: k-searcy@northwestern.edu.

MATERIALS AND METHODS

Source and purification of *C. parvum* oocysts. *C. parvum* oocysts were collected and purified according to procedures described previously by Searcy et al. (31). Fecal samples of dairy calves naturally infected with *C. parvum* were collected in

Tulare County, CA. Samples were rinsed through a series of 40-, 100-, 200-, and 270-mesh sieves, and the fecal suspension was allowed to settle. The resulting suspension was decanted and centrifuged at 1,000 × *g* for 10 min. The supernatant was discarded, and the resulting pellet was resuspended in a 0.2% Tween 20–water solution. A discontinuous sucrose gradient was used for purification of the *C. parvum* oocysts (1). Purified oocysts were stored in a 0.01% Tween 20 solution with antibiotics (penicillin G, streptomycin sulfate, and amphotericin B) at 4°C until they were used for experiments. The concentration of purified oocysts in the suspension was determined through the enumeration protocol described below, and oocysts were used in flume experiments within 2 months of collection.

Enumeration of *C. parvum* oocysts. The concentrations of *C. parvum* oocyst stock solutions and experimental flume samples were determined using a filtration/direct-count method (31). Oocyst samples were vacuum filtered onto a black membrane filter with a pore size of 0.2 μm. A fluorescein isothiocyanate-conjugated monoclonal antibody solution specific for *Cryptosporidium* (Waterborne, Inc., New Orleans, LA) and 0.1% Eriochrome Black T counterstain solution were applied to the filter, which was then incubated in the dark for 30 min. The filter was rinsed of excess staining solution using a phosphate-buffered saline solution and then removed from the filtering tower and transferred onto a microscope slide. Oocysts on the filter were viewed at a magnification of ×160 with a Zeiss Axiophot epifluorescence microscope (Carl Zeiss, Jena, Germany) equipped with an excitation/band-pass filter for fluorescein isothiocyanate. A subset of the oocysts was counted within the square fields of the microscope's ocular grid across a wide area of the filter, and the concentration of oocysts in the sample was calculated based on the number of oocysts counted, the number of fields counted, the field area, and the volume of the filtered sample (31).

Suspended sediment preparation. A stable suspension of colloidal kaolinite was prepared using a protocol described previously by Packman et al. (20). Solid blocks of kaolinite clay (Ward's Scientific, Rochester, NY) were ground with a mortar and pestle and placed in a rolling-ball mill with alumina balls for 24 h. The milled kaolinite was wet sieved through a nylon mesh with a pore size of 50 μm to remove larger quartz impurities. The kaolinite was mixed in a 2 M NaCl solution to convert the kaolinite to homoionic sodium-kaolinite. The sodium-kaolinite was then rinsed several times with deionized water to remove excess salts.

Colloidal iron oxide particles were produced by aging an iron-hydroxide gel (34). To prepare the concentrated gel, equal volumes of 6 N NaOH and 2 M $FeCl_3$ were mixed together and aged in a glass bottle for 72 h at 100°C. Excess salts were removed from the aged gel through repeated dialysis against deionized water. The resulting stable iron oxide suspension was stored in a sealed glass bottle in the dark at 4°C.

Natural sediments were collected from the Des Plaines River and Salt Creek, both of which drain mixed agricultural/municipal/industrial watersheds in northeastern Illinois. Sediment cores (3 cm deep) were extracted from the river bottom and homogenized. The homogenized sediments were wet sieved through a mesh with a 45-μm opening. The sediments that passed through the mesh were concentrated via sedimentation, and the resulting sediment suspension was stored at 4°C.

The concentrations of all sediment stock suspensions were determined by drying a known volume of the suspension at 105°C and weighing the dry sediment. The concentrations of inorganic sediments in experimental samples were measured using a spectrophotometer. A linear relationship was found between the suspended sediment concentration and light absorbance readings for both kaolinite and iron oxide over the concentration range used here, at wavelengths of 300 nm and 560 nm, respectively. Due to the wide size distribution of the natural sediments, light absorbance did not reliably characterize the concentration of the natural sediment suspensions. Therefore, the concentration of natural sediment samples was determined by filtering, drying, and weighing the sediments.

Preparation of oocyst suspensions for flume experiments. Flume experiments were performed with oocysts only and with oocysts in combination with other suspended sediments. For flume experiments containing *C. parvum* oocysts only, a suspension was made by diluting the oocyst stock solution with a 3 mM NaCl solution to a total volume of 1 liter. The total number of oocysts added to the suspension was selected so that after the 1-liter suspension was injected into the flume, the initial concentration within the recirculating water would be 1.5×10^5 oocysts/liter. The suspension was mixed for 1 h prior to injection.

For flume experiments containing both suspended sediments and *C. parvum* oocysts, a suspension of oocysts and suspended sediments was made by diluting the stock solutions of each into a 3 mM NaCl solution to give a final volume of 2 liters. The amount of oocysts and sediments added to the suspension was selected to produce initial concentrations in the recirculating water of 1.5×10^5 oocysts/liter and 200 mg/liter, respectively. The pH of the 2-liter suspension was adjusted to 7.0 with 5 mM NaOH or HCl, and the suspension was mixed on a rotating shaker for 24 h to allow for the oocyst-particle attachment to come to equilibrium prior to injection into the flume.

