Final Report

VIRUS TRANSPORT IN THE VICINITY OF A PUMPING WELL: April 1995 to June 1998

Prepared for

National Water Resources Institute and EPA

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PROJECT SUMMARY
April 1995 to June 1998

A portion of our work is still on going, though the majority of our effort has been competed and has either been accepted for publication or is being prepared for publication. We have chosen a simple format for this report. First a summary of study results and policy implications are presented. Second, research necessary to provide additional foundation for policy is proposed. Third, our published and draft papers are attached to provide details and interpretation of our research efforts

Summary of Results

FRENCHTOWN HIGH SCHOOL SITE.

Research results at the Frenchtown High School site represent the occurrence and transport of virus in a sand, and sand and gravel dominated aquifer with groundwater at 9 to 12 ° C. This work was supported by NWRI, and EPA, and principally by the Montana Water Research Center, USGS Water Center Program. Natural gradient tracer test and samples from wells located in the septic waste plume downgradient from the drainfield. Results showed that:

1. MS2 and PhiX174 moved at the same rate as bromide.

2. Sorption appears to control the majority of virus. (attachment-detachment)

3. A portion of the injected viruses survived for over 9 months in cold groundwater. MS2 titers remained above background in the injection well and in wells within 7.6m of the injection well.

4. Male specific and somatic coliphage concentrations in the school septic tank ranged from 7,000 to 4,000,000 PFU/L. Average concentrations were about 500,000 PFU/L.

5. Enteroviruses in the septic tank were sporadically detected (only being observed twice at concentrations of 4.4 virus/L and 0.26 virus/L).

Policy Implications:

1. High numbers of male specific and somatic coliphage were present in the septic tank. This enhances their use as indicators of viral contamination from sewage. Measurable concentrations were also found in the groundwater contaminated with septic effluent. Coliphage could be found in samples of 4 L or less over a transport distance of 17.4 m. Larger samples would have detected a continued decreasing concentration with distance of transport.

2. Coliphage levels in the tank and in the field did fluctuate, however, indicating that multiple samples taken over several weeks may be required to adequately characterize the source concentrations rather than one single sample.

3. Set back distances between wells and septic system drainfield of 30.5m would result in about 1 coliphage/1000 L, a decrease in virus concentration of about 6 logs. Based on the concentrations of enteroviruses found in the septic tank, concentrations may be about 1 virus/50,000 L at a well located 30.5 m from the drainfield edge assuming no additional vertical migration. These values suggest that enteroviruses promise little utility as indicators of fecal contamination

4. Seeding of coliphage showed possible survival of attached virus for over 9 months.

5. Standard set back distances of 30.5 m may be inadequate to protect public health and safety if wells are completed in a sand and gravel cold water rapidly flowing (1 to 2 m/d) groundwater system.

6. Viruses remain viable within the aquifer after a release has occurred for months. Perturbations such as chemical changes could act to release additional virus into the aqueous phase at some time in the future.

ERSKINE SITE.

Funded by NWRI and EPA

The Erskine site is located on a floodplain of a gravel- bedded river. Gravel and boulders dominate the aquifer. Groundwater is free from sewage, calcium bicarbonate dominated and ranges in temperature from 5 to 12 C. Natural groundwater velocity is 27 m/d. A number of natural gradient slug input multiple virus seeding experiments and pumping experiments were conducted. Four viruses were seeded under both natural gradient and forced gradient conditions: PRD1, PhiX174, MS2 and attenuated polio virus type 1 (Chat Strain).

Natural Gradient Results:

1. Bacteriophage were detected over 40.5 m of transport. Bromide and attenuated poliovirus were traceable over 19.9 m.

2. Attachment of virus to the aquifer material accounted for relative attenuations for MS2, PRD1, PhiX174 and attenuated polio virus of 49, 71, 65 and 99 %, respectively.

3. Virus peaks arrived at the same time as the bromide peak with the exception of the attenuated polio peak that appeared to arrive before the bromide peak. However, this apparent faster transport of the attenuated polio virus was determined to be an artifact of breakthrough peak truncation. This occurs because of the rapid rate of attachment of the polio virus.

4. Virus within the portion of the aquifer impacted by the seeding appear to remain viable for over 9 months. Long tails on breakthrough curves imply continued low level release of viruses.

Pumping Well Capture Zone Study

1. A well pumping between 378 and 408 L/min created a groundwater capture zone. During the pumping of this well, the same four viruses used in the natural gradient experiment were seeded into the aquifer 20 m from the pumping well. Transport rates were enhanced two to three times.

2. Mass balances calculated at the pumping well showed over the 47 hr of sample collections 78% of the bromide, 30% of the PRD1, 16% of the MS2, 3.8% of the PhiX174 and 0.12% of the attenuated polio virus were extracted from the aquifer. A preliminary figure is attached.

3. Preferential flow affected the rate of transport and the concentration at the pumping well. Rapid transport in a high hydraulic conductive zone resulted in concentrations at the pumping well being 2.6 times higher than predicted by assuming average properties of the aquifer.

Policy Implications

1. Transport rates and breakthrough timing, but not magnitudes, of a bromide tracer are good predictors of rates and breakthrough curves of the three coliphage. Attenuated polio virus breakthrough curves are impacted by high attenuation rates and peak concentrations observed in the field may arrive slightly ahead of times predicted by the bromide curve.

2. Viruses attach at different rates. Under the Erskine Site conditions, at natural gradients, bacteriophage attached at lower rates than the attenuated polio virus. The higher resolution pumping experiment showed PRD1 to sorb less than the smaller MS2 and PhiX174. In both natural gradient and forced gradient conditions the attenuated polio virus attached at high rates. This suggests that the used of coliphage as indicators of virus contamination will be conservative relative to the behavior of the attenuated polio virus. The rates of attachment appeared to be directly related to the surface isoelectric points of the different viruses in relation to the net charge of the sediment.

3. Set back distances between wells and virus sources of 30.5 m will most likely be inadequate under Erskine Site conditions to protect public health.

4. A portion of attached viruses remain viable after initial attachment for over 9 months. Thus, a source of virus remains long after a contamination event. As a result, a low level of virus may be continually released into the aquifer.

5. Transport in the vicinity of a pumping well may exceed arrival times and concentrations predicted using average aquifer properties. Set back and well-head protection zones need to be reviewed relative to aquifer characteristics and the likely-hood of preferential flow zones.

RESEARCH IN PROGRESS

Additional funding to support these efforts is needed. As of January 1999 these projects are still in progress and final summaries or papers have not been prepared.

Survival Study

1. Samples at the Erskine Site are being collected from selected wells and analyzed for MS2 to determine if viable virus remain in the portion of the aquifer impacted by the virus seed.

Attachment-Detachment Study (MS Thesis in progress)

1. Two columns were set up and the virus MS2 were seeded under conditions of different velocities.
2. Detachment was then assessed by injecting the columns with virus free water.

Vadose Zone Transport and Attenuation of Virus (MS Thesis in progress)

1. A portion of the Frenchtown High School drainfield has been instrumented with effluent sampling ports, stainless steel suction lysimeters and multi-level monitoring wells. These instruments will be used along with a virus seeding experiment to examine the behavior of virus transport through the unsaturated zone.

Field Mass Balance for Virus and Bacteria (field work completed, analysis underway)

Partial support by NWRI and principle support by Montana Water Center, USGS Regional Program.

1. Within 3 m of a pumping well, MS2 coliphage and bacteria will be seeded into the Frenchtown High School Aquifer. A mass balance at the pumping well will be calculated. The mass of injected microbes remaining on the solid aquifer material will be determined by coring and extraction.

Policy Implications of On-going Research.

1. Predicting the behavior of virus requires field and laboratory data on how changes in groundwater velocity impacts the aqueous virus phase. It also requires input of attachment rates so that maximum concentrations can be appropriately predicted. The on-going work is addressing these issues as tempered by field observations.

2. Currently we can only use data from the saturated zones in predicting how various thicknesses of vadose zones will impact virus movement and survival. Work is needed to develop techniques and methods by which septic system's virus concentrations can be used to predict final groundwater virus concentrations.

ADDITIONAL RESEARCH NEEDED TO PROVIDE SUPPORT OF PROPOSED REGULATIONS:

The Erskine and Frenchtown High School sites provide excellent sites for continued experimentation in the areas listed below.

1. Continuation of long term studies following virus survivals. Currently survival has been followed for three consecutive nine-month intervals and can now be extended for several years if funding is available.

2. Identification of the importance of velocity changes in the vicinity of a pumping well on the magnitude and rate of virus transport.

3. Develop a procedure to evaluate when preferential flow zones should be considered when assessing potential impact in aquifer.

4. Establish the average concentrations of coliphage in individual septic tanks so that standard estimations of effluent virus concentrations can be established.

5. Establish to what degree the percolation of virus through the unsaturated zone beneath a drainfield reduces the effluent virus concentrations entering the groundwater.

6. Develop attachment and detachment rates for viruses to allow simulation of virus transport in relation to the surface isoelectric points of both viruses and sediments.

RESEARCH PAPERS

Figure of Pumping Experiment Results at Erskine: Accumulation of Tracer at Pumping Well.

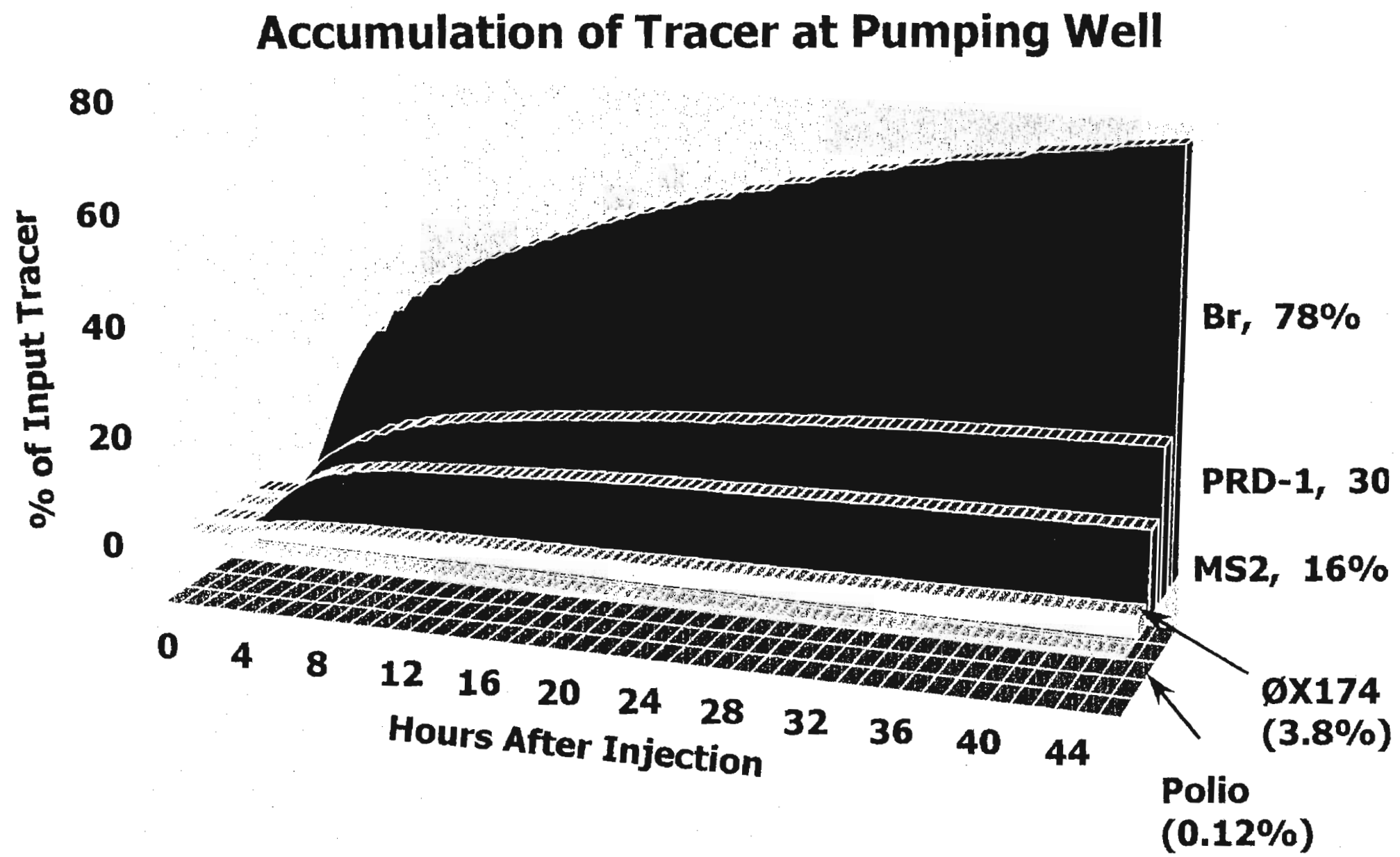
Research Note: Coliphage Prevalence in High School Septic Effluent and Associated Ground Water. 1998 Wat. Res., 32(12), p. 3781-3785.

Research Paper: Virus Occurrence and transport in a School Septic System and Unconfined aquifer. 1998 Ground Water, 36(5), p. 825-834.

Research Paper: Rapid Transport of Viruses in a Floodplain Aquifer. 1999 Accepted for Publication. Wat. Res.

Research Paper: Virus Transport in the Floodplain Groundwater of a Headwater Stream, Western Montana, USA. 1998, IAHC, p. 197-207.

Research Paper: Virus Transport in the Capture Zone of a Well Penetrating a High Hydraulic Conductivity Aquifer Containing a Preferential flow Zone: Challenges to Natural Disinfection. 1998, Source Water Assessment and Protection'98, p. 167-174.


