

Future research needs involving pathogens in groundwater

Scott A. Bradford¹ · Ronald W. Harvey²

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Abstract Contamination of groundwater by enteric pathogens has commonly been associated with disease outbreaks. Proper management and treatment of pathogen sources are important prerequisites for preventing groundwater contamination. However, non-point sources of pathogen contamination are frequently difficult to identify, and existing approaches for pathogen detection are costly and only provide semi-quantitative information. Microbial indicators that are readily quantified often do not correlate with the presence of pathogens. Pathogens of emerging concern and increasing detections of antibiotic resistance among bacterial pathogens in groundwater are topics of growing concern. Adequate removal of pathogens during soil passage is therefore critical for safe groundwater extraction. Processes that enhance pathogen transport (e.g., high velocity zones and preferential flow) and diminish pathogen removal (e.g., reversible retention and enhanced survival) are of special concern because they increase the risk of groundwater contamination, but are still incompletely understood. Improved theory and modeling tools are needed to analyze experimental data, test hypotheses, understand coupled processes and controlling mechanisms, predict spatial and/or temporal variability in model parameters and uncertainty in pathogen concentrations, assess risk, and develop mitigation and best management approaches to protect groundwater.

Keywords Microbial processes · Contamination · Groundwater monitoring · Health · Groundwater/surface-water relations

Introduction

Groundwater constitutes ~95% of the world's usable reserve of fresh liquid water, supplies about half the drinking water in the United States (Maupin and Barber 2005), 75% of the drinking water in Europe, and is the principal source of drinking water for as many as 2 billion people worldwide (Alley 2006). Although traditionally considered less vulnerable to contamination by pathogens than surface water, groundwater is responsible for a disproportionate fraction of reported waterborne disease outbreaks (Jin and Flury 2002). Contributions of groundwater to the global burden of waterborne disease outbreaks are difficult to estimate accurately due to a lack of reliable data; however, where systematic, long-term data were collected regarding the sources of such outbreaks, it appears that pathogen-contaminated groundwater is often a major cause—for example, statistics collected from 1998 to 2012 for a national outbreak surveillance system in Scandinavia indicate that most of the waterborne disease outbreaks having a known water source (76%) were linked to groundwater (Guzman-Herrador et al. 2015). Waterborne disease outbreak surveillance data collected in the US over a 36-year period (1971–2006) indicate that a majority (52%) of the deficiencies associated with drinking-water disease outbreaks involved either untreated or inadequately treated groundwater, compared with 19% for untreated or inadequately treated surface water (Craun et al. 2010). Although there was a marked decrease in outbreak risks associated with surface water in public systems during this period, there was no corresponding

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✉ Scott A. Bradford
Scott.Bradford@ars.usda.gov

¹ USDA-ARS, US Salinity Laboratory, Riverside, CA 92507, USA

² USGS, Boulder, CO 80303, USA

decrease in outbreak risks associated with untreated or inadequately treated groundwater (Craun 2012).

Groundwater-related outbreak statistics in the US relate, at least in part, to the observation that approximately one in six households obtain drinking water from private wells for which there is little regulation (Rogan and Brady 2009; Wallender et al. 2014). In a pooled analysis of groundwater systems in the US and Canada (1990–2013), 15% (216 of 2,210) of groundwater samples tested positive for enteric pathogens (Hynds et al. 2014). More than 150 enteric viruses can contaminate groundwater (Wong et al. 2012) as can many pathogenic bacteria including *Arcobacter butzleri*, *Campylobacter* spp., *Escherichia coli*, *Helicobacter pylori*, *Legionella* spp., *Salmonella* spp., *Shigella* spp., *Vibrio cholera*, and *Yersinia* spp., and pathogenic protists such as *Cryptosporidium* spp., *Encephalitozoon intestinalis*, *Giardia lamblia*, and *Naegleria fowleri* (Tufenkji and Emelko 2011). Table 1 provides a summary of recent waterborne disease outbreaks with known etiology that were caused by consumption of contaminated

groundwater. In addition to quantification of the occurrences of pathogens in well water, more comprehensive epidemiological studies will be needed in the future to quantify groundwater-related health risks (Murphy et al. 2014).