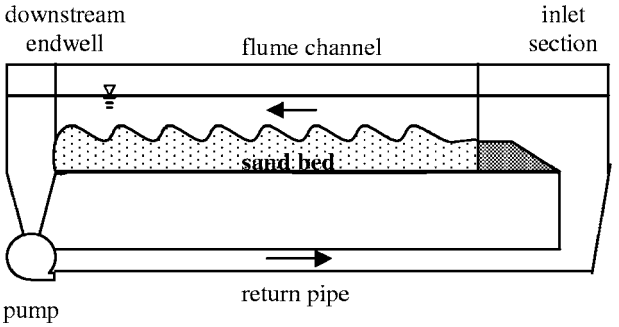

FIG. 1. Schematic diagram of the recirculating flume used for *C. parvum* oocyst deposition experiments.

Flume experiments. Experiments to investigate the stream-subsurface exchange of *C. parvum* oocysts were conducted in a laboratory recirculating flume (Fig. 1). This flume is a closed system with continual recirculation over the sediment bed, which allows transport processes to be observed under steady conditions for extended periods of time. The flume affords good control of physical and chemical background conditions and provides ready access to the system for measurements. The flume used here has a 20-cm-wide channel with a 2.5-m-long test section made of Plexiglas and a downstream endwell made of fiberglass. Water was recirculated to the test section through a 10-cm-diameter polyvinyl chloride return pipe, and the recirculating flow was controlled by a variable speed pump and measured with a vortex-shedding flow meter.

At the beginning of each flume experiment, a sediment bed composed of Ottawa no. 12 Flint silica sand was established in the flume channel. This high-purity sand (99.8% SiO_2) has a mean grain diameter of 486 μm and a total porosity of 0.362. For each experiment, deionized water was filled to a depth of approximately 16.5 cm, and sand was then placed into the main channel of the flume so that the total depth of the sand bed and overlying water was approximately 20 cm. The bed topography was formed by manually creating small sediment mounds and then increasing the water velocity to produce natural dune forms, after which the velocity was reduced to the desired experimental condition. The bedform height, bedform wavelength, water depth, and stream water velocity were measured in detail for each experiment.

Once the sediment bed was prepared, the pH of the recirculating water was adjusted to 7.0 through the addition of HCl or NaOH. Once the pH of the recirculating water had stabilized, a conservative tracer solution (NaCl) was injected into the flume, and the conductivity of the recirculating water was measured over time (ES-12; Horiba, Kyoto, Japan). The conservative tracer provides a basis for evaluating the hydrodynamic stream-subsurface exchange rate for each experiment. The tracer solution was poured into the downstream endwell of the flume over the course of one circulation period, approximately 2 min for each experiment, in order to rapidly bring the entire volume of the recirculating water to the same tracer concentration. The exchange of the surface water with pore water in the sediment bed then caused the NaCl concentration of the surface water to decrease until the system reached equilibrium, yielding a uniform tracer concentration throughout the entire flume (recirculating water and pore water). The NaCl mass was selected to yield a final concentration of 3 mM NaCl throughout the flume for each experiment.

After the NaCl concentration had reached equilibrium, the suspension of oocysts (or oocysts mixed with suspended sediments) was poured into the endwell of the flume over one circulation period. To determine the rate of incorporation of oocysts and sediments into the sand bed, a series of 40-ml samples was extracted from the surface water in the downstream endwell over the course of each experiment. Prior results have shown that in-stream mixing is rapid relative to particle deposition under the conditions used here, so a single surface water sample is sufficient to characterize the bulk average suspended oocyst concentration at each time. An aliquot of the sample was used to measure the suspended sediment concentration for flume experiments in which suspended sediments were used, and the remainder of the sample was preserved in a 10% formaldehyde solution and stored at 4°C until oocysts were counted.

Enumeration of oocysts attached to flume walls. The hydrophobic nature of *C. parvum* oocysts suggested that they could stick to the walls of the flume. The extent and rate of oocyst loss by this mechanism were determined using Plexiglas

sample coupons with a composition identical to that of the channel walls. Coupons (8 cm by 2 cm) were glued to the side of the channel and the endwell prior to each experiment. Following the oocyst injection, coupons were removed from the flume walls at various times throughout the experiment. Coupons were placed in a 50-ml centrifuge tube with 45 ml of 0.01 M phosphate-buffered saline buffer (Sigma Chemical Co., St. Louis, MO), and the oocysts were dislodged by sonication at a frequency of 38.5 to 40.5 Hz for 1 min (Aquasonic ultrasonic cleaner; VWR Scientific Products, West Chester, PA). Following sonication, the tubes were vortexed for 30 s to resuspend the oocysts. This sonication-vortex cycle was repeated three times. Similar methods have been used to effectively transfer surface-attached microorganisms to a suspension in preparation for enumeration without damaging cell integrity (35). The coupons were then removed from the centrifuge tubes, and the suspensions were preserved in 10% formaldehyde at 4°C until oocysts were counted. A flume experiment was conducted to observe oocyst deposition in the absence of a sand bed. The rate of oocyst loss from the recirculating water was fully explained by the observed accumulation on the sample coupons, confirming that oocyst attachment to the channel walls is reliably characterized by the analysis of the emplaced coupons.