Accumulation of Tracer at Pumping Well
% of Input Tracer
80
60
40
20
0
0
4
8
12
16
20
24
28
32
36
40
44
Hours After Injection
Br, 78%
PRD-1, 30
MS2, 16%
ØX174
(3.8%)
Polio
(0.12%)

Pergamon

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RESEARCH NOTE

COLIPHAGE PREVALENCE IN HIGH SCHOOL SEPTIC EFFLUENT AND ASSOCIATED GROUND WATER

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Abstract—At the present time, somatic and male-specific coliphage and human enterovirus groups are being considered as indicators of possible pathogenic human enteric virus contamination from fecal contamination. A primary attribute for any indicator of fecal contamination is its prevalence at the source and in associated ground water. It must be consistently found in the source material at concentrations that are measurable with available techniques. Over a period of ten months, male-specific and somatic coliphage ranged from ~7000 to ~4,000,000 PFU/L in the effluent from a multi-user septic-tank. Unlike the values determined for septic-tank effluent, coliphage concentrations measured in ground water over this same period only varied by five-fold. Coliphage concentration in ground water under the down-gradient edge of the drainfield contained ~1000 PFU/L. This concentration decreased at $-1\ \log_{10}$ 5 m during 17.4 m of ground-water transport. From these data, coliphage concentrations in septic-tank effluent seem sufficient to allow their use as indicators of fecal contamination in ground water.

Key words: coliphage, fecal waste, viral indicators, virus, ground water, ground-water transport, Ground Water Disinfection Rule.

Waterborne disease outbreaks occur each year despite regulations establishing both minimum vertical distances between effluent disposal systems and ground water, and horizontal setback distances between drainfields and water supply wells (Keswick and Gerba, 1980; Moore, 1982; Metcalf *et al.*, 1995). The 1987 Amendments to the Safe Drinking Water Act mandate the United States Environmental Protection Agency (USEPA) develop a set of regulations ensuring ground-water supplies are not contaminated with viruses. Such regulations may require sampling of wells for indicator virus.

At the present time, somatic and male-specific coliphage and human enterovirus groups are being considered as indicators of possible human enteric virus contamination (USEPA, 1996). Arguments for (Borrego *et al.*, 1990; Sobsey *et al.*, 1990; Springthorpe *et al.*, 1993; Hernandez-Delgado and Toranzos, 1995) and against (Yeager and O'Brien, 1977; Nasser *et al.*, 1993) using these viral groups as indicators have been presented. While a positive viral indicator assay demonstrates fecal waste contamination, a negative assay does not necessarily indicate uncontaminated ground water. The level of significance that can be applied to a negative assay depends upon the prevalence of indicator virus in the source and associated ground water, and its detection limits. In this paper we consider these primary attributes of an indicator of fecal contamination — its prevalence and detectability at the source and in associated ground water.

Coliphage groups are considered common in sewage and could be useful surrogates for sporadically occurring pathogenic human viruses (USEPA, 1996). Male-specific coliphage concentrations in raw sewage generated from more than 100 people have been estimated at 10^6 PFU/L (USEPA, 1996). This level is several orders of magnitude higher than concentrations estimated for human viruses (10^2 to 10^4 PFU/L) in sewage effluent (Moore, 1982; Matthess and Pekdeger, 1985). Though some data for the presence of coliphage in sewage and effluent have been reported (USEPA, 1996), no systematic published data are available on average coliphage concentrations in septic-tank effluent sources. Using a large multi-user (350 students, staff and faculty) septic-tank system located at Frenchtown High School in western Montana, we assayed both male-

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Table 1. Male-specific and somatic coliphage concentrations in the Frenchtown High School septic tank effluent[a]

	Coliphage (PFU/L) found in septic tank effluent			
	individual samples		time-weighted average	
Date	male-specific	somatic	male-specific	somatic
12/9/94	3.00E + 05	2.76E + 05	3.00E + 05	2.76E + 05
3/1/95	5.87E + 05	1.73E + 05	5.87E + 05	1.73E + 05
3/15/95	1.16E + 06	3.88E + 06	1.16E + 06	3.88E + 06
5/3/95	1.60E + 05	8.03E + 04	1.60E + 05	8.03E + 04
5/23/95	8.86E + 05	1.80E + 04		
5/25/95	1.34E + 06	2.80E + 04	1.11E + 06	2.30E + 04
6/1/95	9.00E + 04	1.15E + 04		
6/5/95	2.30E + 04	7.73E + 03		
6/7/95	2.64E + 06	7.70E + 03	9.17E + 05	8.98E + 03
6/13/95	1.97E + 06	1.02E + 04		
6/14/95	2.10E + 06	2.56E + 04		
6/15/95	1.23E + 06	2.93E + 04		
6/16/95	1.58E + 06	3.84E + 04		
6/17/95	1.05E + 06	5.44E + 04		
6/18/95	1.18E + 06	8.00E + 04		
6/19/95	7.16E + 05	8.28E + 04	1.40E + 06	4.58E + 04
6/20/95	5.22E + 05	6.28E + 04		
6/21/95	4.18E + 05	7.94E + 04		
6/22/95	3.29E + 05	6.84E + 04		
6/23/95	2.34E + 05	5.54E + 04		
6/24/95	1.92E + 05	1.02E + 05		
6/25/95	1.63E + 05	8.84E + 04		
6/26/95	1.06E + 05	2.08E + 05		
6/27/95	9.95E + 04	1.72E + 05	2.81E + 05	9.48E + 04
7/29/95	1.50E + 04	5.95E + 05		
8/2/95	9.80E + 03	3.40E + 05	4.14E + 04	3.69E + 05
8/21/95	1.70E + 05	1.75E + 05		
8/22/95	1.27E + 05	1.47E + 05		
8/26/95	2.55E + 04	2.47E + 04		
8/27/95	7.10E + 03	3.30E + 04	7.00E + 04	8.15E + 04
8/28/95	2.02E + 04	2.77E + 04		
8/29/95	2.70E + 05	1.18E + 04		
8/29/95	5.38E + 05	1.30E + 04		
8/30/95	5.06E + 05	1.45E + 04		
8/30/95	5.40E + 05	1.24E + 04		
8/31/95	5.94E + 05	1.20E + 04		
8/31/95	9.56E + 05	3.00E + 04		
9/1/95	1.32E + 06	1.09E + 05		
9/2/95	6.20E + 05	2.10E + 05	6.68E + 05	5.16E + 04
9/3/95	4.21E + 05	1.10E + 05		
9/4/95	5.67E + 05	2.00E + 05		
9/5/95	5.18E + 05	1.60E + 05		
9/6/95	1.76E + 06	1.24E + 06		
9/7/95	2.00E + 06	5.55E + 05		
9/8/95	3.40E + 06	7.00E + 05	1.44E + 06	4.94E + 05
Average PFU/L:	7.44E + 05	2.30E + 05	6.79E + 05	4.65E + 05

[a] *Escherichia coli* C and *E. coli* C3000 were used as host bacteria for growth and plaque assays of somatic and male-specific coliphage, respectively.

specific and somatic coliphage in septic effluent and the associated ground water.

From 12/94 to 9/95, coliphage in the septic tank effluent were assayed intermittently by single-layer agar plaque analysis (DeBorde *et al.*, 1998). The range of male-specific coliphage varied from 7.1×10^3 to 3.4×10^6 PFU/L, and from 7.7×10^3 to 3.9×10^6 PFU/L for somatic coliphage (Table 1). All samples that were collected within 7 consecutive days were grouped and averaged as one entry to eliminate close-spaced sampling bias for the calculation of the time-weighted average (Table 1). Male-specific and somatic coliphage had time-weighted averages of 6.8×10^5 and 4.7×10^5 PFU/L, respectively. This data set included frequent samples from two, two-week periods associated with school closing (June 1995) and school startup (August 1995).

The data collected at 12 to 24 h intervals during these two, two-week periods are plotted in Fig. 1. Both somatic and male-specific coliphage concentrations fluctuated greatly over these transition periods. Although the variation was usually one to two orders of magnitude during these periods, the rate of change was gradual over either two-week interval. Therefore, daily sampling appeared sufficient for establishing average coliphage concentrations.

When the data in Table 1 are separated by "school in-session" (Sept.–May) or "school out-of-session" (June–Aug.), the male-specific coliphage levels in-session had a time-weighted average twice that of out-of-session, while the somatic coliphage was 5-fold higher during in-session than out-of-session periods. In spite of this variation, the large average numbers of coliphage found in septic-tank

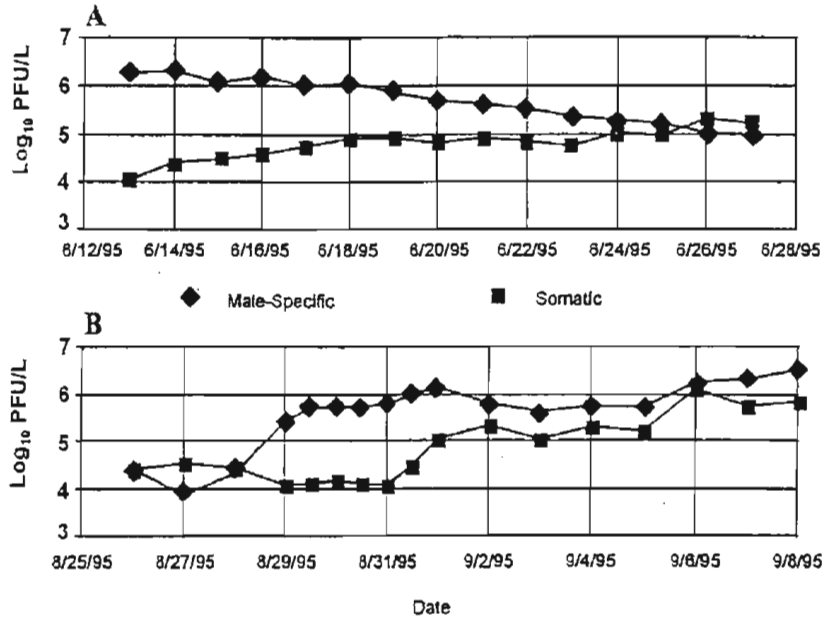

Fig. 1. Concentrations of male-specific and somatic coliphage in effluent in the Frenchtown High School septic tank are plotted over time. Panel (A) = two weeks just after the end of the academic year (June 2, 1995), and panel (B) = a two-week period that includes the start of the new school year on August 30, 1995. 95% confidence limits are ±15%. Plotted limits are obscured by the data series symbols.

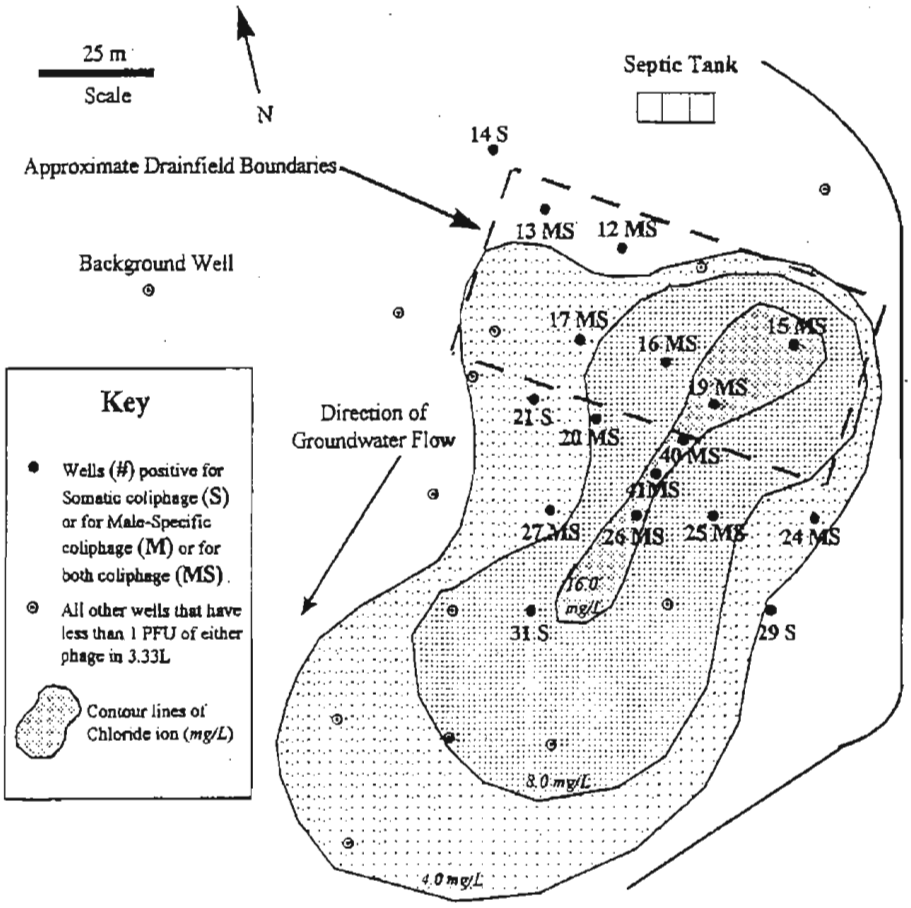

Fig. 2. Site map of the Frenchtown High School septic system, and associated chloride and virus impacted ground water. Some close set wells in the septic plume area have been left off the map for clarity.

effluent increases the probability that they will be detectable in ground water and hence useful as markers of sewage contamination.

Septic-tank effluent percolates from the drainfield laterals through 1.5 m of medium sand to the water table. Ground-water temperature is 10°C and flows at 1 to 2.9 m/day (DeBorde *et al.*, 1998). The 31 monitoring wells (Fig. 2), perforated in the upper 2 m of the sand and gravel unconfined aquifer, were used to characterize the distribution of male-specific and somatic coliphage. These phage were monitored by sampling all wells at the start and end of this project. Selected wells (#19, 40, 41, and 26) that were located within the main plume of septic effluent-impacted ground water were frequently monitored during the summer. As needed, MPN (most probable number) assays employing ten replicates at three different volumes (1, 10, and 100 ml) or (3, 30, and 300 ml) (DeBorde *et al.*, 1998) were used to increase sensitivity over that of the 10 ml direct plaquing assay. The wells that tested positive for either coliphage are indicated on the site map (Fig. 2) together with the septic plume represented by elevated chloride concentrations (DeBorde *et al.*, 1998). Concentrations ranged from as little as 1 phage per 3.3 L in well #14 to approximately 1000 PFU/L at wells #15 and #19. The relative location of wells testing positive for coliphage and concentration data showed good correlation with the chloride plume (DeBorde *et al.*, 1998) (Fig. 2).