The transport and fate of pathogens from dispersed fecal sources through the heterogeneous subsurface to groundwater wells depend on a wide variety of incompletely characterized physical, chemical, and microbiological factors, and considerable knowledge gaps exist (Bradford et al. 2013). Because pathogens impair more than 2 million ha of lakes and over 480,000 km of shoreline in the US alone (US EPA 2010a), more research is clearly needed concerning the roles of surface-water–groundwater exchanges in pathogen transmission to wells. Furthermore, there is concern that the risk of pathogen contamination to groundwater may increase in the future because of ever-increasing demand for potable groundwater; extreme weather events associated with climate change, land use alterations, and ecological disturbances; waterborne diseases of emerging concern; and the proliferation of managed

Table 1 Selected recent waterborne disease outbreaks with known etiology that were caused by consumption of contaminated groundwater

Pathogen(s)	Location	Year	Cases	Deaths/hospital	Reference
Viruses					
Norovirus genogroup 1 and 2	Jeju Island, S. Korea	2004	194	NR	Kim et al. 2005
Norovirus genogroup 1.2	S. Korea	2011	4,451	NR	Cho et al. 2014
Norovirus genogroup 1.2	Sicily, Italy	2011	156	NR	Giammanco et al. 2014
Norovirus	New Mexico, USA	2011	119	0/0	Beer et al. 2015
Norovirus genogroup 1.2	Wisconsin, USA	2012	19	0	Beer et al. 2015
Norovirus genogroup 1.2	Wisconsin, USA	2007	229	0/6	Borchardt et al. 2011
Norovirus	Wyoming, USA	2001	230	NR	Blackburn et al. 2004
Bacteria					
<i>Campylobacter jejuni</i> , & <i>Yersinia enterocolitica</i>	Alaska, USA	2001	12	NR	Blackburn et al. 2004
<i>Campylobacter</i> sp.	Roros, Norway	2007	105	0/7	Jakopanec et al. 2008
<i>E. coli</i> O157:H7 & <i>Campylobacter jejuni</i>	Walkerton, Canada	2000	2,300	7/160	Howard 2006
<i>E. coli</i> O157:H7 & O121	Arizona, USA	2011	56	0/2	Beer et al. 2015
<i>Legionella pneumophila</i>	Washington, USA	2011	3	1/3	Beer et al. 2015
<i>L. pneumophila</i>	Ohio, USA	2011	11	1/11	Beer et al. 2015
<i>L. pneumophila</i>	Pennsylvania, USA	2011	6	1/5	Beer et al. 2015
<i>L. pneumophila</i>	Pennsylvania, USA	2012	2	1/2	Beer et al. 2015
<i>L. pneumophila</i>	Maryland, USA	2011	7	1/6	Beer et al. 2015
Protozoa					
<i>Cryptosporidium</i> sp.	Indiana, USA	2001	10	NR	Blackburn et al. 2004
<i>G. intestinalis</i>	Utah, USA	2012	28	0/0	Beer et al. 2015
<i>G. intestinalis</i>	Florida, USA	2001	6	NR	Blackburn et al. 2004
<i>N. fowleri</i>	Arizona, USA	2002	2	2/2	Gerba et al. 2009
Multiple types					
<i>Campylobacter coli</i> , Norovirus, & Rotavirus group A	Gourdon, France	2000	202	0/6	Gallay et al. 2006
<i>Giardia</i> sp., <i>Salmonella typhimurium</i> , & Norovirus	Ohio, USA	2004	1,540	NR/21	Fong et al. 2007

aquifer recharge and on-land waste disposal. Consequently, more directed studies will be needed to meet future challenges regarding the safety of groundwater sources.

The purpose of this paper is to identify and discuss some of the more critical knowledge gaps and potential directions for future studies involving the transport and fate of pathogens in groundwater. This paper is not meant as a comprehensive review. Factors that influence the transport and fate of pathogens in soils and aquifers cover a vast body of published literature and the interested reader is referred to more detailed information in the following reviews: Schijven and Hassanizadeh 2000; Ginn et al. 2002; Jin and Flury 2002; Bradford et al. 2013, 2014; Molnar et al. 2015.

Selected critical issues needing additional research

Microbial pathogens such as viruses, bacteria, and protozoan parasites are found in the fecal wastes excreted by humans, domesticated animals, wildlife, and insects (Gerba and Smith 2005). Contamination of groundwater can originate from point or non-point sources. Although well-defined point sources can facilitate the study of field-scale pathogen fate and transport, highly variable non-point sources can be major sources of groundwater contamination (Sobsey et al. 2001). Additional research is needed to identify, treat, and manage pathogen sources of groundwater contamination. This management includes better on-land disposal practices regarding domestic wastewater, agriculture practices, and storm-water runoff. For the latter topic, optimized design of engineered infiltration systems could substantially reduce the pathogen and indicator loading from urban areas.

