and fourth most populous states. There is significant interchange between surface and subsurface features, fluids, and waterborne materials. In many unconfined karstic aquifers, groundwater and surface water behave as a single body of water (2). The Edwards and Trinity aquifers are the only significant water sources for semiarid south central Texas, including San Antonio, the eighth largest city in the United States. The Edwards aquifer is one of the most permeable and productive carbonate aquifers in the world. It provides a public water supply to more than 2 million people, water for agriculture and industry, and discharges into major springs. These springs support recreation and business, provide flow to downstream users, and are a habitat for several threatened and endangered species. Its high porosity and permeability result in part from the development of secondary porosity and fracturing. The Trinity aquifer, by contrast, is much less permeable or productive than the Edwards. Its lithologies contain significantly more clay minerals and other siliciclastics, as well as minor evaporites. Nevertheless, it supplies water to scattered communities, ranches, and individual wells north of the Edwards in an area of rapid urbanization and growth.

Karstic limestone aquifers are characterized by three primary types of porosity: intergranular matrix porosity, fracture porosity, and the development of cavern conduits (3). A conduit is defined as any interconnected pathway for water of sufficient size to permit turbulent flow. Resistance to flow in conduits is much less than in the adjacent matrix, and, as a consequence, most regional flow is concentrated in conduits. Similarly, most local matrix flow is toward the nearest conduit. Thus, hydrodynamic gradients can vary significantly between conduit recharge and regional flow.

Contaminant dispersion would take place by a variety of methods using this model. Contaminants within conduits travel much more rapidly than those within the matrix, which follow Darcy's law and the advection–dispersion equation. However, exchange of contaminants between the two can occur under a variety of naturally occurring cycles, such as floods versus droughts. Thus the system should be modeled as a network of conduits embedded in a porous matrix (4). The complex depositional environments that produce carbonate rocks, their pronounced digenetic susceptibilities, and the fabric controls related to tectonic alteration of large carbonate units result in groundwater systems that are highly heterogeneous and poorly understood. Regional bedrock karstic limestone aquifers cannot be understood without critical information on the geologic boundary conditions.

CRYPTOSPORIDIUM

Cryptosporidiosis has been recognized as a human disease since 1976 (5). The first diagnosed waterborne outbreak of cryptosporidiosis in the world occurred in Braun Station, Texas, in 1984 (6). The first report of the disease associated with a contaminated municipal water supply was in 1987 in Carrollton, Georgia, where 13,000 became ill (7). This water system met all state and federal drinking water standards. In Milwaukee, Wisconsin, in 1993, municipal drinking water infected 400,000 people with *Cryptosporidium* and resulted in approximately 50 deaths (8,9). Subsequently, the Texas Department of Health reported a more recent water outbreak in Brushy Creek, Texas, in 1998. Overall, there have been 12 documented waterborne outbreaks of cryptosporidiosis in North America between 1985 and 1997; in two of these (Milwaukee and Las Vegas), mortality rates in the immunocompromised ranged from 52% to 68% (10).

Cryptosporidium parvum is a protozoan tissue parasite and is an agent of enterocolitis in mammals (11). *Cryptosporidium* has a complicated and extensive life cycle. The environmental stage is an oocyst, which is a metabolically dormant protective phase (12). The oocysts are nearly spherical and have a diameter of 4.5 to 5.5 μm. Their surfaces are slightly negative to neutrally charged in natural waters (13,14), and their density is close to that of water at 1.025 to 1.070 (14). The oocyst encases four sporozoites, each capable of infecting a host cell. Ingestion of as few as 10 oocysts can lead to infection; the feces of infected mammals may contain as many as 10^7 oocysts/mL (15).

Cryptosporidium can enter the environment via human and animal wastes. It has been found in marine water and bathing beaches in the vicinity of a nearby sewage outfall (16). Cryptosporidiosis has been reported in many domestic animals, especially cattle. An infected calf can excrete 10^{10} oocyts per day. In a study of farm drains, Kemp et al. (17) found 0.06 to 19.4 oocysts per liter, which can result in contamination of surface waters. Typical concentrations of *Cryptosporidium* in untreated domestic wastewaters are between 1 and 10 oocysts/mL (18) and in polluted streams, are between 0.1 to 100 oocysts/mL (19). *C. parvum* forms a hardy oocyst that can survive chlorine disinfection as commonly practiced in conventional water treatment (20,21). Furthermore, Chauret et al. (22) concluded oocysts exposed to environmental conditions are as resistant to inactivation by chlorination as freshly shed oocysts. *C. parvum* oocysts have also survived for weeks in surface waters (23).

Mawdsley et al. (24) quantitatively monitored the movement of *Cryptosporidium parvum* oocysts from livestock waste through low-permeability silt clay loam soil in laboratory column and box studies. In their box studies, livestock waste was applied to a portion of the surface of 20 cm deep by 80 cm long blocks of undisturbed low-permeability silt clay loam soil. The soil was contained within a tilted box and water was applied for 70 days. Oocysts were found in leachate in numbers ranging from 10^4 to 10^6 oocysts. Postsoil core analysis found decreasing numbers of oocyst with distance from inoculation.