The relationship of coliphage concentration in ground water with transport distance was plotted in Fig. 3. Each point was the average of five or six individual measurements at each well, taken over the summer of 1995. The results for any one well varied no more than five-fold during this period. Thus, by the time the coliphage had entered the ground water below the drainfield the large variations in concentrations seen in the septic tank effluent had been greatly diminished. The variation at a 95% confidence level for replicates sampled and processed on the same day was ±15%. In this sewage-impacted setting, both coliphage concentrations are essentially identical, decreasing approximately one order of magnitude with every 5 m of transport (17.5 m/3.5 logs).

Due to their high numbers in the septic-tank effluent, male-specific and somatic coliphage will be far more likely to be found in effluent impacted aquifers than the human enteric viruses, and thus, be more sensitive indicators of fecal contamination. Though this study focused on a large, multi-user septic tank (56,700 L) it may be possible to relate coliphage concentrations to the typical 3780 L, four-person household septic tank. On a per user basis, the school's effluent is approximately 6 times more concentrated than that found in a domestic system. At sites of similar hydrogeology, household septic tank discharge would result in ground-water concentrations of approximately 1 coliphage/1000 L at a typical setback distance of 30.5 m. This concentration is close to the limit of detection for coliphage and enteroviruses (USEPA, 1994). Based on the ratio of coliphage to enterovirus concentrations (10^5–10^6 PFU/L to 5.5 PFU/L, respectively) found in the septic effluent (DeBorde *et al.*, 1998), we would predict that enterovirus concentrations would be four to five logs lower than coliphage at this typical setback distance, and hence, not useful as fecal indicators. Note also that in our field setting, background coliphage values were very low, no matter which host *E. coli* was used. At our levels of background detection in non-effluent impacted areas, no differences in prevalence for either male-specific or somatic coliphage were observed.

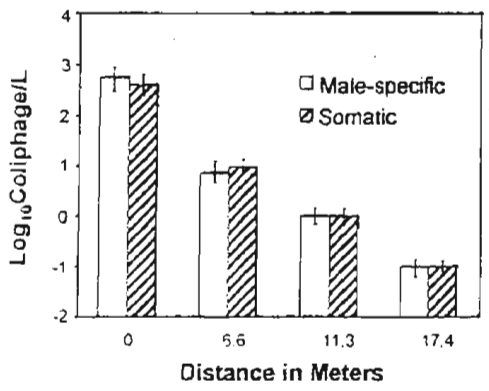

Fig. 3. Male-specific and somatic coliphage concentrations at wells #19, 40, 41, and 26, located at 0, 5.6, 11.3, and 17.4 m, respectively. These wells are centered in the septic system plume and are aligned in the direction of ground-water flow. The vertical bars represent the 95% confidence limits for each average.

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Virus Occurrence and Transport in a School Septic System and Unconfined Aquifer

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Abstract

Federal efforts to establish reliable natural disinfection criteria for ground water supplies require the identification of appropriate indicator viruses to represent pathogenic viruses and an understanding of parameters affecting virus survival and transport in a variety of hydrogeologic settings. A high school septic system and the associated fecal waste-impacted unconfined sand and gravel aquifer were instrumented to: (1) evaluate if the concentrations of enterovirus and coliphage in this system were sufficient to allow their use as indicator viruses; (2) establish viral transport rates, transport distances, and concentrations in a highly conductive cold water aquifer. Enteroviruses were found in only two of eight assays of the septic tank effluent (0.26 and 4.4 virus/L) and were below detection in eight ground water samples. Male-specific and somatic coliphage were detectable in both the septic tank effluent (averaging 674,000 and 466,000 coliphage/L, respectively) and in the impacted underlying ground water, decreasing to detection limits beyond 38 m of the drainfield. Virus transport parameters in this aquifer were measured by seeding high numbers of MS2 and ∅X174 coliphage into the ground water and documenting their transport over 17.4 m. A portion of the seeded virus traveled at least as fast as the bromide tracer (1 to 2.9 m/d). Proposed natural disinfection criteria would not be met in this aquifer using standard 30.5 m setback distances. In addition, the virus sorption processes and long survival times resulted in presence of viable seed virus for more than nine months.

Introduction

Domestic septic system effluent typically contains about 3×10^7 coliform bacteria/100 mL and, following some types of human viral infections, up to 1×10^7 virus/L (Canter and Knox 1985). In rural areas of the United States, where residents, schools, gas stations, and other businesses depend on ground water for their potable water and on septic systems for waste disposal (Bitton and Gerba 1984), more than three trillion liters of septic effluent leaves rural drainfields and percolates to the underlying ground water annually (Yates 1985). Even with county, state, and federal efforts to minimize exposure of rural ground water users to pathogenic organisms, more than 42% of water-associated disease outbreaks in this population can be traced to the consumption of untreated, sewage-impacted ground water (Keswick and Gerba 1980).

As concern over the contamination of ground water by viruses grows, the U.S. EPA is attempting to promulgate requirements for disinfection of ground water sources and systems found to be contaminated or vulnerable to contamination (Macler 1995). One part of this effort is the establishment of natural disinfection criteria that would identify ground water supplies protected from microbiological contamination by their hydrogeological setting. No other forms of disinfection would be required where natural disinfection criteria were met (Macler 1995). These criteria will require understanding the occurrence and behavior of viruses in various hydrogeologic settings.

Natural disinfection criteria will be based on the physical conditions at an individual site, including the sediment type, ground water velocity and temperature, and the vertical and/or horizontal separation of a water supply well from a contaminant source (Macler 1995). An acceptable criteria will avoid pathogenic virus contamination regardless of the virus size, charge, protein coat properties, and survival rate. These physical and biological factors affect the time of travel between a source and ground water supply and allow for the reduction of viral concentration by physical dispersion, adsorption, and inactivation (Bales et al. 1995; Gerba 1983; Keswick and Gerba 1980). However, studies of virus movement and survival at hydrogeologic sites that have been extensively characterized are limited. In addition, the characterization of viral properties that impact transport and survival are incomplete for most viruses (Alhajjar et al. 1988; Bales et al. 1989; Borrego et al. 1990; Corapcioglu and Haridas 1985; U.S. EPA 1986; Vaughn et al. 1983; Yates and Jury 1995; Yeager and O'Brien 1977). While it would be impractical to perform extensive viral transport and survival experiments at every well site being considered for natural disinfection, a more reasonable approach is to generate a database of a limited number of ground water-viral studies that span the range of hydrogeological settings, conditions, and viruses.

The generation of such a database is challenging from both hydrogeological and biological aspects. While standard site characterization procedures will frame the hydrogeologic setting, characterizing the occurrence and behavior of viruses is more difficult

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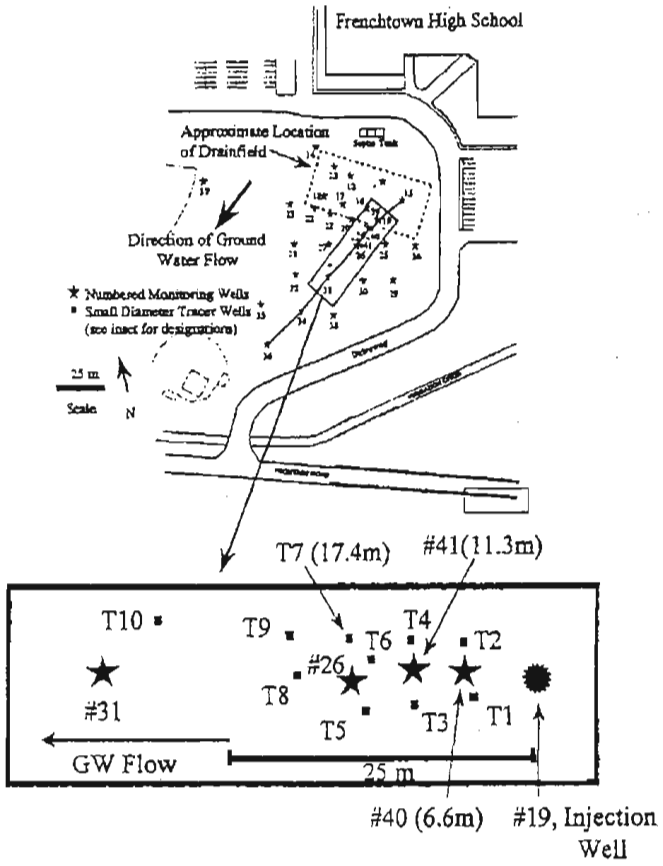

Figure 1. Site map of the Frenchtown High School research site showing the locations of all main monitoring wells (indicated by numbers) and small-diameter tracer wells (indicated by a capital T). The rectangular inset shows the location of the network of monitoring and tracer wells for the virus seeding experiment. Well #19 is the main injection well. The numbers in parentheses by the well names in the inset are the distances in meters from the injection well. A section line between well #15 to well #36 locates those wells shown in Figure 2.

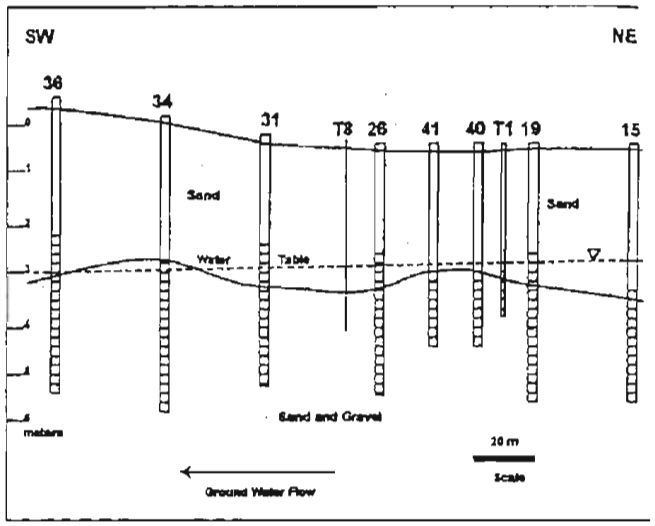

Figure 2. Cross section (SW to NE) centered between wells #36 and #1 (Figure 1). The upper zone is fine to medium sand that is underlain b sand and gravel which forms the unconfined aquifer. The base of th sand and gravel is located 10.7 m below land surface. Wells indicate with numbers are constructed with 5.08 cm diameter PVC placed i hollow stem augered boreholes. T1 is a 3.2 cm diameter PVC sand poin and T8 is a 1.3 cm diameter polyethylene tube. Perforated intervals ar indicated by series of horizontal lines.

Direct knowledge of the behavior of human pathogenic viruses in ground water is poor because: (1) human viruses are present in fecal waste only when the source population is infected, requiring frequent sampling over long times of sources such as individual septic systems; (2) there are many different pathogenic viruses; (3) assay techniques for human pathogenic viruses are complex, costly, and nonexistent for some viruses; and (4) permission to inject pathogenic viruses into an aquifer is extremely difficult, if not impossible, to obtain. Thus, it is desirable to identify a single virus or group of viruses to act as viral indicators that are consistently found in septic waste and for which established assay techniques are available.

Many of the same factors that plague research on pathogenic viruses also affect characterization of pathogenic bacteria in ground water. As a result, coliform bacteria, which occur in high numbers in fecal waste, are used as indicators of the potential presence of bacterial pathogens (U.S. EPA 1989). Coliforms are not reliable indicators for viruses, however, due to the physical differences between bacteria and viruses (Gerba et al. 1979; Marzouk et al. 1980). Both human enteroviruses and coliphage (viruses that infect coliform bacteria found in the human intestinal tract) have been proposed as indicator candidates (Kott et al. 1974; IAWPRC 1991; U.S. EPA 1992; Wentzel et al. 1982). Testing of natural disinfection criteria will require monitoring for appropriate viral indicators at specified setback distances. If possible, these indicator viruses should span the range of surface properties determined for the pathogenic viruses much as the chosen hydrogeologic sites should span the range of hydrogeological characteristics of aquifers.

We examined the presence, abundance, and distribution of these groups of indicator viruses, both human enteroviruses and coliphage, in a large, multiuser septic system and in the associated fecal waste-impacted sand and gravel aquifer at the rural Frenchtown High School (FHS) in western Montana. We also injected MS2 and ØX174 coliphage directly into the impacted ground water system to more accurately determine viral transport rates, concentration changes with distance, and virus fate. The data from these experiments allowed us to (1) evaluate if human enterovirus or coliphage were present in sufficient concentrations to be useful as naturally occurring indicator virus groups; and (2) establish viral transport rates, transport distances, and concentrations in a cold water, highly conductive sand and gravel aquifer.

This study extends previous work by using a high school population as a community source for viral indicators of fecal contamination (Borrego et al. 1990; Vaughn et al. 1983; Wentzel et al. 1982; Yates 1985). The hydrogeological conditions at this site are characteristic of aquifers most apt to allow virus survival and rapid movement in the United States. Finally, unlike other studies reported in the literature (Bales et al. 1995; Rossi et al. 1994; Yeager and O'Brien 1977), we were also able to revisit the site over time and observed viral persistence.

Site Description

A research site was established at the rural Frenchtown High School located 25 km west of Missoula, in western Montana. Approximately 12,180 L/d of sewage effluent produced by 350 students and staff is disposed of by a three-chambered 56,700 L septic tank and 1860 m^2 drainfield (Figure 1). The drainfield is constructed of perforated 10.2 cm diameter Schedule 20 PVC pipe with 26 laterals buried in trenches 0.6 m below land surface and surrounded by washed 5 cm diameter cobbles. A fine to medium sand is present at land surface and extends to a depth of 2.4 to 3.4 m. The

sand is underlain by approximately 7.6 m of sand and gravel, which is saturated and forms the water table aquifer (Figure 2). Some residents use the shallow aquifer for water supply. A confined sand and gravel aquifer, separated from the unconfined system by 30 m of fine sand, is the drinking water source used by the school and the majority of rural residents in the area (not shown in Figure 2).