Quantitative determination of pathogen concentrations in complex environmental samples such as groundwater is costly and time consuming. Pathogen abundances in environmental samples are often below the detection limit using current methods, so large volumes of water frequently need to be filtered before analysis (Toze 1999; Sobsey et al. 2001; Lazcka et al. 2007). The recovery efficiency of pathogens from filters and environmental samples is often low and variable (Sobsey et al. 2001), and it may therefore be a critical limiting step in determining quantitative pathogen concentrations that has received limited research attention. Typical methods used to assess pathogen concentrations (fluorescent stains, fluorescence in situ hybridization, monitoring of specific activity, or molecular approaches such as qPCR that amplify selective gene sequences in extracted nucleic acids) require specialized equipment and personnel, and frequently only produce semi-quantitative concentrations for a single pathogen (Toze 1999; Sobsey et al. 2001). Consequently, more research is needed to measure in a cost-effective and timely manner the abundances of pathogens in groundwater environments. New, more sensitive and (or) efficient methods

of pathogen detection are needed to more accurately determine the distribution of pathogens in groundwater used as sources of drinking water. Techniques such as solid-state cytometry (Stevenson et al. 2014), comprehensive droplet digital detections (Kang et al. 2014), and advanced molecular methods (Ramírez-Castillo et al. 2015) may facilitate future studies by providing more accurate and timely information.

Due to the high costs and extreme difficulties in accurately determining pathogen concentrations, regulations to protect public health from pathogens (US EPA 2006, 2010b) only require the periodic monitoring for easily measured indicator microorganisms such as total or fecal coliform (FC), *Enterococcus*, and *E. coli*. However, recent studies indicate that current indicators such as coliform bacteria often do not correlate well with pathogens of interest. For example, in a study involving contaminated groundwater in Bangladesh, it was found that culture-dependent indicators of fecal contamination failed to predict total bacterial pathogens (Ferguson et al. 2012). Furthermore, little research attention has been paid to differences in the source, transport, and fate of indicators and pathogens, although pronounced differences are expected. *Bacillus subtilis* spores were recently found to serve as a conservative surrogate for the transport, retention, and release of *Cryptosporidium parvum* oocysts (Headd and Bradford 2016; Bradford et al. 2016). Further investigations are needed to select indicators that are representative of other pathogen sources, transport, and fate.

Emerging infectious diseases, most of which are caused by zoonotic pathogens, constitute a major threat to public health (Daszak et al. 2007). Groundwater contamination caused by “emerging pathogens” or “pathogens of emerging concern” will continue to be an important research topic in the future. Increasing detections in groundwater of emerging bacterial pathogens such as *H. pylori* (Ryan et al. 2014), only recently linked to ulcers and gastrointestinal cancers, and *Arcobacter*, which has been linked to a number of gastrointestinal diseases (Hsu and Lee 2015), suggest additional research is needed on this topic. In particular, more and better data from the field will be needed in order to develop required inputs for a quantitative microbial risk assessment in order to establish guidelines that would safeguard human health while considering sources of uncertainty (Ryan et al. 2014).

The public health challenges presented by pathogens in groundwater are exacerbated by recent increases in reported instances of antibiotic resistance (Böckelmann et al. 2009; Economides et al. 2012; Bech et al. 2014), which make waterborne diseases harder to treat. Indeed, antibiotic resistance in the environment has been identified as one of the more important infectious disease issues in the United States (Ashbolt et al. 2013), with intensive use of antibiotics in agricultural activity (Bech et al. 2014) and overprescribing of antibiotics (Binder et al. 1999) being major contributors to this problem. The report that 23 of the 25 *V. cholerae* O1 strains

isolated from stream and well water samples in Cameroon exhibited multiple antibiotic resistance (Akoachere et al. 2013) underscores the seriousness of antibiotic resistant bacteria (ARB) in groundwater. Future studies need to account for selective pressures that facilitate ARB, identify and quantify the rates of horizontal gene transfer in ARB propagation, and modify outdated dose–response methodologies for application to ARB (Ashbolt et al. 2013).