Brush et al. (25) and Harter et al. (11) performed laboratory column studies of *Cryptosporidium parvum* oocyst transport and fate. Brush et al. examined oocyst transport through columns containing glass beads, well-sorted sand, and shale aggregates. In these short-duration studies, approximately 50% of the oocysts were retained in the sand and roughly 40% in the glass beads and shale. Despite the losses within the column, oocysts were eluted during the same timeframe as the conservative tracer chloride in the sand and glass beads. The velocity of *C.*

parvum oocyst through the shale aggregate was slightly faster than chloride ions. Brush et al. (13) suggest that this was due to size exclusion and not charge exclusion because of the nearly neutral surfaces of the oocysts. Brush et al. modeled the movement of oocyst with a one-dimensional convective–dispersion transport equation. Sorption was described as instantaneous equilibrium sorption where the relationship between sorbed and aqueous concentrations was linear. All loss processes, including decay, sieving, impingement, and settling, were modeled as a single (i.e., lumped) first-order rate process. The results of their modeling efforts indicated that oocysts experienced less shear and turbulence than dissolved chloride ions and that the oocysts did not adhere to the porous media.

Harter et al. (11) investigated the influence of pore-water velocity and sand grain size on the transport and fate of *Cryptosporidium parvum* oocysts. They used 10 cm long columns, groundwater of medium ionic strength (100 to 150 mg/L TDS), and bovine *Cryptosporidium parvum* oocysts. Their results indicated no trend between sand size and oocyst recovery in the three studies. They found that oocysts arrived before chloride and the elution of oocysts continued after chloride for all column studies. In a limited number of extended studies, tailing of oocyst concentration was observed until the end of the experiment (250+ pore volumes). The early breakthrough, as with Brush et al., was attributed to size exclusion. The tailing in oocyst elution was attributed to reversible deposition.

Harter et al. (11) used a more complex one-dimensional transport model to simulate their data. Filtration was modeled as a first-order irreversible process calculated from the physical properties of the soil (grain size, porosity, and bulk density), water (density, viscosity, pore velocity), and microbial colloid (density, size, diffusion coefficient). Sorption was modeled as a first-order, rate-limited reversible process. Half of the loss was attributed to irreversible filtration, but rate-limited reversible sorption could account for the early breakthrough and partially account for the extended tailing. Harter et al. called for further experimental and theoretical research to measure and explain the long-term elution behavior of *Cryptosporidium parvum* oocysts.

GIARDIA

Giardia lamblia is the cause of the most frequently identified intestinal disease in the United States (26). Humans become infected by ingesting giardia cysts, which are the environmentally resistant stage of giardia (27). Giardia cysts can survive for prolonged periods. For example, Bingham et al. (28) documented cysts surviving in distilled water for 77 days at 8 °C. In another study, cysts of *Giardia muris* (a species that infects mice but is often used as analogue for *Giardia lamblia*), survived 28 and 56 days in lake water at depths of 4.5 meter (19 °C) and 9 m (6.6 °C), respectively. In cold river water (0–2 °C), cysts survived for 56 days (28). Typical concentrations of *Giardia* are between 1 and 100 cysts/mL in untreated domestic wastewater (18) and about 1 cyst/mL in polluted

The *Giardia* cysts, which are 8–16 μm in diameter, are somewhat resistant to typical levels of wastewater treatment methods. During primary settling, only 0–53% of cysts are removed. Secondary treatment with clarification can remove 98.6–99.7% of cysts (28). Advanced tertiary treatment can further reduce the numbers of cysts by physical filtration and precipitation.

MICROSPORIDIUM

Microsporidia are unicellular protozoan parasites that infect a wide variety of animals, from insects and fish to every class of mammal, including humans. The vehicle for transmitting these organisms is its spores, which are shed from infected individuals (animal and human) via the urine, feces, respiratory sputum, and upon death and decay. Human infections are of concern in immunodeficient patients, especially those who are HIV+, and may infect a broad range of tissues. *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are the most common species of microsporidia isolated from patients with chronic diarrhea attributed to microsporidiosis (29,30). Exact routes of transmission for human microsporidial infection have not been confirmed, but considerable evidence supports fecal–oral, sexual, respiratory, and waterborne routes (29–31).

The potential for waterborne transmission of spores is the focus of our research. At least two published studies have detected the presence of *E. bieneusi* (32) or *E. bieneusi*, *E. intestinalis*, and *Vittaforma corneae* (another human pathogenic microsporidium) (33,34) in surface water using the polymerase chain reaction (PCR). Hutin et al. reported in a case-controlled risk factor analysis of HIV+ individuals in France that the greatest risk for intestinal microsporidiosis was use of a swimming pool in the prior 12 months.