Methods

Site Instrumentation

Thirty-one, 5.08 cm diameter PVC monitoring wells, extending to a depth of 4.6 to 6.1 m with the lower 1.5 to 3.0 m slotted (30 slot), were placed in an array including the drainfield and the surrounding area (Figures 1 and 2). Wells were installed in the 11.4 cm diameter hollow stem of a 20.3 cm diameter auger flight. Auger flights were withdrawn and the borehole allowed to collapse around the well screen. The hole was backfilled with drill cuttings above the water table and sealed with bentonite at the surface. Well boreholes located in the drainfield area were backfilled with bentonite from a depth of 1.2 m to land surface. Wells were developed by surge block, bailing, and pumping. Soil samples collected during well construction were described and sieved. Monthly water level measurements by electric tape were used to determine the depth to ground water and characterize the direction of flow. Pumping tests and a bromide tracer test were used to determine the hydraulic conductivity of the sand and gravel aquifer (Fetter 1994).

Ten additional small-diameter wells (T1 through T10) were installed to depths of 3.6 to 4.9 m parallel and perpendicular to a flowpath extending from the drainfield edge 35 m downgradient (Figures 1 and 2). Wells T1 to T7 were constructed of 3.2 cm diameter Schedule 80 PVC pipe with 0.61 m of hand-slotted perforations (20 slot). After coring the approximately 3.3 m of sand with a 3.8 cm diameter Geoprobe core barrel, the well was driven to between 3.7 and 4.3 m. The borehole was backfilled with cuttings and sealed at the surface with bentonite. Sampling points T8 through T10 were constructed using a 2.54 cm diameter steel pipe with a carriage bolt loosely fit in the end that was driven to a depth of 3.6 to 4.9 m. A 1.3 cm diameter polyethylene tube with the bottom 0.61 m slotted and wrapped with a fine nylon mesh screen was inserted in the steel pipe and the pipe withdrawn (Figure 2). Cuttings were used to backfill the hole and bentonite was used to form a surface seal.

Chemical Indicator Sampling

Water quality samples were collected from the wells and the septic tank using site dedicated 1.3 cm diameter, acid-washed polyethylene tubing fitted with a short section of sterilized C-FLEX tubing (Cole-Parmer Instrument Co., Vernon Hills, Illinois) that attached to a peristaltic pump. At each monitoring well, sample tube intakes were positioned within the perforated portion of the well, usually 0.6 to 1 m below the measured water table. The sample intake tube in the septic tank was set approximately 1 m below the liquid surface in the third and final septic tank compartment. Each well was purged of approximately one to two well volumes prior to sample collection using a peristaltic pump. During well evacuation, field measurements of temperature, pH, electrical conductivity, and dissolved oxygen (DO) were recorded. Samples were passed through a 0.45 µm filter, preserved as required, and packed on ice for transport to the University of Montana analytical laboratory (U.S. EPA 1986). Standard analytical procedures using inductively coupled argon plasma emission spectrophotometry (Jerrell Ash) and ion chromatography (Dionex, AS4A column) were applied to determine the general inorganic chemistry of septic effluent, background ground water, and septic effluent-impacted ground water.

Sampling for Enterovirus and Coliphage

Using the same basic procedures described for the chemical indicators previously outlined, septic tank effluent samples were collected in 100 mL sterilized polypropylene bottles, or by pumping 90 to 400 L of sample through autoclaved 1MDS Virosorb filter cartridges (CUNO, Meriden, Connecticut) and prefilter setups (U.S. EPA 1994). Ground water sample volumes of 0.1 to 4 L were collected into sterile polypropylene containers. One to two bore volumes of ground water were removed prior to sample collection. When it was anticipated that virus concentrations would be below detection in small grab sample volumes, 1000 to 2000 L were filtered through the 1MDS filter-prefilter setup. The filter housings were packed in ice and shaded from direct sunlight during filtration. After the desired sample volume had been filtered, the excess water was drained from the housing and openings covered with sterile aluminum foil. The sealed unit was placed on ice and returned to the laboratory.

Any adsorbed viruses were then eluted from the 1MDS filters following standard procedures with the following modifications (U.S. EPA 1994). The 1MDS filters were always eluted within four to eight hours after collection. Small volumes (on the order of 40 to 100 mL each) were taken from the initial beef extract eluate, pH 7 to 7.5, for archive and coliphage analyses. The archive sample was frozen at −70°C while the coliphage sample was held at 4°C for no more than one to three hours before the plaque analyses were performed. Volume measurements and calculations of virus concentrations were performed as described in the ICR protocol (U.S. EPA 1994).

Coliphage Assays

Grab samples and 1MDS eluates were assayed by single-layer agar gel technique (Grabow and Coubrough 1986) using the appropriate hosts. The 1MDS eluates were filtered through a 0.45 µm filter that had been treated with the sterile 3% beef extract, 0.05 M glycine (BEG) solution at pH 7.5 to prevent nonspecific binding of the virus and to remove contaminating bacteria. Both somatic (*Escherichia coli* C) and male-specific (*Escherichia coli* C3000) host bacteria were used (U.S. EPA 1994). When the concentration of coliphage/mL was too low to be reliably assayed in 10 mL or less of sample (>3-5 PFU/mL), then Most Probable Number (MPN) assays were performed using total sample volumes of approximately 1.1 to 3.3 L as described by DeBorde et al. (1997).

Enterovirus Assays

Buffalo Green Monkey (BGM) kidney cell culture plaque and MPN analyses were used to monitor the levels of enteroviruses in both grab and filtered samples (Berg 1984; U.S. EPA 1994).

Bromide Seeding Experiment

One week before the coliphage seeding experiment, a sodium bromide tracer test was initiated. We did not co-inject the bromide tracer and phage to avoid initial high salt conditions that could affect the adsorption of the coliphage to aquifer sediment. Bromide tracer, 132 L at a concentration of 4900 mg/L, was injected over 15 minutes into two 5.08 cm diameter wells, #19 and #38. Well #38 is within 0.6 m of well #19 and was used to broaden the slug source. Samples

were collected from the wells within the tracer network at 12- to 24-hour intervals for the next seven days. Bromide analyses were completed by ion chromatography. Aquifer longitudinal dispersivities were determined by analyzing breakthrough curves (Sauty 1980).

Coliphage Seeding

MS2 and ØX174 coliphage were grown to high titers in broth cultures, and cell debris removed by low-speed centrifugation (4°C, 15 minutes at 3500 × g) in a Beckman J6 centrifuge. The coliphage suspensions (< 1 L) were added to 136 L of ground water from well #19 that had been pumped into a clean holding tank. The concentration of this injectate was 1×10^9 PFU/mL for MS2 and 1.12×10^6 PFU/mL for ØX174. The 136 L of mixed virus and water was gravity-fed back into well #19 over 15 to 20 minutes. Samples of ground water were taken from the injection well and the downgradient wells for the next 28 to 32 days, with sampling frequencies depending upon the well location (e.g., sampling of the nearest well in the flowpath (#40) began with 12-hour sampling intervals, and after four days the sampling intervals were lengthened from 12 to 24 hours, and then after 12 days to 48 hours).

Coliphage Survival Study

Survival studies of the two seeded viruses were performed on site and in the lab to determine if inactivation (die-off) was significant during the seeding experiment. Water from well #19, collected just after coliphage injection, was placed into two 30 mL polypropylene Oakridge centrifuge tubes and sealed. One tube was held in the laboratory at 4°C, while the other tube was sealed and suspended below the water table in well #19 (average temperature about 10 to 11°C).

Table 1
Selected Site Hydrogeological Characteristics

Parameter	Sand	Sand and Gravel
Thickness of zone (m)	0 – 2.4	7.6
Mean grain size (mm)	0.14	2.4[a]
Uniformity coefficient	1.8	22.4
Estimated porosity (%)	30	20
Hydraulic gradient	N.A.[b]	0.002
Hydraulic conductivity (m/d)	N.A.	240 – 300
Ground water velocity (m/d)	N.A.	1 – 2.9

[a]Large particles excluded in sieve analysis.
[b]Not applicable because the sand unit is unsaturated.

Table 2
Selected Water Quality Data

Parameter	Background Ground Water	Septic Tank	Impacted Ground Water
Temp. °C	6 – 12	13 – 22	9 – 12
pH	6.6 – 7.2	6.7 – 7.3	6.0 – 6.4
DO, mg/L	3.4 – 6.4	0.2 – 3.1	<0.1 – 3.0
Conductivity, μmhos/cm²	311 – 375	525 – 843	323 – 790
Cl^-, mg/L	2.3 – 3.9	13.8 – 50	13.8 – 42.9
NH_3-N, mg/L	<0.1	11.8 – 74.8	1.1 – 23.1
NO_3-N, mg/L	0.8 – 1.1	<0.05 – 0.1	0.2 – 16

Results

Site Hydrogeologic Properties

The septic tank effluent discharges to the drainfield where it percolates through less than 2.8 m of uniform medium sand with an estimated porosity of 30% (Morris and Johnson 1967). The fluvially derived underlying 7.6 m thick sand and gravel unit transmits the majority of the shallow ground water (Figure 2). The material contains some cobble clasts exceeding 5 cm in diameter and is extremely nonuniform (uniformity coefficient 22.4). This mixture of fine and coarse grained particles has an estimated porosity of 20% (Morris and Johnson 1967). The water table occurs between 2.4 and 3.6 m below land surface and is highest in the spring and summer and lowest in late winter. Based on interpolation of head data, ground water flow is from the northeast to the southwest (Figure 1). The flow direction remains relatively constant throughout the year. The sand and gravel aquifer has a hydraulic conductivity of 240 to 300 m/d determined by analyses of pumping and tracer tests. Based on bromide tracer test results, the velocity ranges from 1 to 2.9 m/d. Site hydrogeologic properties are summarized in Table 1.

The inorganic chemistry of the septic effluent, background ground water, and effluent-impacted ground water is summarized in Table 2. Samples were taken over a two-year period. Background ground water is cold, DO-rich, and calcium bicarbonate dominated. Septic effluent is typically warmer, lower in DO, and higher in dissolved constituents than the native ground water. Water samples from wells immediately beneath and adjacent to the drainfield (to the southwest) show evidence of degradation from percolating septic effluent. Ground water is elevated with constituents typically found in high concentrations in septic effluent. The plume of chloride emanating from the septic tank is shown in Figure 3. The center of the plume encompasses wells #15, #19, #40, #41, #26, and #31, and the tracer network wells (T1 through T10).

Enteroviruses in the Septic Tank Effluent and Ground Water

Five 1MDS filtered septic tank effluent samples and eight 1MDS filtered ground water samples were collected over the

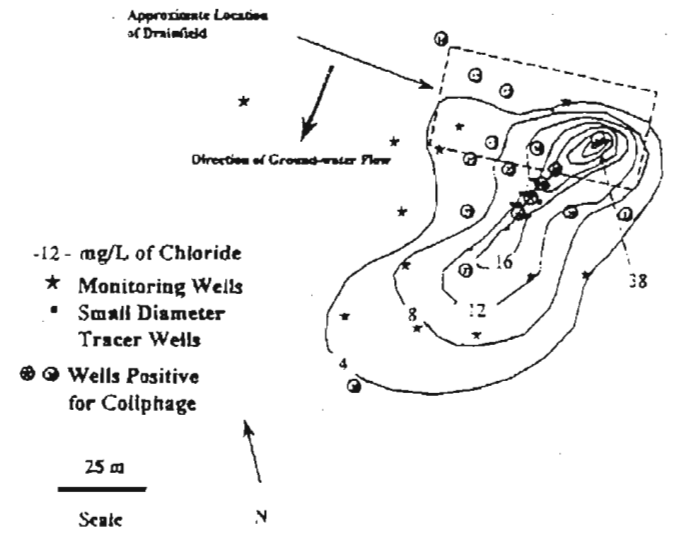

Figure 3. Site map showing ground water chloride plume (mg/L). Wells with ground water samples that had >1PFU/L of coliphage in either beginning or ending screening are shown as circled numbers (Tables 4 and 5). Wells with ground water samples that were found positive (>1PFU/L) at other times prior to seeding experiment are indicated by circled stars.

Table 3
Enterovirus Concentration in 1MDS Filtered Samples

Sample Location	Date	Sample Size (L)	MPN (virus/L) or Detection Limit[a]	PFU Detection Limit
Septic tank	9/13/94	140	4.42	NA
Septic tank	12/9/94	180	NA[b]	<1 in 45 L
Septic tank	3/15/94	342	<1 in 134 L	<1 in 134 L
Septic tank	6/1/95	94.5	NA	<1 in 31 L
Septic tank	6/7/95	90	0.26	NA
Well #15	3/15/95	1927	<1 in 733 L	<1 in 733 L
Well #16	3/1/95	726	<1 in 284 L	<1 in 95L
Well #16	6/7/95	2407	<1 in 1429 L	NA
Well #19	6/1/9	1941	NA	<1 in 638 L
Well #25	3/15/95	2139	<1 in 823 L	<1 in 823 L
Well #26	3/1/95	603	<1 in 247 L	<1 in 82 L
Well #31	8/1/95	2709	<1 in 1440 L	NA
Well #37	8/1/95	2635	<1 in 1496 L	NA

[a]Detection limit volumes equal that part of the total filtered sample that was represented in the assay.
[b]NA, not assayed.

Table 4
Coliphage Concentrations[a] in Wells on March 1, 1995

Well #	Phage Grown on Male-Specific Host (PFU/L)	Phage Grown on Somatic Host (PFU/L)	Total Phage (PFU/L)
12	5000	1000	6000
13	29500	45000	74500
14	<1 in 3 mL[b]	500	500
15	13500	16000	29500
16	56000	71500	127500
17	5000	4500	9500
19	6000	2500	8500
36	330	<1 in 3 mL	330

[a]Data determined by plaque analysis. Wells that had no plaques or positive MPN results from either host were not included in the table.
[b]No plaques in 3 mL.

course of one and one-half years (Table 3). In addition, three septic tank effluent grab samples varying from 0.1 to 4 L were assayed by direct plaquing, and no enteroviruses were detected. Of these 16 samples, only two septic tank effluent samples showed the presence of detectable enterovirus. The septic effluent virus concentrations calculated by the MPN method for the two positive samples were 4.4 virus/L and 0.26 virus/L. These values were lower than anticipated based on the large number of fecal waste contributors. In the eight ground water samples, neither plaques nor positive MPN samples were observed at wells located within the septic plume and at background well #37. Our detection limit for the ground water assays was about 1 virus/1000 L. These ground water results were not surprising, because measurable amounts of enterovirus in the septic tank had proven to be low and infrequent. Thus, it was not possible to use septic waste-associated enteroviruses to measure virus transport rates and distances in this hydrogeologic setting.