Determination of oocyst settling velocities. The effective settling velocity is an input parameter for the stream-subsurface exchange model used to analyze oocyst deposition in flume experiments. Settling column experiments were performed to determine the average settling velocity of free oocysts and oocysts attached to a variety of suspended sediments, as described previously by Searcy et al. (31). A 1-liter suspension of oocysts (1,000 oocysts/ml) and suspended sediments (200 mg/liter) were mixed in a 3 mM NaCl (pH 7.0) solution for 24 h on a rotating shaker. After the mixing period, two 7-ml samples were taken to determine the initial concentrations of oocysts and sediments, and the remainder of the suspension was poured into a 1-liter settling column. A syringe was used to extract samples through septum-filled ports in the side of the settling column. Ten 10-ml samples were extracted at a constant depth in the column over time. A portion of each sample was used to determine the suspended sediment concentration, and the remainder of the sample was stored in 10% formaldehyde at 4°C until oocysts were counted.

Theory. The model described previously by Packman et al. (21) for the advective stream-subsurface exchange of solutes and colloidal particles was applied to analyze NaCl exchange and *C. parvum* deposition. This model has been shown to successfully predict the deposition of a variety of inorganic colloids (20, 25–27). The work presented here represents the first application of this type of process-based model to the stream-subsurface exchange and deposition of microorganisms. *C. parvum* is an ideal organism to use in this initial investigation, because *C. parvum* occurs in natural waters only in the form of oocysts, which are passively transported. Thus, complicating factors such as growth and motility do not have to be considered.

The essential elements of the theory for stream-subsurface exchange will be presented here. Stream-subsurface exchange flow carries dissolved and suspended substances into and through the streambed (9, 10, 36). Advective flow into and out of the streambed is controlled by the dynamic pressure variation induced at the bed surface by stream flow over bedforms. The piezeometric head, $h(x)$, over dunes can be closely approximated by a sine function:

$$h = h_m \sin(kx) \tag{1}$$

where h_m is the half-amplitude of the head variation over the dune, k is the bedform wavenumber ($k = 2\pi/\lambda$, where λ is the dune wavelength), and x is the longitudinal coordinate along the streambed. The following empirical estimate of h_m was developed previously by Elliott and Brooks (10):

$$h_m = 0.28 \frac{U_2}{2g} \begin{cases} \left(\frac{H/d}{0.34}\right)^{3/8} & H/d \leq 0.34 \\ \left(\frac{H/d}{0.34}\right)^{3/2} & H/d \geq 0.34 \end{cases} \tag{2}$$

where U is the mean stream velocity, H is the dune height (trough to crest), d is the mean stream depth, and g is the acceleration due to gravity. Equations 1 and 2 can be used as a boundary condition to calculate the flow field in the porous bed and the resulting flux through the bed surface. The resulting pore water velocity distribution is given as follows:

$$u = -kKh_m \cos(kx)\,[\tanh(kd_b)\sinh(ky) + \cosh(ky)] \tag{3}$$

$$v = -kKh_m \sin(kx)\,[\tanh(kd_b)\cosh(ky) + \sinh(ky)] \tag{4}$$

where u and v are the horizontal and vertical Darcy pore water velocities, K is the hydraulic conductivity of the bed sediment, and d_b is the depth of the bed. It can be seen from equations 3 and 4 that the maximum pore water velocity occurs just at the bed surface, and this characterizes the overall rate of stream-subsurface exchange. Equations 1 to 4 can be implemented in a numerical model to predict stream-subsurface exchange of conservative solutes based on all measured input parameters, as described previously by Elliott and Brooks (10).

Advective stream-subsurface exchange carries suspended colloidal particles into the subsurface, where they can be permanently retained in the sediment bed through settling or filtration mechanisms (14, 20, 24). Although *C. parvum* oocysts have a negligible settling velocity due to their low specific gravity, oocysts can also associate with suspended particles present in the surface water (19, 31). This association can increase the effective size and specific gravity of oocysts, thus increasing their settling velocity and downward transport both in the stream and by pore water flow. Once in the streambed, oocysts or sediment-oocyst aggregates are also subject to deposition by filtration and straining mechanisms (5, 12, 37). Filtration is the physicochemical process that causes the attachment of transported colloids to larger, stationary sediment grains, while straining refers to the capture of colloids in pore throats with a diameter smaller than that of the transported particles. When the colloid settling velocity, filtration, or straining is high, very little of the suspended matter that is transported into the bed will return to the surface water. This phenomenon has been termed "complete trapping." The *C. parvum* oocysts and kaolinite and iron oxide particles used here have sufficiently high filtration coefficients under our flume experimental conditions (12, 20, 24), and the natural sediments have a sufficiently high settling velocity (21), to yield deposition of all suspended material carried across the stream-subsurface interface. Therefore, complete trapping was assumed for modeling the stream-subsurface exchange of both oocysts and the background suspended sediments in our flume experiments.

When complete trapping occurs, the rate of oocyst accumulation in the bed can be described as follows:

$$\frac{dN_b}{dt} = \bar{q}_p A C(t) \tag{5}$$

where N_b is the number of oocysts deposited in the bed, $\bar{q}_p$ is the average downward velocity of oocysts across the stream-subsurface interface, A is the bed surface area, and $C(t)$ is the concentration of oocysts in the recirculating water at time t. The average rate of delivery of oocysts from the surface water to the sediment bed, $\bar{q}_p$, is calculated as the sum of an advective hydrodynamic exchange term obtained by averaging equations 3 and 4 over the bed surface and a gravitational settling term:

$$\bar{q}_p = \frac{u_m \tanh(kd_b)}{\pi} + \frac{\theta v_s}{2} \tag{6}$$

where $u_m = kKh_m$ is the maximum pore water velocity at the bed surface, θ is the porosity of the bed sediment, and v_s is the average oocyst settling velocity (21).