The ultimate distance that pathogens can be transported in porous media depends on survival and reversibility of retention; however, the retention rate is typically much higher than the decay (death/inactivation) rate. Hence, retention is viewed as the major contribution to protection of drinking-water aquifers from pathogen contamination; furthermore, porous medium surfaces potentially could be designed to remove pathogens near the source or downgradient at the wellhead. Numerous studies of pathogen retention and release, particularly at the interface-, pore-, and column-scales, have demonstrated extreme sensitive to a multitude of physical, chemical, and microbiological factors. At the field-scale, many of these properties are subject to large spatial and temporal variability that collectively hamper accurate quantification of retention and release parameters and their distribution in situ. Consequently, there is a critical need to be able to predict more accurately the influence of physicochemical factors on pathogen retention and release in the subsurface. Factors that affect retention, enhanced release, and diminish retention capacity (e.g., transients in ionic strength, pH, solution composition, temperature, and colloids and solutes that compete for the same retention sites as pathogens) are of special interests and deserve more research attention because they can increase the risk of pathogen transport. Also important are factors that lead to non-exponential declines in retention with transport distance such as straining, surface, size, or morphologic heterogeneity within a microbial population, variability in hydrodynamic conditions, and blocking.

A number of theoretical approaches have been developed to quantify pathogen retention and release in the subsurface under various physicochemical conditions. Filtration theory considers that the retention rate depends on the mass transfer and immobilization of pathogens on the solid surface. A number of correlation equations have been developed to predict the mass transfer of microbes to the solid-water interface (SWI) under highly idealized pore geometries. New research is needed to study microbe mass transfer to the SWI under conditions that are more representative of natural pore space geometries with wide ranges in grain size distributions, grain-grain contact characteristics, and pore-throat diameters and geometries.

Pathogen immobilization and release from the SWI depends on Brownian, adhesive, and hydrodynamic forces and/or torques that collectively act on the nearly neutrally buoyant microbe. Additional research is warranted to quantify

more accurately these forces and torques, especially the influence of pathogen shape, roughness, chemical heterogeneity, and short-range (e.g., nm-scale) interactions in adhesive forces. Computer simulations that have explicitly accounted for these forces and torques indicate that nanoscale chemical and roughness heterogeneity control immobilization at low velocity and high ionic strength, especially for smaller microbes (Pazmino et al. 2014; Bradford and Torkzaban 2015). Conversely, surface straining (entrapment) at microscopic roughness locations and grain-grain contacts has been found to control immobilization under low ionic strength and high velocity conditions, especially for larger microbes, by substantially increasing and decreasing the lever arms associated with adhesive and hydrodynamic torques, respectively. These findings are generally consistent with micromodel observations of colloid and microbe retention in porous media, and observed differences of retention in batch and column studies (Treumann et al. 2014; Torkzaban and Bradford 2016). Simulation results also indicate that nanoscale heterogeneity creates spatial variability in depths of interaction energy (primary or secondary) minima on the SWI that can induce short- or long-term immobilization at a particular location. These observations demonstrate the complexity of boundary conditions for pathogen immobilization at the SWI, and challenge filtration theory assumptions of a constant sticking efficiency and transport at a constant average pore-water velocity. Additional research is needed to resolve better these issues at the interface- and pore-scales, and to upscale simulation results to physically meaningful continuum-scale parameters under steady-state and transient physicochemical conditions.

Rates of microbial retention are generally greater than those of decay (death and inactivation), although there are some notable exceptions (Schijven and Hassanizadeh 2000). It is often difficult to quantify separately the processes of liquid-phase decay and irreversible retention; also, solid-phase decay can hamper accurate determination of reversible retention. Improved experimental procedures are needed to separately quantify decay and retention processes and obtain mass balance, especially in the field. Conditions that enhance pathogen survival are of special concern when assessing their environmental fate and more research is needed on persistent subpopulations (Proctor et al. 2006; Headd and Bradford 2016), regrowth of bacterial pathogens (Zaleski et al. 2005), transformations between active and vegetative phenotypes (Lewis 2007; Veening et al. 2008), and interactions between fecal pathogens and indigenous microbial populations (Hall-Stoodley et al. 2004). However, microcosm studies only consider a limited set of natural conditions, and are therefore not always representative of in situ field measurements of pathogen survival (Sidhu et al. 2015). Consequently, additional research is needed to predict more accurately field-scale survival and their uncertainties.