Coliphage Levels in the Septic Tank Effluent and Underlying Ground Water

From December 1994 through September 1995, 45 grab samples were taken of the septic tank effluent at irregular intervals. Male-specific coliphage had a time-weighted average of 674,000 phage/L and somatic coliphage had a time-weighted average of 466,000 phage/L. Measured concentrations of coliphage never fell below 7000 phage/L.

Table 5
Coliphage Concentrations[a] in Wells on May 30, 1996

Well #	Phage Grown on Male-Specific Host	Phage Grown on Somatic Host	Total Phage
13	0.66	0.66	1.3
14	0.31[c]	<1 in 3.33L[d]	0.31
15	20000	29000	49000
16	19	18	37
17	4.0	1.4	5.4
19	100000	23000	123000
20	0.31	5.0	5.3
21	<1 in 3.33 L	1.0	1.0
24	15	2.0	17.0
25	9.0	1.0	10
26	200	1.4	200
27	3.0	83	86
29	<1 in 3.33 L	0.31	0.31
31	<1 in 3.33 L	17	17
37	0.31	<1 in 3.33 L	0.31
40	7500	7.0	7507

[a]Data determined by both MPN and plaque analysis. Data given as coliphage/L. Wells that had no plaques on either host were not included in the table.
[b]Wells #19, #40, #26, and possibly #31, still show the influence of seeded MS2 injected in well #19 on August 26, 1995.
[c]This value, 0.31, represents the lowest possible positive MPN result using a total of 33330 mL of sample.
[d]No phage growth in any MPN dilution.

Coliphage in ground water were monitored by two general screenings of all wells and by frequent monitoring during the summer of 1995 from wells that appeared to be directly impacted by septic effluent. The general monitoring of all wells occurred at the beginning and at the end of the project. All numbered wells indicated by stars in Figure 1 were assayed in each general screening. Background concentrations of coliphage were obtained from well #37 and additional piezometers adjacent to the drainfield. Ground water from these wells was always either negative or just at our limit of detection of one positive MPN sample in 3330 mL. This background virus concentration did not bias our results.

The first general survey (March 1995) assayed 2 to 3 mL of ground water per sample for plaque-forming units (Table 4). These first virus assays were performed in conjunction with an initial sampling to establish the inorganic ground water chemistry. The wells that were positive for both male-specific and somatic coliphage were all directly under the drainfield, except for two wells, #14 and #36. Well #36, the furthest well from the drainfield in the flowpath, was sampled several more times during the course of the study, but no further coliphage were found.

In an attempt to achieve more confidence in the measured levels of coliphage in the assays, larger ground water samples were used after the first survey. The final survey (May 30, 1996) was performed nine months after this area had been used for the virus injection experiment (August 26, 1995). It was hoped that this nine-month interval would allow for inactivation and dispersal of any residual coliphage from the seeding experiment. The assay data are presented in Table 5. Using large sample MPN assay, the range of measurable virus concentrations was a thousand-fold more sensitive than the original general survey (Table 4). Due to this increased sensitivity, it was possible to detect the coliphage at greater distances beyond the drainfield edge. The relative location of phage originating from the septic tank, and entering the ground

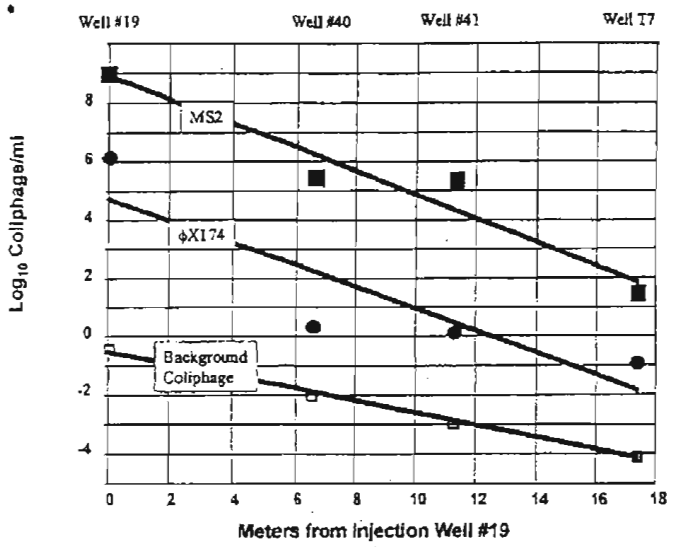

Figure 4. The peak concentrations of seeded MS2, ∅X174, and average background concentrations of male-specific and somatic coliphage at wells #19, #40, #41, and T7 plotted as a function of distance from well #19. The best exponential fit to each series of points indicates the rate of virus concentration loss over distance. The concentrations of background coliphage were taken during the summer months before the seeding experiment.

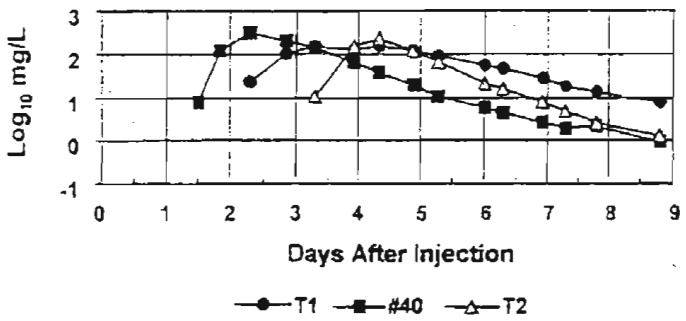

Figure 5. Breakthrough curves for bromide at the transect located 6.6 m from the injection site (Figure 1). The variation in peak arrival times reflects aquifer heterogeneities.

water appears to have remained much the same as that measured in March 1995 (Table 4) and shows excellent correlation with the chloride indicator plume (Figure 3). Three of the wells, #19, #40, and #26, contained high concentrations of male-specific coliphage compared to surrounding wells. As indicated by the data in Table 5, the ratios of male-specific to somatic coliphage were unusually one-sided at these wells, suggesting that even after nine months these wells were still impacted by the MS2 coliphage injected at the start of the seeding experiment.

Between the first general survey and the controlled seeding experiment, ground water samples were collected from four wells centered in the effluent plume (#19, 40, 41, and 26) to evaluate the change in coliphage concentration with distance along the ground water flowpath (Figure 4). The plotted concentrations of male-specific and somatic coliphage represent an average of five to six individual measurements from each well, taken during the summer of 1995. The range of variation within any one well was less than fivefold during this time period. In this setting, the background coliphage concentration changes are essentially identical for both male-specific and somatic coliphage declining one $\log_{10}$ of concentration with every 5 m of transport.

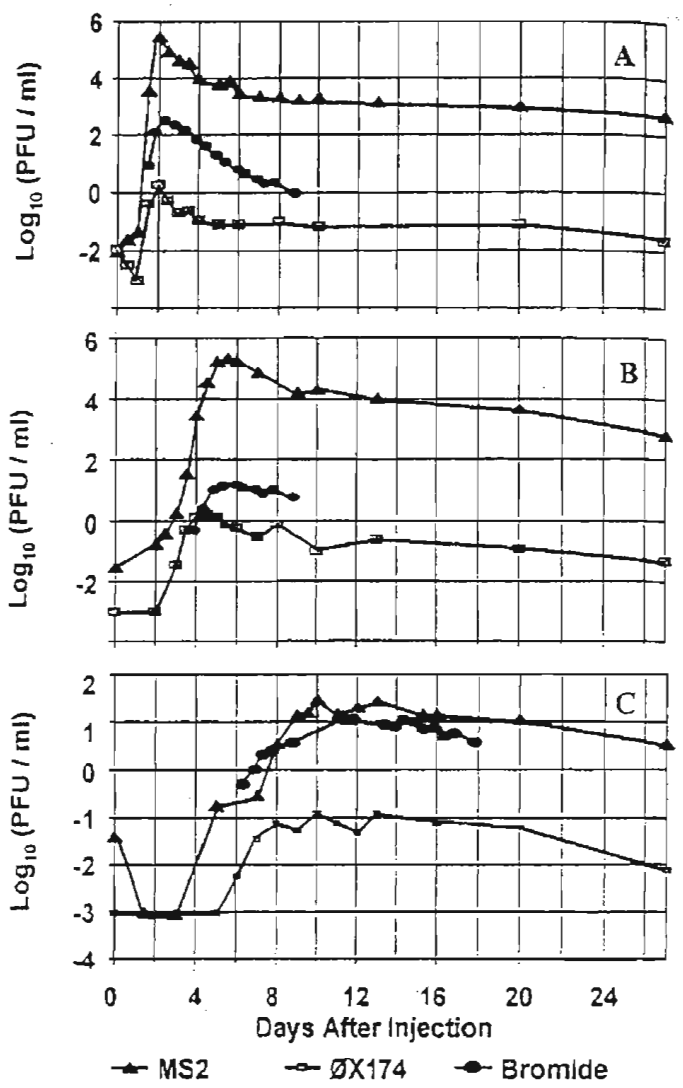

Figure 6. Breakthrough curves for MS2, ∅X174, and bromide are plotted for wells #40 (a), #41 (b), and T7 (c) (see Figure 1 for well locations). Coliphage measurements below 1×10^{-1} were determined by MPN analyses; those above this limit were measured by plaque assays.

Bromide and Coliphage Seeding Experiments

One week prior to the virus seeding, a bromide tracer test was conducted to document the ground water flowpath associated with injection at well #19, aquifer dispersion properties, and ground water velocities. The limited project budget prevented installation of an extensive multilevel sampler network; thus, existing wells were used to assess bromide and virus behavior in the upper 0.6 to 1.6 m of the sand and gravel aquifer. The 4900 mg/L bromide solution may have created sufficient density contrasts to allow for some vertical plume migration (Istok and Humphrey 1995). However, bromide data were not used for mass balance calculations and bromide peaks were observable at monitoring wells. The bromide distribution and breakthrough curves at wells T1, #40, and T2 located perpendicular to the ground water flow at 6.6 m from well #19 are shown in Figure 5. The arrival of the highest bromide peak at well #40 indicates this well is more centered in the ground water flowpath than either well T1 or T2. However, the later arrival of bromide peaks at the two adjacent wells suggests flow rates and paths downgradient of the injection point are not uniform; thus, a classic elliptic tracer slug does not form at this site. Bromide breakthrough data at well #40, #41, and T7 were compared with coliphage breakthrough curves (Figure 6).

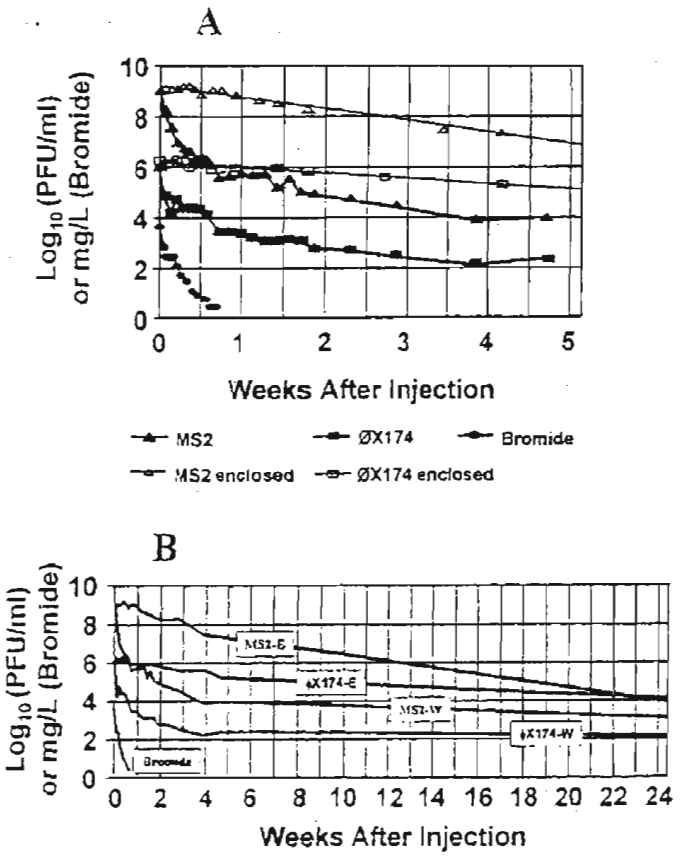

Figure 7. Comparison of die-off rates and/or removal rates. MS2, ØX174, and bromide concentrations in the ground water are plotted as a function of time at injection well #19. In addition, the MS2 and ØX174 survival data from the enclosed Oakridge tubes, suspended below the water table in well #19, are also plotted against time. For clarity, marker symbols were left off the time expanded graph in 7b. A "-E" indicates coliphage data from the enclosed Oakridge tubes, while "-W" indicates that the sample was ground water from well #19.

The seeding of high concentrations of MS2 and ØX174 allowed us to accurately determine transport rates and the variation of coliphage concentration with distance in the ground water. Concentrations at well #19 immediately after injection were 1×10^9 PFU/mL of MS2 and 1.1×10^6 of ØX174. After injection, sampling efforts were concentrated on wells #40, #41, and T7 as they appeared to be within or near the center of the bromide plume. Some wells, further away from the drainfield edge, were sampled intermittently after coliphage breakthrough occurred at well T7. MS2 was found occasionally at T10 (34 m) and at well #31(38 m), but concentrations were too low and the sampling too infrequent to establish clear breakthrough curves. The background of existing septic effluent virus had no significant effect on our results as the MS2 and ØX174 titers at any well were always at least tenfold higher than background concentrations (Figure 4).

Breakthrough curves for bromide, MS2, and ØX174 coliphage at wells #40, #41, and T7 are presented in Figure 6. The bromide breakthrough curves at these wells imply ground water velocities along the 17.4 m flowpath ranging from 1 to 2.9 m/d. The most rapid transport appears to occur between the injection well #19 and well #40. However, the apparent velocity from well #40 to well #41 and from well #41 to well T7 is about 1 m/d. This observed variation in velocity may be a function of the heterogeneous nature of the aquifer (true velocity changes) or a result of poor resolution of the peak concentrations and corresponding arrival times because of well locations not perfectly centered in the plume (apparent velocities). Using methods described by Sauty (1980), longitudinal dispersivities of 0.08 to 0.27 m were determined.