The recirculating flume is a closed system for conservative solutes, suspended sediments, and oocysts. By applying the principle of mass conservation to analyze the redistribution of *C. parvum* in this system, we obtained the following equation:

$$N_o = C(t)V + N_b(t) + L(t) \tag{7}$$

where N_o is the number of oocysts initially introduced to the flume, V is the volume of recirculating water in the flume, and $L(t)$ is the total number of oocysts attached to the flume walls at time t. Equations 5 to 7 were used to predict the accumulation of oocysts in the streambed, $N_b(t)$, and the resulting change in the in-stream oocyst concentration, $C(t)$, from independent measurements of the stream velocity, system geometry, sediment properties, and effective oocyst settling velocity measured for the particular solution conditions and background suspended sediments occurring in each experiment. Equation 7 was also used to relate the decrease in the in-stream oocyst concentration observed in each experiment to the extent of oocyst accumulation in the bed. Oocyst loss to the walls is a confounding factor that obscures the relationship between in-stream concentrations and deposition in the streambed. The wall loss term in equation 7 was quantified by direct measurement of oocyst deposition on sample coupons attached to the channel walls, as described above. There was negligible attachment of oocysts to the flume walls in experiments conducted with background suspended sediments, but there was substantial loss to the walls in experiments with oocysts alone. To allow consistent comparisons between both types of experiments, results of experiments with oocysts only were converted into equivalent curves of $N_b(t)$ that would have been observed without any loss to the walls.

Statistical analysis of results. Experimental results will be presented as the fraction of oocysts accumulated in the streambed over time, $N_b(t)/N_o$. Linear regressions of the fraction of oocysts deposited as a function of time were

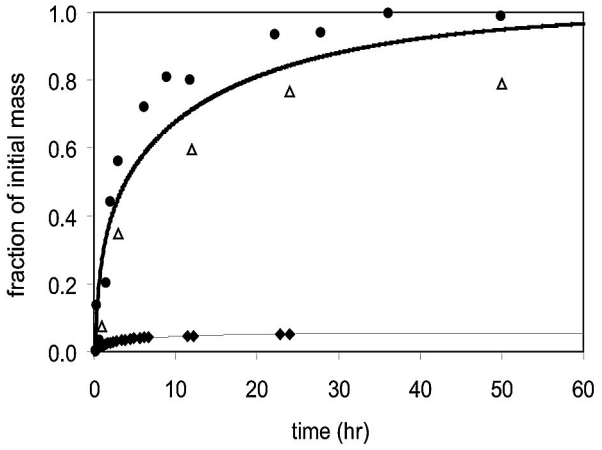

FIG. 2. Comparison of the fraction of NaCl (solid diamonds) and *C. parvum* oocysts (solid circles) removed from the surface water of the recirculating flume over time. The open triangles show the fraction of injected oocysts that became attached to the flume walls over time. The experimental data are compared to predictions of the stream-subsurface exchange model for NaCl (thin line) and oocysts when their attachment to the flume walls is taken into consideration (thick line).

performed, and a comparison of linear regression coefficients was used to demonstrate the effects of various suspended sediments on the deposition of oocysts (32). The regression was implemented for the data in the time interval required for 60% of the suspended oocysts to be transferred to the sediment bed, i.e., until $N_b(t)/N_o$ equaled 0.6. The significance of differences between the deposition rate of free oocysts and the deposition rate of oocysts mixed with suspended sediments was evaluated using one-sample t tests (32).

RESULTS

Stream-subsurface exchange of *C. parvum* oocysts without suspended sediments. A flume experiment in which NaCl and *C. parvum* oocysts were injected into the recirculating water without any additional suspended sediments was conducted. Figure 2 shows the fraction of the conservative tracer, NaCl, and the fraction of *C. parvum* oocysts removed from the surface water of the flume over the course of the experiment. NaCl is transferred from the stream to pore water because of hydrodynamic exchange with the sediment bed. Figure 2 shows that stream-subsurface exchange is reasonably fast, and only a few hours are required for the conservative solute to become completely mixed throughout the system. The in-stream solute concentration decreased by 5% during the course of the experiment. This was expected, as the pore water represents 5% of the total water volume in the flume. In contrast, almost all of the *C. parvum* oocysts injected into the recirculating water were removed from the suspension by the end of the experiment (Fig. 2, closed circles). However, by extracting experimental coupons from the side of the flume walls during the course of the experiment, it was determined that 79% of the oocysts removed from the surface water had become attached to the sides of the flume walls (Fig. 2, open triangles). Similar behavior was observed in experiments conducted without a sand bed (results not shown).