At least one outbreak of microsporidiosis has been described and attributed to contaminated drinking water originating from a particular treatment plant. According to Cotte et al. (31), an outbreak of intestinal microsporidiosis in the summer of 1995 affected 200 people (all apparently HIV+), or about 1% of the HIV+ population in the study area. Additionally, 15% of the 361 patients from whom microsporidia were identified during the 3-year study had no known immunodeficiency condition. The clustering of residences of infected individuals in the town of Lyon, France, led the authors to suggest that a water treatment facility serving the area may have been a contributing factor to these cases. This plant employs flocculation, ozoflotation, and filtration but not chlorination in the treatment process and uses surface water as a water source.

A few studies have described the susceptibility of human-pathogenic microsporidial spores to drinking water treatment methods and environmental conditions. Laboratory studies by Kucerova-Posisilova et al. (35) indicate that *E. intestinales* spores retain infectivity for at least 2 weeks at temperatures up to 33 °C. However, information on the viability of spores concentrated from a chlorine concentration of 2 mg/L with exposure for at least 16 minutes resulted in a 99.9% reduction in the viability of *E. intestinalis* spores. Microsporidia were detected in four recreational water samples from Arizona; one was confirmed as *E. intestinalis*. In addition to the three recreational water samples, three irrigation water samples from Mexico and two from Arizona were positive for microsporidia. One of the samples from Mexico was confirmed as *E. intestinalis*. *V. corneae* spores were identified from irrigation water from Costa Rica and from secondary sewage from Tucson, Arizona (37).

In summary, mounting evidence demonstrates that microsporidia are found in water, may originate from human and animal reservoirs, and need to be considered as potential waterborne pathogens. As such, more information is needed on the incidence and survival of spores in the environment and through the treatment process.

BACTERIOPHAGE

Several bacteriophage transport studies have been conducted under well-characterized conditions [see recent review by Schijven and Hassanizadeh (38)]. The bacteriophages PRD-1 and MS-2 are often employed as surrogates for viruses of concern in human health (39,40) because of the hazards and costs associated with human viruses.

CALCULATING FLOW IN KARSTIC LIMESTONE AQUIFERS

In karstic limestone aquifers, groundwater often flows through highly permeable flow paths formed by dissolution along faults, fractures, bedding plane partings, or stratigraphic features. Compared with diffuse flow through granular aquifers, groundwater velocities in karstic systems can be very high, often in the range of miles per day. Consequently, karstic aquifers require a much larger wellhead protection area than common for wells in sand and gravel.

Multiple options exist for modeling flow and transport in karstic aquifers. For simple applications requiring only global water balances in steady-state conditions, existing modeling tools such as MODFLOW have served adequately. For modeling responses to storms or contaminant movement, more advanced models involving explicit flow features (40), irregular grids, and/or multiple interacting flow systems (41) are needed. Software tools necessary for explicit modeling of karst features have recently appeared (42).

TRANSPORT MODELS

Models for microbe transport and of colloid transport in general are analogous to solute transport models nonideal sorption terms account for rate limitations in the attachment/detachment processes. The relevant physical processes include advection, dispersion, attachment/detachment, physical filtration, inactivation, and advection facilitated by sorption on other types of colloids (38). Early models of microbe transport in saturated porous media used the equilibrium sorption assumption and an empirical distribution coefficient to model microbe attachment/release (43,44). These equilibrium sorption models performed poorly in case studies (45) and fail to reproduce the results of experiments that show unretarded breakthrough and slow (nonequilibrium) release (50). Modern models of microbe transport and of colloid transport in general use kinetic models for attachment/release. Single-site kinetic models are often used (46–49). Bales et al. (50) use a two-site model with one set of sites in equilibrium and the other kinetically controlled. Bhattacharjee et al. (55) and Schivjen et al. (56) use two-site models wherein attachment to both types of sites is kinetically controlled but with different rate constants. Schivjen et al. (56) analyze several laboratory experiments and clearly demonstrate that a two-site kinetic model is necessary to reproduce the measured breakthrough curves.

Most previous studies use the first-order rate law. Second-order rate laws have been used to model sorption of inorganic colloids (49) and microbes (55). In either case, attachment rates may be empirical or determined from mechanistic models of colloid filtration (46,49). Combinations of empirical rates and mechanistic rate models have also been used (48). When mechanistic models are used to calculate the attachment rate, models for colloid filtration in packed-bed reactors (50) are the usual choice.

Transport facilitated by other colloids is another process to be considered. Schivjen and Hassanizadeh (38) note that the removal rate for viruses declines with increasing travel distance, and that this nonlinear removal may be due to partial attachment to other colloidal particles. Colloid-facilitated transport is of particular concern in karst systems that often have large amounts of suspended sediments whose attributes are favorable for facilitating transport (51,52). Two studies (48) have addressed the effect of random spatial variability of hydraulic conductivity on microbe transport. These studies clearly provide important insights into the effect of spatial variability on microbe transport but are better suited to granular aquifers, where spatial variability can be more readily represented as a simple random space function.

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