At well #40, all three tracers broke through at the same time (within the limits of the sampling frequency). Bromide, MS2, and ØX174 coliphage peaks arrived together at all the wells, indicating that any difference in their rates of transport was not observable over the distances sampled. However, when the ratio of the initial concentrations in well #19 are compared to the peak concentrations at well #40, 6.6 m downgradient, the bromide tracer shows a change of one log in concentration where the viruses show a decrease in concentration of 3.5 logs. Once the coliphage peak moved beyond a monitoring well (Figure 6a), its concentrations decreased more slowly than the corresponding bromide concentration. This same relationship was also observed at the injection well (Figure 7). This difference between the behavior of bromide and coliphage indicates that coliphages, unlike bromide, are not conservative (i.e., viable phage bind to the aquifer material and then slowly and continually release back into the water column). When the peak concentrations of MS2 and ØX174 are plotted against transport distance, their titers declined at the same rate, approximately $-1 \log_{10}/2.5$ m (Figure 4). This rate is twice the loss rate noted for the background coliphage.

Both coliphage showed little inactivation when incubated in septic waste-impacted ground water over 32 days at 4°C: MS2 decreased less than threefold, while ØX174 had no discernible loss (data not shown). MS2 and ØX174 survival over time in the sealed tube suspended in the ground water (10 to 11°C) at well #19 and the aqueous concentration in the aquifer are plotted in Figure 7a. Interestingly, sampling the phage in ground water at well #19, which should be affected by both dispersion and die-off, showed a reduction in concentration that was less than that measured in the sealed Oakridge tube, especially over a long time (Figure 7b). When extended out to nine months, MS2 concentrations in well #19 were higher than predicted by inactivation alone. These data indicate that sediment bound viruses may act as a source of viable virus that has a much slower inactivation rate than suspended viruses.

Discussion

This research effort provided us with an opportunity to evaluate the occurrence, distribution, and transport rate of viruses in a productive sand and gravel aquifer. The FHS site, which met all legal siting criteria, provided us with a large septic-waste source generated by 350 students and staff, and a hydrogeologic setting that was anticipated to permit virus survival and transport (Figure 1; Tables 1 and 2). We attempted to answer the following questions: At what concentrations are the enterovirus and coliphage present in the septic system and underlying ground water? How fast and far can viruses move upon reaching the ground water before losing activity? What was their inactivation rate? How does adsorption affect their survival and movement? Would either of these viral groups be appropriate indicators of fecal waste contamination at current source-to-well separation distances and thus be useful as viral indicators to test natural disinfection criteria?

Concentration of Enterovirus in the Septic System

We anticipated that the multiuser septic system would contain more frequent loading of enteroviruses than a single household system. However, the concentrations of enteroviruses at the site were generally below our detection limits (Table 3). Enteroviruses were

found twice in the tank effluent, but at such low concentrations that finding them in the aquifer, even with 1MDS filtering of 1000 to 2000 L, would be unlikely. This expectation proved true as sampling of wells #15, #16, #19, #25, and #26 (Table 3) did not detect enteroviruses even though ground water at these locations contained both coliphage and chemical indicators of septic effluent. Possible factors for the low level of enterovirus concentrations in this school are: (1) our recovery and assay methods were not effective in this septic waste-impacted field setting; (2) the high school students were primarily healthy young adults, not often infected with enteroviruses; (3) the sick students stayed home; and (4) the main enterovirus transmission season was during the summer months (Moore 1982), when the school is unattended.

To examine if our low enterovirus concentrations were a result of poor methods, we determined the recovery efficiency for the 1MDS filtration technique coupled to either the MPN or PFU analysis. At other sites, our experience filtering unimpacted ground water recovered 30 to 50% of control polio virus seeded into the sample discharge line before the filter. Laboratory tests filtering 113 L of FHS septic tank effluent seeded with control polio virus compared the viral recovery efficiency from septic waste effluent to that of unimpacted ground water. Both MPN and PFU analyses of the eluted samples gave recovery values of 5 to 6%. We also tested the concentrated septic tank effluent samples for virucidal activity, in case the reduction in viral numbers occurred due to the action of some unknown virucidal agent during sample collection and processing. Recoveries of virus mixed with septic waste, and then assayed directly by either PFU or MPN analyses were better than 85%, indicating that no substantial virucidal activity was present. The results from these two control experiments provided evidence that for septic tank samples, our recovery and assay systems were working, although at a low level of efficiency (5 to 6%). While substantially lower than recoveries from unimpacted ground water, we should have seen significant levels of enteroviruses, had they existed. Based on our recovery experiences and the apparent absence of virucidal activity in the samples, recovery values for the enteroviruses in the ground water most likely ranged between 5 and 30%. The upper end represented our lowest efficiency value for unimpacted ground water and the lower limit represented the efficiency determined in septic tank effluent.

The health of the high school students and staff was not monitored during our study. Certainly the low enterovirus concentrations detected in the septic effluent may reflect the absence of enterovirus-infected students. We assumed that some portion of the infected population would be attending school and, hence, detectable concentrations of enterovirus would be present. It is also possible that the frequency of enterovirus infections was low during the school year. Thus, without health monitoring data, the contributions of factors 2, 3, and 4 to the lack of measurable enterovirus cannot be resolved.

Our original study plan focused on finding a grade school septic system to evaluate. Lack of school board permission and physical layout constraints prevented instrumentation of a grade school system during this study period. Repeating the study at an elementary school might improve our chances of detecting enterovirus in both the septic source and in ground water under similar hydrogeologic conditions, because the level of enterovirus infections should be higher in this age group. This potential could be tested initially by collecting and assaying monthly septic tank effluent samples from such a site prior to well instrumentation.

Coliphage Concentrations in the Septic Tank and Ground Water

Unlike enterovirus, coliphage was found consistently in the s tic effluent at high concentrations. These concentrations varie the septic tank effluent ranging between 7×10^3 PFU/L and $5 \times$ PFU/L. Both types of coliphage had similar time-weighted avera during our study period of 674,000 phage/L for male-specific c iphage and 466,000 phage/L for somatic coliphage (DeBorde et 1997).

Coliphage were also detected in the unconfined aquifer. As effluent leaves the drainfield at this site, it rapidly percolates thou the uniform, thin unsaturated zone and enters the underlying grou water. Inorganic chemistry of the ground water indicates a detecta "plume" of mixed effluent and ground water extends about 90 downgradient from the edge of the drainfield (well #19 to well #. Figure 3). As shown by our general surveys of septic waste-as ciated coliphage in ground water, these viral indicators track well with the chemical indicators of septic waste (Tables 4 and Figure 3). The highest concentrations of coliphage were coincid with the maximum concentrations of inorganic septic waste co stituents. Coliphage could be consistently found in samples of - or less along 17.4 m of ground water flowpath beyond the drainfie Coliphage were sporadically detected out to 38 m, e.g., the peak somatic coliphage seen in well #31 (Table 5). Whether these sp radic occurrences are the end result of high transient inputs of c iphage to the septic effluent or coliphage that were mobilized some change in water chemistry (Bales et al. 1995) is unknov Based on the concentrations found in the ground water at 17.4 from the drainfield, if larger sample volumes were taken fro wells beyond this distance, we would most likely have found ev decreasing concentrations of coliphage.

Sampling of wells #19, #40, #41, and #26 prior to the coliphc seeding experiment provided us with information as to the avera change in septic waste-associated coliphage concentrations as a fun tion of transport distance (Berg et al. 1984). Both classes of c iphage behaved similarly. At sites where coliphage were consisten found (out to 17.4 m), their concentrations were reduced at a r of approximately -1 $\log_{10}$ / 5 m of transport in this ground water sy tem. While these septic waste-associated coliphage concentratio can provide an overview of virus occurrence and distribution, th cannot be used to determine transport rates or virus survival rat

Coliphage Seeding

The seeding experiment allowed us to: (1) measure vir transport rates; (2) compare the behavior of seeded coliphage w the septic waste-associated coliphage; and (3) examine the pers tence of infectious coliphage in the ground water system.

Virus Transport

Even though highly adsorptive, the two seeded and cloned c iphage strains had some individuals that move through the grou water at least as fast as conservative bromide tracer (Figure 6). The fast-moving virus particles have not yet adsorbed to the aquifer se iment, which would have slowed their movement. This small fra tion of unbound viruses may represent a portion of the source th by chance has not yet encountered the aquifer matrix, or it may re resent a subset of the main population that has different surface ch acteristics. This fraction of fast-moving infectious viruses migr ing with or ahead of the conservative tracer constitutes the high density of virus/mL to impact downgradient wells. In a high-vel

ity ground water system, such as the one we evaluated, this virus peak can easily arrive at a well before inactivation has occurred, and thus represents the most serious public health concern following a contamination event.

Some authors have also reported "faster transport" of coliphage compared with fluorescent "conservative tracers" (Alhajjar et al. 1988; Corapcioglu and Haridas 1985; Rossi et al. 1994). Coliphage transport rates faster than the average bromide rates may occur by the same mechanism as seen in pore-exclusion gel chromatography. Virus-sized particles (about 25 to 30 nm diameter) can find fewer but shorter paths. Adsorption may also effectively cause an apparent shift forward in the breakthrough curve peak location. Neither mechanism can be distinguished with these data.

Comparison with Naturally Occurring Background Coliphage

By plotting the peak concentration data from the monitoring wells against their distance downgradient from the injection well, the rates of concentration change for seeded MS2 and ØX174 coliphage can be compared with similar background coliphage data. The concentrations of MS2 decreased at twice the rate (-1 $\log_{10}$/2.5 m) determined for the background male-specific coliphage (-1 $\log_{10}$/5 m). This difference may result from either (1) physical dissimilarities between the septic waste-associated coliphage (composed of collections of somatic and male-specific viruses) and the cloned MS2 and ØX174 marker phage, or (2) a contrast in the method of virus input: the seed phage entered the aquifer as a one-time slug, while septic waste-associated phage were entering as a nearly continuous source. It is likely that both of these factors influenced virus concentrations.

Long-Term Virus Survival

A second public health concern is the long-term survival and release of infectious virus bound to aquifer sediment. It appears that a substantial portion of the input virus becomes bound to the sediment in the vicinity of the injection site. The persistence of viruses seen at downgradient monitoring wells long after the seeded peak has passed (Figure 6), indicates that adsorbed virus must slowly desorb from the aquifer materials and re-enter the ground water flow. Repeat sampling in wells #19, #40, and #41 after the seeding study revealed concentrations of MS2 above background during a nine-month period (Table 5). While not truly identical to site conditions, our closed-tube survival study showed that the seeded coliphage have low die-off rates at the ambient ground water temperature.

Figure 7b clearly displays that the rate of decrease in seeded virus concentrations over long time periods is less than the die-off rate determined using the enclosed tube data. Thus, the enclosed samples overestimated the actual rate of inactivation in the natural system. The magnitude of this overestimation may be even higher than it appears because the seeded coliphage are being removed from the injection well site area by two mechanisms: die-off and ground water transport. If pathogenic viruses act similarly, they may also be removed from the ground water by binding to the aquifer material and survive longer in this state (Gerba 1984; Goyal and Gerba 1979). The rate at which these bound viruses can be remobilized may be enhanced if a change in the chemical or physical conditions occurs (Bales et al. 1995). These bound viruses provide a source of infectious viruses that can enter the ground water long after the initial contamination event.

Viral Indicators and Natural Disinfection Criteria

The continuous presence of coliphage in the septic effluent and impacted ground water make this group of viruses reasonable candidates as viral indicators. While not pathogens of humans, many are present in human waste, are similar in size to pathogenic human virus, and have comparable adsorptive properties and chemical components (Goyal and Gerba 1979; IAWPRC 1991). The observed average concentration of the coliphage in the septic tank effluent indicates that they would be at least four orders of magnitude more sensitive indicators of fecal waste contamination than the enteroviruses. Based on the distribution of these septic waste-associated coliphage in the ground water flowing from under the septic system drainfield (Figure 4), we would predict their concentration would decrease by approximately six logs during saturated zone transport over a standard drainfield/well setback distance of 30.5 m. Using this prediction, and the concentration of background coliphage in the ground water at the outer edge of the drainfield, filtration of at least 1000 L of ground water would be required to begin to detect background coliphage at standard setback distances in this sand and gravel aquifer.

Our work found that enteroviruses occurred sporadically and at such low levels in the school septic tank effluent that none were detectable in the underlying ground water. Using the previously stated values, it would appear that septic waste-associated coliphage could occur at concentrations of about 1 virus per 1000 L at a water supply well located at the minimum 30.5 m setback distance. A typical septic tank effluent containing 10,000 pathogenic viruses/L as suggested by the U.S. EPA (1992) represents 2% of the average concentrations we observed for coliphage (500,000 PFU/L). Thus, if the pathogenic viruses exhibit similar transport characteristics to the coliphage through the 2 m sand vadose zone and the sand and gravel aquifer, then pathogenic viruses would reach the 30.5 m setback distance at 2% of the concentration of the coliphage, or approximately 1 virus per 50,000 L. Using risk assessment, the U.S. EPA proposed that if the health goal for waterborne virus infections was set at less than one infection/10,000 people/year of water use, then the allowable concentration of virus at a wellhead would be less than 1 in 10,000,000 L (U.S. EPA 1992). Meeting such a requirement would only require three logs of additional virus loss, which would mean extending the setback distance an additional 15 m in this aquifer. Thus, standard setback distances of 30.5 m would be considered inadequate to meet natural disinfection criteria in this hydrogeologic setting. However, for similar sand and gravel aquifers with higher ground water temperatures, and hence a significant virus inactivation component, such a highly protective criteria may already be met with existing setback requirements. Rational natural disinfection criteria will need to be based on a series of hydrogeologic studies at sites with a wide range of physical and chemical properties, and at which characterization of the occurrence, distribution, and fate of background and/or seeded coliphage has been examined. Additional foundation for natural disinfection criteria could be gained by permitting controlled coliphage and selected vaccine virus seeding experiments in representative hydrogeologic settings. Realistically, natural disinfection criteria will be based on coliphage sampling of ground water at sites proposed to meet natural disinfection criteria and additional transport studies at the field scale using seeded coliphage.