Stream-subsurface exchange of *C. parvum* oocysts in the presence of suspended sediments. The deposition of suspended *C. parvum* oocysts in the presence of suspended sediments is shown in Fig. 3. In flume experiments with suspended sediments, there was negligible attachment of the oocysts to the flume walls. Therefore, the results from the experiments shown in Fig. 3 were compared to the baseline oocyst exchange rate from the experiment with no suspended sediments, which is the rate that would have been observed had there been no loss to the walls. The removal rate of oocysts when mixed with kaolinite, iron oxide, and Des Plaines River sediments (Fig. 3A, B, and C) is faster than that of oocysts not mixed with sediments based on the comparison of regression coefficients ($P < 0.01$). This result was expected, because results from settling column experiments showed that oocysts have a much higher settling velocity in the presence of these suspended sediments (Table 1) (31). The removal of oocysts in association with Salt Creek sediments (Fig. 3D) was not significantly greater than that of oocysts alone, most likely due the small increase in the effective oocyst settling velocity in the presence of Salt Creek sediments (Table 1).

Model predictions of tracer and oocyst transfer to the streambed. Solute transport and oocyst deposition with and without various suspended particles were quantitatively analyzed using the stream-subsurface exchange model. The model input parameters used to simulate flume experiments are listed in Table 1. As mentioned previously, all of these model inputs were determined independently. The model accurately predicted the stream-subsurface exchange of the NaCl tracer in all experiments reported here, as shown in Fig. 2. Model predictions of *C. parvum* deposition to the sediment bed are shown in Fig. 2 and 3. The calculated hydrodynamic stream-subsurface exchange rate, the rate of oocyst delivery to the streambed due to settling, and the net oocyst deposition rate for each experiment are listed in Table 2. These variables are related by equation 6. The model was generally accurate in describing the rate of oocyst deposition when the appropriate effective oocyst settling velocity was used. In the flume experiment with Des Plaines River sediments (Fig. 3C), the model underestimates the deposition of oocysts at the beginning of the experiment and overestimates their removal towards the end. This pattern has been previously observed for particles with a broad or bimodal size distribution, because the model uses a single average settling velocity to describe colloid sedimentation, and this does not provide a good representation of the settling behavior of particles with complex size distributions (27). Therefore, the most likely explanation for the discrepancy in modeling the results of the experiment conducted with the Des Plaines River sediments is the wide settling velocity distribution of oocysts associated with this sediment.

DISCUSSION

Through a series of laboratory flume experiments, we have demonstrated that *Cryptosporidium parvum* oocysts are carried into the subsurface by a combination of advective stream-subsurface exchange and particle settling, leading to extensive oocyst deposition within the streambed and drastically reducing the oocyst concentration in the overlying water column. This mode of deposition is generally not considered when predicting the fate of *C. parvum* in the environment, and oocysts are normally thought to be transported conservatively in surface waters because of their very low settling velocity. However, the pressure differential created by stream flow over channel topography drives oocysts into the sand bed, and the high filtration of oocysts in the subsurface prevents their return