Pathogens can be transported by advection through soil and groundwater when retention and decay are collectively insufficient to completely remove them from the pore-water. Because an increase in flow velocity produces greater advective transport and less retention of pathogens in porous media, high velocity zones pose an increased risk of rapid pathogen transport at the field-scale. In addition, transport of pathogens can be more rapid than that of solutes because of size exclusion from fine soils and smaller pore spaces with lower or stagnant flow conditions. Many aquifers are characterized by a high degree of physical heterogeneity and exhibit substantive spatial changes in permeability and flow field as a result of layers and lenses of varying soil texture. Much less is known about preferential pathogen transport in fractured rock and karst aquifers and soils characterized by unstable flow, dynamic capillarity, soil structure, macropores from decaying plant roots, burrowing earthworms and animals, and cracks in clayey soils. Experiments in undisturbed soil columns, lysimeters, tile-drains, and the field have frequently revealed that preferential transport pathways are a major contributor to the overall transport of microbes (Zhang et al. 2012; Bradford et al. 2013). More field-based research and modeling is needed to understand better the influence of preferential flow paths on pathogen transport to drinking-water wells and how the resulting information can be used to determine risk assessment more accurately.

A variety of continuum-scale models have been developed to simulate deterministically the transport and fate of pathogens in the subsurface (Bradford et al. 2013, 2014; Molnar et al. 2015). Deterministic models are valuable to analyze experimental data resulting from small-scale transport studies, to test hypotheses regarding factors that control the fate of the pathogens, to improve our understanding of complex processes, and to identify conditions of enhanced risk. Additional model improvements are needed to predict the dependency of pathogen transport on physicochemical conditions, and to quantify and assess the implications of coupled pathogen transport and fate processes. Furthermore, there is still no consensus about the proper conceptual and mathematical description of many transport and fate processes, including the following: size-exclusion; migration adjacent to the solid phase; a non-constant sticking efficiency; reversibility of retention; and release due to variability in physicochemical conditions. Deterministic models can also be employed to simulate field-scale pathogen migration by explicitly accounting for subsurface heterogeneities and the coupling between retention, release, and survival parameters on physicochemical factors. However, deterministic models neglect the considerable uncertainties in the processes controlling transport and fate that are needed to assess risks accurately. Stochastic modeling approaches have been developed to account for uncertainty in flow, transport, and fate processes (Rehmann et al. 1999; Maxwell et al. 2007). Accurate descriptions of field-scale

flow, transport, and fate processes and parameters remain a critical challenge for both deterministic and stochastic modeling approaches that seek to improve predictions of field-scale pathogen migration, to advance quantitative microbial risk assessment, and to develop mitigation and best management approaches.

Concluding thoughts

Figure 1 and the following summarize key concepts and research gaps involving pathogens in groundwater. A variety of enteric pathogens are found in fecal wastes. These wastes serve as point or non-point sources of groundwater contamination that has been associated with disease outbreaks. Pathogen concentrations in groundwater are proportional to their abundance in the contamination source. Management and treatment of pathogen sources, therefore, serve as important first barriers to prevent microbial contamination of groundwater; however, existing methods to determine pathogen concentrations in environmental samples are currently laborious, expensive, require specialized equipment and expertise, and only provide semi-quantitative information. Consequently, easily measured microbial indicators are