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RAPID TRANSPORT OF VIRUSES IN A FLOODPLAIN AQUIFER

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Abstract—An unconfined floodplain aquifer near Missoula, MT, was instrumented with 89 monitoring wells and 20 four-port multilevel samplers. Bromide, bacteriophages MS2, PRD1 and ØX174 and the attenuated enterovirus, polio virus (type-1 CHAT strain), were seeded into the aquifer as slug injections. Bromide transport rates ranged between 22–29 m/d. Input concentrations of the tracers and the placement of monitoring wells limited detection of bromide and polio virus to 19.4 m and the detection of three bacteriophage to 40.5 m downgradient from the injection point. After 7.5 m of transport, the calculated relative attenuations [Harvey R. W and Garabedian S. P. (1991) Env. Sci. Tech., 25, 178–185] for MS2, PRD-1, ØX174 and attenuated polio virus were 49, 71, 65 and 99%, respectively. During the 72-h experiment, die-off was negligible (less than 1%) and attachment of virus to sediment surfaces resulted in the overall differences in bromide and virus behavior. Although relative attenuations at downgradient monitoring wells indicated that the virus tracers were attaching to aquifer material along the flowpath, virus peaks arrived at observation wells at rates similar to the bromide peak. The high collision efficiency of the attenuated polio virus resulted in breakthrough curve truncation. Natural attenuation of slug input virus over a "typical" source–supply set-back distance of 30.5 m would most likely not reduce virus concentrations to proposed acceptable risk levels in this or a similar cold-water high-velocity groundwater system. © 1998 Elsevier Science Ltd. All rights reserved

Key words: virus, aquifer, MS2, ØX174, PRD1, attenuated polio virus, groundwater, transport.

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INTRODUCTION

Microbial contamination of groundwater supplies causes over half the waterborne disease outbreaks in the US (Keswick and Gerba, 1980). Protecting wellheads from microbial contamination, especially by viruses, has been a major topic of research in recent years (Wellings et al., 1975; Bitton et al., 1983; Pekdeger [illegible]; Bitton and Gerba, 1984; Mathess and Pekdeger, 1985; Yates et al., 1985; Jansons et al., 1989a,b; Rossi et al., 1994; Bales et al., 1995; Pieper et al., 1997) and a focus of US federal agencies (Macler, 1995). These studies and others identified temperature as an important factor for virus survival (Yates and Yates, 1987; Yahya et al., 1993). Viral surface properties, groundwater quality and sediment surface charges primarily affect the degree of virus attachment during transport (Goyal and Gerba, 1979; Gerba, 1984; Bales and Li, 1993; Penrod et al., 1996; Dowd et al., 1998). Though these basic transport and survival processes have been documented for indicator bacteriophages and some strains of polio virus in laboratory settings, relatively few multiple virus seeding experiments have been conducted at the field scale (Noonan and McNabb, 1979; Alhajjar et al., 1987; Jansons et al., 1989a,b; Bales et al., 1989, 1995, 1997; Rossi et al., 1994).

The affect of different hydrogeologic settings on virus transport is also poorly documented. Difficulties in characterizing sand and gravel and cobble dominated aquifers has especially limited our understanding of virus transport in these generally high velocity groundwater systems. Virus seeding studies in coarse-grained aquifers have observed viruses traveling over 900 m (Noonan and McNabb, 1979). Rates of virus transport in sand and gravel aquifers, as indicated by monitoring peak concentrations from slug injections, have been reported at 0.2 to 1 m/d in the glacial outwash of Cape Cod (Bales et al., 1995; Pieper et al., 1997), 1–2.9 m/d in fluvial sand and gravel near Frenchtown, MT. (DeBorde et al., 1998a,b), 11–132 m/d in a floodplain aquifer in the Emme Valley, Switzerland (Rossi et al., 1994) and over 300 m/d in highly permeable alluvial aquifers of the Canterbury Plains of New Zealand (Noonan and McNabb, 1979). Unfortunately, other field-based studies often contain incomplete hydrogeologic data making transferability of results difficult.

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ABSTRACT

An unconfined floodplain aquifer near Missoula, MT, was instrumented with 89 monitoring wells and 20 four-port multi-level samplers. Bromide, bacteriophages MS2, PRD1, ØX174, and the attenuated enterovirus, polio virus (type-1 CHAT strain), were seeded into the aquifer as slug injections. Bromide transport rates ranged between 22-29 m/d. Input concentrations of the tracers and the placement of monitoring wells limited detection of bromide and polio virus to 19.4 m, the detection of three bacteriophage to 40.5 m. After 7.5 m of transport, the calculated relative attenuations for MS2, PRD-1, ØX174, and attenuated polio virus were 49, 71, 65, and 99 %, respectively. During the 72 hr experiment, die-off was negligible (less than 1%) and attachment of virus to sediment surfaces resulted in the overall differences in bromide and virus behavior. Although the virus tracers appeared to be attaching to aquifer material along the flowpath, virus peaks arrived at observation wells at rates similar to the bromide peak. The high collision efficiency of the attenuated polio virus resulted in breakthrough curve truncation. Natural attenuation of slug input virus over a "typical" source-supply set-back distance of 30.5m would most likely not reduce virus concentrations to proposed acceptable risk levels in this or a similar cold-water high-velocity ground-water system.

Key Words: Virus, aquifer, MS2, ØX174, PRD1, attenuated polio virus, groundwater, transport

INTRODUCTION

Microbial contamination of groundwater supplies causes over half the waterborne disease outbreaks in the United States (Keswick and Gerba, 1980). Protecting wellheads from microbial contamination, especially by viruses, has been a major topic of research in recent years (Wellings et al., 1975; Mathess and Pekdeger, 1981; Pekdeger and Mathess, 1983; Bitton and Gerba, 1984; Bitton et al., 1983; Yates et al., 1985; Jansons et al., 1989a,b; Bales et al., 1995; Rossi et al., 1994; Pieper et al., 1997) and a focus of United States federal agencies (Macler, 1995). These studies and others identified temperature as an important factor virus survival (Yahya et al., 1993; Yates and Yates, 1987). Viral surface properties, ground-water quality, and sediment surface charges primarily affect the degree of virus attachment during transport (Penrod et al, 1996; Dowd et al., 1998; Bales et al., 1993; Goyal and Gerba, 1979; Gerba, 1984). Though these basic transport and survival processes have been documented for indicator bacteriophages and some strains of polio virus in laboratory settings, relatively few multiple virus seeding experiments have been conducted at the field scale (Alhajjar et al., 1987; Jansons et al., 1989a,b; Bales et al., 1989; Bales et al., 1995; Rossi et al., 1994; Bales et al., 1997; Noonan and McNab, 1979).

The affect of different hydrogeologic settings on virus transport is also poorly documented. Difficulties in characterizing sand and gravel and cobble dominated aquifers has especially limited our understanding of virus transport in these generally high velocity groundwater systems. Virus seeding studies in coarse-grained aquifers have observed viruses traveling over 900 m (Noonan and McNabb, 1979). Rates of virus transport in sand and gravel aquifers, as indicated by monitoring peak concentrations from slug injections, have been reported at 0.2 to 1 m/d in the glacial outwash of Cape Cod (Bales et al., 1995; Pieper et al., 1997), 1 to

2.9 m/d in fluvial sand and gravel near Frenchtown, MT. (DeBorde et al., 1998a,b), 11 to 132 m/d in a floodplain aquifer in the Emme Valley, Switzerland (Rossi et al., 1994) and over 300 m/d in highly permeable alluvial aquifers of the Canterbury Plains of New Zealand (Noonan and McNabb, 1979). Unfortunately, other field-based studies often contain incomplete hydrogeologic data making transferability of results difficult.

Ideally, lab and field efforts would be used to generate viral transport models that would appropriately predict viral concentrations down-gradient from a source and allow determination of safe well setback distances under a variety of hydrogeologic conditions. Unfortunately, these attempts have generally been unsuccessful (Yates and Jury, 1995; Macler, 1995). Yates and Jury (1995) cite uncertainty in aquifer parameters and varying individual viral attributes as common components in failed models. Because few field experiments have been performed in aquifers dominated by gravel and cobbles, predicting virus fate and transport under such conditions is highly uncertain. Reduction of uncertainty will occur as additional field scale multi-virus seeding experiments are conducted over a range of hydrogeologic environments.

This research attempts to characterize the movement and survival of four viruses (the bacteriophages MS2, PRD1, and ØX174, and attenuated polio virus type-1 (CHAT strain)) during rapid transport through 40.5 m of a gravel-dominated floodplain aquifer. The experiment's design and site conditions allowed for: 1) rapid collection of tracer data (within 72 h); 2) minimization of virus inactivation (not a significant component over the duration of the experiment), and 3) detailed resolution of the virus plumes and peak travel times. The behavior of viruses during transport are compared and contrasted, and the relation of study results to proposed natural disinfection criteria is discussed.

METHODS

Site Description. The study was conducted in the grassland floodplain of the Clark Fork River at the Erskine Fishing Access near Missoula, MT (Figure 1). The shallow, unconfined, aquifer contains clast-supported cobbles and gravel with a medium- to coarse-grained sand matrix to a depth of 6 m, where the aquifer material fines and becomes predominantly sand. The porosity of the coarse portion of the aquifer was estimated at 0.15 (Johnson, 1967). During most of the year, the water table varied between 2.1 to 2.6 m below ground surface. Throughout the site, groundwater flow is from the east and to the west. The hydrologic properties were determined from tracer tests and aquifer tests using standard procedures (Table 1). The 10° C groundwater is a calcium bicarbonate type (Table 1).

Field Methods. A study area of 240 m by 285 m was instrumented with 89 monitoring wells and 10 staff gauges in low lying areas and sloughs to characterize the groundwater flow system(Figure 1). A final monitoring well network was designed after numerous preliminary bromide and rhodamine-wt tracer experiments so that virus seeded at well I4 would pass through the arcs of multi-level monitoring wells located at distances of 7.5, 19.4, 30, and 40.5 m from the injection point (Figure 2). Each multi-level monitoring well was constructed with 0.5 cm diameter high-density polyethylene (HDPE) tubing affixed to a 1.3 cm diameter PVC pipe. These sampling ports were 1.8, 2.7, 3.6, and 4.5 m below the surface. The tubing ends were perforated over 5 cm and screened with nylon mesh. A dedicated 0.3 m long flexible tubing attached to each HDPE tube port allowed sampling with a battery powered peristaltic pump equipped with a MASTERFLEX quick release head (Cole-Parmer, Vernon Hills, IL).

The multiple virus seeding was preceded one week by a bromide tracer test. Water level monitoring before, during and after both tests showed no measurable change in the hydraulic gradient. At the time of the seedings, the water table was 2.1m below land surface. In each test, groundwater from a background well up gradient of the injection well was used to create the tracer solution, 18.9 L of bromide and 37.8 L of virus. The solution was gravity drained into injection well I4 over a period of 10 to 12 minutes. No elevated water levels at or in the vicinity of the injection well were observed during or immediately following the injection. Initial concentrations of bromide and the four viruses as measured at I4 at time zero (just after injection) are shown in Table 2. The CHAT strain of polio virus is highly attenuated for humans. Prior to virus injection, the use of the selected viruses was approved by the University of Montana Biohazards Committee, Missoula City-County Health Department, Montana Department of Environmental Quality, and Region 8 EPA. In addition, an Environmental Assessment (Montana Environmental Policy Act) was submitted at the request of the land steward, Montana Department of Fish, Wildlife, and Parks.

Sampling for the tracer experiments covered a 36 h period for bromide, and a 72 h period for the virus seeding. Samples were collected from I4 and the multi-level monitoring well ports located at 0.6, 1.5, and 2.4 m below the water table. A sampling schedule based on results of previous tracer tests was implemented that captured tracer peak arrivals at each arc of wells. Wells were sampled from expected lowest concentration to expected highest concentration to further reduce the risk of cross contamination. Bromide samples were collected in clean HDPE 50 ml bottles. Virus samples were collected in sterile 50 ml polypropylene vials. Both bromide and virus samples were immediately placed on ice, and transported in ice-filled coolers to the appropriate laboratory at the University of Montana where they were stored at 4° C until analysis

was completed, usually within 48 h. Some samples were re-assayed later within seven days of the sample collection. Previous work had shown that our marker bacteriophage held in ground water at 4C had no detectable loss in infectivity over a 30 day period (DeBorde et al., 1998a,b).

Virus inactivation that occurred after sample collection was determined by analyzing a groundwater sample containing a stock virus that was held at 4° C. A similar control sample was collected from a vial filled with groundwater containing a known concentration of seeded virus that was immersed in an unused well for the duration of the experiment. Daily samples were collected from this vessel and analyzed using viral techniques described below.

Analytical Methods. Bromide samples were filtered (0.45 μm) in the lab and analyzed within 24 hours of collection using a Dionex ion chromatograph (AS4A column) and standard procedures (Pfaff, 1993). An analytical error of 4% was calculated. Comparison of results from duplicates collected during field sampling showed a 95% confidence limit (CL) of ±14%. Bromide concentrations were reported in mg/L to an instrument detection limit of 0.1 mg/L.

MS2, PRD1, and ØX174 coliphage were assayed using host bacteria specific to each virus. The single-layer agar plaquing method used is described in DeBorde et al., (1998a). Replicate field samples were used to determine an overall 95% CL of ±15%. Only when the total number of plaques in an individual assay were low did the 95% CL increase (Eaton et al., 1995). The detection limit is approximately 0.1 PFU/ml.

For polio virus determination, 5 to 7 ml of field sample were filtered through a 0.45 micron filter and diluted in Earle's minimal media supplemented with lactalbumin hydrolysate (Sigma Chemical Co., St. Louis, MO) at a 1:1 dilution. Filtration of polio samples was necessary to remove indigenous bacteria that would contaminate and ruin the assay. The filters were

pretreated with 3% beef extract solution to prevent loss of polio virus by non-specific attachment to the filters (USEPA 1984). These samples were stored in 15 ml polypropylene tubes at -70° C until host cells were ready. The use of controls showed that the freezing had no detrimental effects on the virus recovery and did not lower the titer.