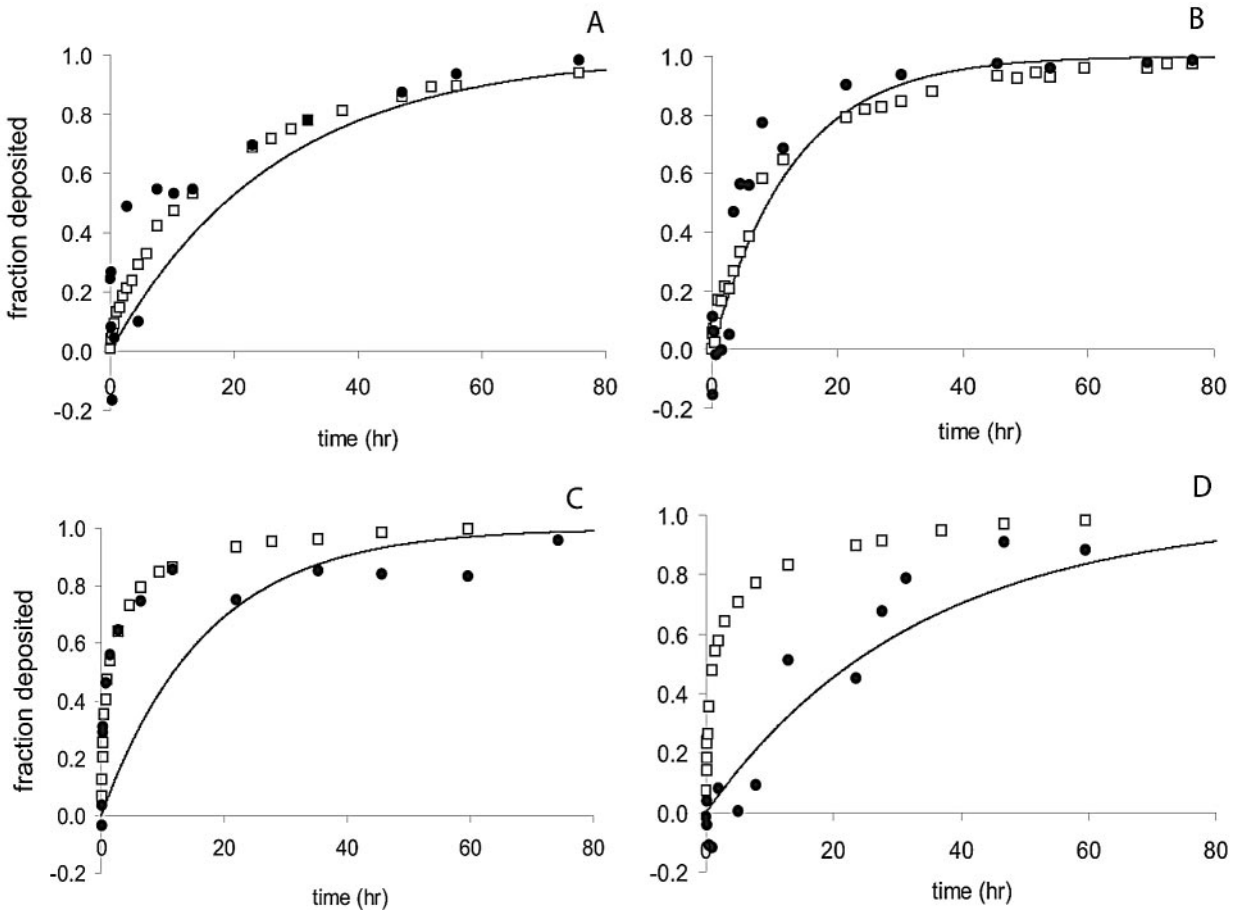

FIG. 3. The fraction of suspended sediments (open squares) and oocysts (dark circles) deposited in the sediment bed of the recirculating flume over time. The line represents the stream-subsurface exchange model prediction for the removal of oocysts in flume experiments with kaolinite particles (A), iron oxide particles (B), Des Plaines River sediments (C), and Salt Creek sediments (D).

to the water column. A variety of suspended solutes and inorganic colloids have been shown to deposit by this mechanism (9, 20, 25), and this work is the first to demonstrate the importance of hyporheic exchange in the environmental transmission of water-borne disease.

We also observed that the rate of *C. parvum* deposition increased in the presence of background suspended sediments. *C. parvum* oocysts have been shown to associate with a variety of inorganic and organic suspended matter, which increases the oocysts' effective size, specific gravity, and settling velocity (19, 31, 40). Oocyst-particle association and the resulting increase in effective settling velocity cause a substantial increase in oocyst deposition by sedimentation. In the natural environment, *C. parvum* oocysts typically enter surface water systems with large quantities of other suspended particles originating from wastewater treatment plants, overland runoff, or sediment bed resuspension (2, 7, 39). The results presented here show that it is important to consider the association of oocysts with suspended particles when predicting the transport and fate of *C. parvum* in surface water systems.

TABLE 1. Measured experimental conditions for all flume experiments

Expt	Suspended sediment	Effective oocyst settling velocity (μm/s)	Stream velocity (cm/s)	Bedform length (cm)	Bedform height (cm)	Stream depth (cm)	Bed depth (cm)
1	None	0.8	14.7	16.4	1.2	10.3	9.8
2	Salt Creek	4.2	14.6	16.5	1.5	10.6	9.5
3	Kaolinite	12.6	14.5	16.7	1.0	10.5	9.6
4	Des Plaines River	32.7	14.4	15.0	1.0	10.4	9.6
5	Iron oxide	53.3	14.4	15.5	1.2	10.8	9.3

TABLE 2. Calculated rates of oocyst delivery to the sand bed by transport mechanisms

Expt	Suspended sediment	Rate of oocyst delivery to bed (cm/s)[a]		
		By advective exchange	By settling	Total
1	None	1.70	0.04	1.74
2	Salt Creek	1.67	0.28	1.95
3	Kaolinite	1.41	0.82	2.23
4	Des Plaines River	1.63	2.13	3.76
5	Iron oxide	1.55	3.48	5.03

[a] To allow a direct comparison, all exchange rates have been converted to units of velocity as in equation 6.

The colloid stream-subsurface exchange model described previously by Packman et al. (21) accurately described the deposition of *C. parvum* oocysts. This model has been used to predict the transport of a variety of inorganic colloids (20, 25, 26), but this is the first study to demonstrate that it can also be used to describe the stream-subsurface exchange and deposition of microorganisms. The increase in oocyst deposition that was observed in the presence of other suspended particles was successfully predicted by using the effective oocyst settling velocity as a model input parameter. The settling velocities of oocysts can vary with the background sediment type and can also differ from the average settling velocity of the sediment itself (31). Simple settling column experiments can be performed to determine the effective settling velocity of oocysts in the presence of other suspended sediments, and this information can then be used to predict the rate of oocyst sedimentation in streams and other surface water bodies. The colloid transport model used here distinguishes the relative importance of advective hyporheic exchange and gravitational settling in net oocyst deposition. Table 2 shows that while the rate of oocyst delivery to the sand bed by advective hyporheic exchange was similar in all experiments, oocyst flux to the streambed by sedimentation increased substantially as oocysts became associated with particles that settled faster. The model confirms that this process was directly responsible for the observed increase in the rate of oocyst removal from the water column in the presence of background suspended matter.