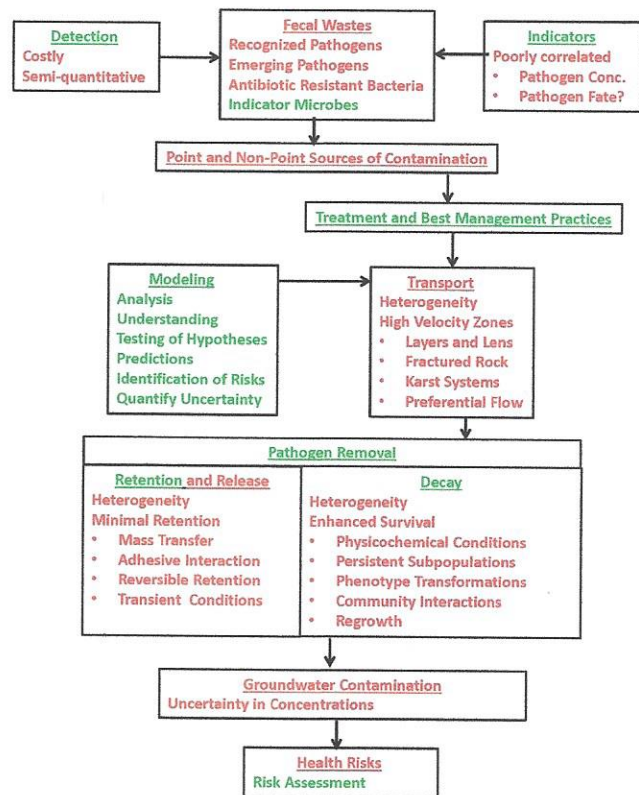


Fig. 1 A schematic illustrating keys knowledge gaps that were identified in this paper. *Green-colored font* indicates conditions or tools that minimize health risks, whereas *red font* indicates conditions that enhance risks

sought for various pathogens. The major conundrum is that some indicators exhibit little correlation with pathogen concentrations in contaminated groundwater and, consequently, should not be used to assess their environmental fate; furthermore, increasing presence of antibiotic resistant bacterial pathogens in groundwater is a topic of emerging concern. Adequate removal of pathogens during passage through soils is, therefore, a critical consideration for safe groundwater production.

Elimination of pathogen threats during soil passage can occur as a result of immobilization on the surface of porous or fractured media, or as a result of death and/or inactivation. The pathogen retention rate is commonly found to be larger than the decay rate, so retention is viewed as the main removal mechanism. Many laboratory-scale research studies have been conducted in order to isolate more effectively the influence of specific processes and factors that contribute to pathogen removal. Results demonstrate that pathogen removal is highly dependent on poorly characterized interactions with diverse environmental surfaces and a multitude of physical, chemical, and microbiological factors. Processes that diminish pathogen removal such as reversible retention and enhanced survival, are of particular concern because they substantially increase the risk of groundwater contamination, but remain incompletely investigated. Improved theory and modeling tools are needed to assess the applicability of laboratory data to field application, test hypotheses, understand coupled processes and controlling mechanisms, and to identify conditions of risk.

Natural soil and aquifer environments exhibit great spatial and/or temporal heterogeneity in the physical, chemical, and microbiology properties that strongly influence pathogen transport and fate. This heterogeneity substantially increases the level of complexity and uncertainty in pathogen transport and fate; however, most of the relevant studies involve laboratory column and microcosms. Few studies have attempted to characterize this uncertainty in pathogen transport or removal at the field scale, and approaches to upscale laboratory information to the field-scale are almost nonexistent. Enhanced risks of pathogen transport and groundwater contamination are expected in high velocity zones with little removal. These rapid velocity zones occur in the presence of lenses and layers of high permeability sands and gravels, in rock fractures, in karst systems, and/or due to preferential flow of recharge water through macropores in the vadose zone. Because climate change has been reported to induce more extreme precipitation (recharge) events, the frequency and severity of such preferential flow events are likely to increase pathogen contamination of groundwater, particularly in systems characterized by a high degree of preferential flow. Mathematical models need to account accurately for variability in field-scale flow, transport, and fate processes in order to quantitatively determine risks of pathogen contamination, and

to develop mitigation and best management approaches to protect groundwater.

In closing, this paper was written from the perspective of scientists concerned with predicting pathogen transport and fate in groundwater; however, the vast majority of published research on this topic has been conducted at the interface-, pore-, or column-scales. There is frequently a disconnection between findings deriving from laboratory-scale studies and the needs of stakeholders concerned with what happens at the field-scale. For example, drinking water utilities need to safely and cost-effectively provide water, farmers need irrigation water of a quality that does not result in pathogen-contaminated fruits and vegetables, public health officials and government regulators need to be able to monitor groundwater for the presence of pathogens and assess the associated risks to human health, and engineers and consultants often need to site water-supply wells at locations that ensure pathogen removal under highly variable, field-scale conditions. Groundwater flow and processes controlling pathogen retention and release are expected to change spatially and temporally because of field-scale heterogeneity. Consequently, the scientific community needs to do a much better job of upscaling laboratory results to the field-scale, and developing field-scale modeling tools that better account for heterogeneity and uncertainty. Conversely, stakeholders frequently do not adequately communicate their research needs and field-scale observations with research scientists. These stakeholders need to be willing to recognize potential problems through open communication and collaboration with research scientists, proactive monitoring and/or transport modeling, and implementation of best management practices.

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