The net effects of these processes will be illustrated by model simulations of oocyst deposition. For this comparison, we will consider two representative streams: a typical headwater agricultural stream with a mean depth of 15 cm, velocity of 15 cm/s, and discharge of 0.05 m^3/s, and a moderately sized river with a depth of 80 cm, velocity of 20 cm/s, and discharge of 3 m^3/s. We will compare net oocyst deposition resulting from three mechanisms: classic sedimentation of free oocysts, stream-subsurface exchange of free oocysts, and stream-subsurface exchange of oocysts associated with background sediments. Model input parameters for oocyst removal are taken from the laboratory results presented here. In the headwater stream, a distance of 7.3 km would be required for each log removal of free oocysts by sedimentation. When considering stream-subsurface exchange of free oocysts, this distance decreases to 1.1 km, and when including deposition of oocysts associated with background sediment matter, this distance decreases still further to 0.37 km. In the larger river, these distances become 52 km for free oocyst sedimentation, 7.6 km for free oocyst exchange, and 2.6 km for oocyst deposition in association with other suspended matter. It can therefore be seen that the processes of stream-subsurface exchange and attachment to background suspended matter are expected to greatly alter the migration of *C. parvum* oocysts through surface water bodies.

This study demonstrated that stream-subsurface exchange and associated deposition processes cause *C. parvum* oocysts to be transferred from the surface water to sediment beds during downstream transport. Not only do these interactions reduce oocyst concentrations in surface waters, they also concentrate oocysts in sediment beds, which then represent a reservoir for pathogens that can be released during high-flow events. We believe that these processes play an important role in regulating the concentrations of *Cryptosporidium* and other pathogens including bacteria and viruses in streams and rivers and that interaction with bed sediments should generally be considered in studies that attempt to assess the distribution, transport, and fate of pathogens in aquatic systems.

ACKNOWLEDGMENTS

This project was supported by USDA National Research Initiative grant 2001-35102-10773. This work was conducted in part under the auspices of the Bernice Barbour Communicable Disease Laboratory, with financial support from the Bernice Barbour Foundation, Hackensack, NJ, as a grant to the Center of Equine Health, University of California—Davis.

We thank Lingling Hou, Ken Kido, and Jimmy Hook for their assistance in the collection and purification of *C. parvum* oocysts and Matthew Parsek for the use of the epifluorescence microscope.

REFERENCES

1. **Arrowood, M. J., and C. R. Sterling.** 1987. Isolation of *Cryptosporidium parvum* and sporozoites using discontinuous sucrose and isopycnic Percoll gradients. J. Parasitol. **73:**314–319.
2. **Atherholt, T., M. LeChevallier, and J. Rosen.** 1998. Effect of rainfall on crypto and Giardia. J. Am. Water Works Assoc. **90:**66–80.
3. **Atwill, E. R., R. Phillips, M. Das Gracas, C. Pereira, X. Li, and B. McCowan.** 2004. Seasonal shedding of multiple *Cryptosporidium* genotypes in California ground squirrels (*Spermophilus beecheyi*). Appl. Environ. Microbiol. **70:**6748–6752.
4. **Bencala, K. E., and R. A. Walters.** 1983. Simulation of solute transport in a mountain pool-and-riffle stream: a transient storage model. Water Resour. Res. **19:**718–724.
5. **Bradford, S. A., and M. Bettahar.** 2005. Straining, attachment, and detachment of *Cryptosporidium* oocysts in saturated porous media. J. Environ. Qual. **34:**469–478.
6. **Chauret, C., K. Nolan, P. Chen, S. Springthorpe, and S. Sattar.** 1998. Aging of *Cryptosporidium parvum* oocysts in river water and their susceptibility to disinfection by chlorine and monochloramine. Can. J. Microbiol. **44:**1154–1160.
7. **Crockett, C. S.** 2004. The significance of streambed sediments as a reservoir of Cryptosporidium. Ph.D. thesis. Drexel University, Philadelphia, Pa.
8. **Dworkin, M. S., D. P. Goldman, T. G. Wells, J. M. Kobayashi, and B. L. Herwaldt.** 1996. Cryptosporidiosis in Washington State: an outbreak associated with well water. J. Infect. Dis. **174:**1372–1376.
9. **Elliott, A. H., and N. Brooks.** 1997. Transfer of nonsorbing solutes to a streambed with bed forms: laboratory experiments. Water Resour. Res. **33:**137–151.
10. **Elliott, A. H., and N. Brooks.** 1997. Transfer of nonsorbing solutes to a streambed with bed forms: theory. Water Resour. Res. **33:**123–136.
11. **Hansen, J. S., and J. E. Ongerth.** 1991. Effects of time and watershed characteristics on the concentration of *Cryptosporidium* oocysts in river water. Appl. Environ. Microbiol. **57:**2790–2795.
12. **Harter, T., S. Wagner, and E. R. Atwill.** 2000. Colloid transport and filtration of *Cryptosporidium parvum* on sandy soils and aquifer sediments. Environ. Sci. Technol. **34:**62–70.
13. **Hayes, E. B., T. D. Matte, T. R. O'Brien, T. W. McKinley, G. S. Logsdon, J. B. Rose, B. L. P. Ungar, D. M. Word, P. F. Pinsky, M. L. Cummings, M. A. Wilson, E. G. Long, E. S. Hurwitz, and D. D. Juranek.** 1989. Large community outbreak of cryptosporidiosis due to contamination of a filtered public water-supply. N. Engl. J. Med. **320:**1372–1376.
14. **Huettel, M., W. Ziebis, and S. Forster.** 1996. Flow-induced uptake of particulate matter in permeable sediments. Limnol. Oceanogr. **41:**309–322.
15. **Jellison, K. L., H. F. Hemond, and D. B. Schauer.** 2002. Sources and species of *Cryptosporidium* oocysts in the Wachusett Reservoir watershed. Appl. Environ. Microbiol. **68:**569–575.
16. **Kistemann, T., T. Classen, C. Koch, F. Dangendorf, R. Fischeder, J. Gebel, V. Vacata, and M. Exner.** 2002. Microbial load of drinking water reservoir tributaries during extreme rainfall and runoff. Appl. Environ. Microbiol. **68:**2188–2197.
17. **Korich, D. G., J. R. Mead, M. S. Madore, N. A. Sinclair, and C. R. Sterling.** 1990. Effects of ozone, chlorine dioxide, chlorine, and monochloramine on *Cryptosporidium parvum* oocyst viability. Appl. Environ. Microbiol. **56:**4123–4128.
18. **MacKenzie, W. R., N. J. Hoxie, M. E. Proctor, M. S. Gradus, K. A. Blair, D. E. Peterson, J. J. Kazmierczak, D. G. Addiss, K. R. Fox, J. B. Rose, and J. P. Davis.** 1994. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. N. Engl. J. Med. **331:**161–667.
19. **Medema, G. J., F. M. Schets, P. F. M. Teunis, and A. H. Havelaar.** 1998. Sedimentation of free and attached *Cryptosporidium* oocysts and *Giardia* cysts in water. Appl. Environ. Microbiol. **64:**4460–4466.
20. **Packman, A. I., N. Brooks, and J. Morgan.** 2000. Kaolinite exchange between a stream and streambed: laboratory experiments and validation of a colloid transport model. Water Resour. Res. **36:**2363–2372.

21. **Packman, A. I., N. Brooks, and J. Morgan.** 2000. A physiochemical model for colloid exchange between a stream and a sand streambed with bed forms. Water Resour. Res. **36:**2351–2361.
22. **Park, C. H., and P. M. Huck.** 2003. A conceptual model of *Cryptosporidium* transport in watersheds. Water Qual. Res. J. Can. **38:**77–113.
23. **Parker, J. F. W., and H. V. Smith.** 1993. Destruction of oocysts of *Cryptosporidium parvum* by sand and chlorine. Water Res. **27:**729–731.
24. **Ren, J., and A. I. Packman.** 2005. Coupled stream-subsurface exchange of colloidal hematite and dissolved zinc, copper and phosphate. Environ. Sci. Technol. **39:**6387–6394.
25. **Ren, J., and A. I. Packman.** 2002. Effects of background water composition on stream-subsurface exchange of sub-micron colloids. J. Environ. Eng. **128:**624–634.
26. **Ren, J., and A. I. Packman.** 2004. Modeling of simultaneous exchange of colloids and sorbing contaminants between streams and streambeds. Environ. Sci. Technol. **38:**2901–2911.
27. **Ren, J., and A. I. Packman.** 2004. Stream-subsurface exchange of zinc in the presence of silica and kaolinite. Environ. Sci. Technol. **38:**6571–6581.
28. **Robertson, L. J., A. T. Campbell, and H. V. Smith.** 1992. Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. Appl. Environ. Microbiol. **58:**3494–3500.
29. **Roefer, P. A., J. T. Monscvitz, and D. J. Rexing.** 1996. The Las Vegas cryptosporidiosis outbreak. J. Am. Water Works Assoc. **88:**95–106.
30. **Schijven, J. F., and A. M. de Roda Husman.** 2005. Effect of climate changes on waterborne disease in The Netherlands. Water Sci. Technol. **51:**79–87.
31. **Searcy, K. E., A. I. Packman, E. R. Atwill, and T. Harter.** 2005. Association of *Cryptosporidium parvum* with suspended particles: impact on oocyst sedimentation. Appl. Environ. Microbiol. **71:**1072–1078.
32. **Sokal, R. R., and F. J. Rohlf.** 1981. Biometry, 2nd ed. W. H. Freeman and Company, New York, N.Y.
33. **Solo-Gabriele, H., and S. Neumeister.** 1996. US outbreaks of cryptosporidiosis. J. Am. Water Works Assoc. **88:**76–86.
34. **Sugimoto, T., and D. Sakata.** 1992. Preparation of monodisperse pseudocubic α-Fe_2O_3 particles from condensed ferric hydroxide gel. J. Colloid Interface Sci. **152:**587–590.
35. **Teitzel, G. M., and M. R. Parsek.** 2003. Heavy metal resistance of biofilm and planktonic *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. **69:**2313–2320.
36. **Thibodeaux, L. J., and J. D. Boyle.** 1987. Bedform-generated convective transport in bottom sediment. Nature **325:**341–343.
37. **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. **338:**5932–5938.
38. **Walker, F. R., and J. R. Stedinger.** 1999. Fate and transport model of *Cryptosporidium*. J. Environ. Eng. **125:**325–333.
39. **Walker, M. J., C. D. Montemagno, and M. B. Jenkins.** 1998. Source water assessment and nonpoint sources of acutely toxic contaminants: a review of research related to survival and transport of *Cryptosporidium parvum*. Water Resour. Res. **34:**3383–3392.
40. **Young, P. L., and S. J. Komisar.** 2005. Settling behavior of unpurified *Cryptosporidium* oocysts in laboratory settling columns. Environ. Sci. Technol. **39:**2636–2644.