The attenuated polio virus was assayed using 3 to 5 day old Buffalo Green Monkey Kidney (BGM) cells that were grown in 25 cm^2 tissue culture flasks (Smith and Gerba, 1982; USEPA, 1984). The detection limit of this method was approximately 1 virus in 1 ml of sample.

Analytical errors were calculated for the infectious assay of attenuated polio virus with the same methods used for the bacteriophages. In most cases the most significant error in the polio virus determinations came from the total number of plaques counted for each sample. As this number approaches our detection limit, the 95% CL becomes very large, e.g. a sample containing 1 PFU/ml would have 95% confidence range of 0.4 - 1.71 based on the total number of plaques counted (Eaton et al., 1995).

A mass balance was attempted using 8h data for the virus and bromide plumes. As no tracer was detected at the 1.5 m sampling ports, 0.6 m was used as the saturated thickness impacted by the tracers. An area bounded by two lines of known concentration was calculated. The area and thickness data were multiplied by an estimated porosity of 0.15 (Johnson, 1967) to obtain the impacted volume of water. The tracer mass was generated by combining the volume data with the median concentration value defined between lines of equal concentration plotted using $\log_{10}$ intervals.

The relative virus breakthrough (RB) at monitoring wells and the degree of virus attenuation by attachment to the aquifer material was calculated using the procedure described by Harvey and Garabedian (1991). The RB is calculated using concentration verses time data from a

sampling point centered in the tracer plume. It is a comparison between the ratio of the measured and source virus concentration with the similar ratio of the conservative tracer (bromide):

$$RB = \int_{t_0}^{t_f} \frac{C_t}{C_0}\, dt \Big/ \int_{t_0}^{t_f} \frac{Br_t}{Br_0}\, dt \qquad (1)$$

Where C_0 and Br_0 are the initial virus and bromide concentrations at the injection well (PFU/ml (Plaque Forming Units) and mg/L), C_t and Br_t are the concentrations at a monitoring well at some time t after the tracer injection, and t_0 and t_f are the times representing the beginning and end of the breakthrough curve. The percent of relative attenuation (RA) is derived by converting RB to a percent and subtracting the result from one hundred (RA = 100-RB).

In addition to calculation of RB, the collision efficiency factor, α, a parameter in filtration theory representing the collision between particles (virus) and collector grains was determined. α represents the ratio of the rate of collisions resulting in attachment to the total rate of collisions (Harvey and Garabedian, 1991). The collision efficiency factor was defined as follows:

$$\alpha = d\{[1-2(\alpha_L/x)\ln(RB)]^2 - 1\}/\, 6(1-\theta)\eta\alpha_L \qquad (2)$$

where d is the average grain diameter (L), α_L is the longitudinal dispersivity (L) , x is the transport distance (L), θ is the porosity and η is the single collector efficiency (dimensionless). The value η was determined as presented by Harvey and Garabedian (1991) and defined by Pieper et al.(1997):

$$\eta = 0.9\, A_s^{1/3}\, [(k_B T/\mu d_p dv)]^{2/3} \qquad (3)$$

where A_s is the Happel sphere-in-cell model correction factor, k_B is the Boltzmann constant (1.38×10^{-23} J $mol^{-1}K^{-1}$), T is absolute temperature (K), μ is the dynamic viscosity (mass/(Lt)), d_p

is the virus diameter (L), d is the average grain diameter (L) and v is the fluid velocity (L/t). A_s is calculated where $\varepsilon = (1-\theta)^{1/3}$:

$$A_s = 1 - \varepsilon^5 / (1 - 1.5\varepsilon + 1.5\varepsilon^5 - \varepsilon^6) \qquad (4)$$

RESULTS

A bromide tracer was injected at the water table using well I4 on September 22, 1996. The seeding of viruses MS2, PRD1, ØX174, and attenuated polio virus type-1 (CHAT strain) followed on October 2, 1996. The plume centers for both injections appeared to pass through wells M2, M7, M14, and M17 (Figure 2).

The transport of viruses through groundwater is controlled by all the hydrologic properties of the aquifer, the surface properties of the virus as a function of water chemistry, and the physical and chemical properties of the individual aquifer grains. The viruses moving through the aquifer that did not attach to the aquifer material were principally affected by mechanical dispersion. Based on the bromide breakthrough data for well M2, located 7.5 m from well I4, the longitudinal and transverse dispersivities were determined to be 1.6 m and 0.24 m by calibrating a two dimensional solute transport model, MT3D (Waterloo Hydrogeologic, 1997)with field concentration data (Woessner et al., 1998).

The predetermined 2 h sampling frequency and careful choice of the multi-level monitoring wells for sampling permitted identification of plume distribution, peak arrivals, and determination of transport rates. Daily analysis of viral concentrations from the closed survival vial showed no significant change in viral concentration over 72 h (less than 1 %).

Peak arrival times at a given well were similar for the four viruses (Figure 3), while the bromide peak appeared to arrive slightly after the virus peaks. Because error bars for both

bromide and virus concentrations often overlap, discrimination of peak arrival time was difficult. At all wells, virus breakthrough curves had their highest concentrations near their leading edge, while the bromide curves increased more symmetrically. Data collected at well M2 suggest that the polio virus peak arrived 2 h prior to the bromide and bacteriophage peaks, but at well M7 overlapping error bars prevent separation of the tracer peaks. The three bacteriophage were also measured at wells M14 and M17, but bromide and attenuated polio virus breakthrough curves could not be constructed in these wells due to paucity of data (Figure 4). Specific peak arrival times for each bacteriophage tracer could not be distinguished due to the overlap of the 95% CL error bars. Based on breakthrough curves, transport rates for bromide and the viruses were calculated (Table 3). These rates are reported as ranges when a single peak assay point could not be differentiated from neighboring points due to overlapping error bars.

Plume sizes and shapes differed among viruses mainly in relation to initial injection concentrations. Virus plumes exceeding 40 m in length and 16 m in width were observed throughout the well network at the end of the 72 h sampling period. These plumes followed the same flowpath and had similar distributions in map and cross sectional view. In comparison, after 36 h of transport, the bromide plume was only detected between the injection well, and M7. The PRD1 plume can be used to represent the distribution of all viruses (Figure 5). An aerial plume was defined at a depth of 0.6 m below the water table (Figure 5A) essentially at the depth of injection. Concentrations at 0.6 m below the water table, are higher than those at 1.5 m below the water table, with the exception of those measured at well M13 where highest concentrations were observed at 1.5 m. The highest observed concentrations are at the injection well throughout the experiment, suggesting tracer is being held up at the injection site either by adsorption and attachment or hydrogeological conditions.

At injection well I4 the concentration of bromide declined one log in 28 h, where the polio virus concentration dropped one log in 5 h (Figure 6). The ØX174, MS2, and PRD1 concentrations each declined one log after 14, 15, and 17 h, respectively. Assuming bromide is acting conservatively and virus die-off over the sampling period is negligible, virus appear to be removed from the aqueous phase by attachment to the aquifer material immediately surrounding the injection well. Attempts at calculating mass balances based on 8 h plume distribution were hampered by the lack of detailed concentration data between I4 and M2. Depending on how the distribution of a component was formulated, mass representing more than that injected (>100%) or less than that injected could be obtained. Thus, a mass balance was not calculated.

Table 4 shows the relative percent breakthrough and relative percent attenuation of the seeded virus at monitoring well locations M2 and M7. Based on 95% confidence limits the relative percent attenuation levels were similar for all coliphage. The coliphage were transported over the 19.4 m (M7) with less attenuation than polio virus. Almost all of the injected polio virus was attenuated over the first 7.5 m of transport. Compared to bromide, all biological tracers continually attached to aquifer sediments over the entire flowpath.

The collision efficiency values for each virus are also shown on Table 4. Because of the difficulty in obtaining a representative sample of the coarse grained aquifer material (sand to boulders), a range of median grain sizes, 0.00125 m (coarse sand) and 0.012 m (medium pebbles), was selected to represent the character of aquifer material in contact with the tracers. This sediment is dominated by metasedimentary quartzites and argillites with a minor fraction of granitic pebbles and lacks iron coatings. Sands are principally quartz, originating from the weathering of metasedimentary rocks and granite. The collision efficiencies were highest for the polio virus increasing with the larger grain size. Coliphage values were about 2.5 to 11 times

lower than those for the polio virus. Collision efficiencies appear to be similar at both the 7.5 m and 19.4 m transport distances for MS2, ØX174, however, they are slightly lower for PRD1 and polio virus.

DISCUSSION

The highest concentrations of tracers were measured in wells I4, M2, M7, M14, and M17. Bromide tracer tests and aquifer tests performed in the well field suggest that there is a zone of preferential flow characterized by an extremely high hydraulic conductivity, ranging from 6,000 to 13,500 m/d. This zone intersects the injection well I4 and monitoring wells M2 and M7. This high velocity zone, 22 to 29 m/d, is most likely composed of clast supported channel or gravel bar deposits. Such zones are characteristic of high energy, fluvial, gravel deposits (Miller, 1991; Smith, 1992; Rossi et al, 1994). Thus, the Erskine Site represents a hydrogeologic setting with characteristics that are between those of both Cape Cod (Bales et al., 1995) and Frenchtown, MT (DeBorde et al., 1998a), and the more rapid groundwater transport sites described by Rossi et al. (1994) and Noonan and McNabb (1979).

Comparison of Virus and Bromide Distribution. While bromide and viruses follow the same flow path; virus plumes were detected over a more extensive area. In part, this difference is due to the wide range of measurable virus concentrations compared to four logs of measurable bromide concentrations. In an effort to avoid density effects (Isotok and Humphrey, 1995), bromide was injected at 10^3 mg/L and it was detectable to 0.1mg/L. Bacteriophages were injected at 10^7-10^{10} PFU/ml and they were detectable to 0.1 PFU/ml. Attenuated polio virus were injected at 10^6 PFU/ml and were detectable to 1 PFU/ml. Because of the rapid dispersion of the bromide tracer

in a hydrogeologic setting such as ours, the use of bromide to map areas of possible virus occurrence at similar sites would most likely underestimate the actual distribution of a viral plume.

Comparison of Transport Rates. Analyses of breakthrough curves reveal two facts, a portion of the seeded viruses traveled at rates similar to that of the bromide tracer, while the majority attached to the aquifer material in the vicinity of the injection well and along the flow path (Figure 3).

The initial breakthrough of the seeded virus at monitoring points suggests a portion of the virus population did not attach to aquifer sediments prior to arriving at the monitoring well. These virus represent that portion of the seeded virus that were not collected by the aquifer grains. It has been suggested that the surface properties of a subset of the seeded virus may be different than the majority of the population and that these are the virus traveling the farthest in the aquifer (Goyal and Gerba, 1979). Our work did not examine this possibility.

Though a portion of the virus appear to act conservatively, virus attachment to the aquifer material is also an important process affecting virus transport. Examination of Table 4 and the C/C_o curves (Figure 7) indicates the attenuated polio virus attached at proportionally higher rates than the other coliphage. Thus, a significant portion of the virus mass is attached to the aquifer material. Collision efficiencies were slightly higher for PRD1 than the other coliphage during the 7.5 m of transport. Calculated efficiencies are similar to those reported by Pieper et al.(1997) for PRD1 transport in a the uncontaminated sand dominated Cape Cod aquifer and other biocolloids studied in the Cape Cod aquifer (Harvey et al, 1989; Harvey et al., 1995). Poliovirus RA and α values are much larger than for the coliphage representing more effective collector grain virus capture. The hydrogeological setting was quartz-dominated, with ground

water of pH 7.2. The isoelectric points of the quartz dominated aquifer sediment and the seeded virus are: quartz, 2-3.5; MS2, 3.9; PRD1, 4.2; ØX174, 6.6; and polio, 7.5, 4.5 (Gerba 1984; Dowd et al., 1998). This implies a greater number of opposite charges on amino acids in the proteins on the surface of the polio virus particles compared to the surface charge on the sediment grains, and is probably the reason polio virus has a higher effective RA than the other viruses. The use of the medium pebble grain diameter increased the collision efficiency value 40 to 50 times. These increases were expected as the collision efficiency is directly proportional to the grain diameter and inversely proportional to the collector efficiency. However, relationships derived using the smaller mean grain size were preserved.

The breakthrough curves presented in Figure 3 show that the polio peak appears to arrive before the other virus peaks and the bromide peak. However, the error associated with peak concentration determination tempers this observation. Similar relationships have been reported in the literature (Rossi et al., 1994) and observed in the field in additional experiments completed at this site (not presented here). This phenomenon has been reported for laboratory experiments using coliphage MS2 and f2 in which transport rates in columns was interpreted to be 1.6 to 1.9 times that of bromide (Bales et al., 1989). We would offer an explanation for the apparent faster movement of the polio virus. Hydrogeologic conditions at the Erskine site and the similar behavior of the various sizes of the bacteriophage (from 30 nm (MS2, ØX174) to 65 nm (PRD1)) do not favor pore exclusion process postulated at some field sites (Pekdeger and Mathess, 1983). Instead, we propose that the high collision efficiency of the poliovirus in this environment results in a truncation of the poliovirus breakthrough curve even though the poliovirus are being transported at the same rate as the coliphage and bromide. Conceptually, the high polio virus attachment rate during the early hours of transport results in a truncation of the virus

breakthrough curve causing an apparent peak to arrive before the non-reactive Br peak or the coliphage. Note that the polio virus breakthrough curve declines more sharply than the other breakthrough curves (Figure 3). This truncation process can be illustrated by applying a simple algorithm to the conservative Br (C/C_0) data (Figure 8) that results in a peak shift to the left [Bromide adjusted (BrADJ)]:

$$Br_tADJ = [Br_t - (K*t*Br_t)], \quad (5)$$

where, Br_t = bromide concentration (C/C_0) at time t (h); t = time of arrival at well M2 (h); K = net virus attachemtn value/h; and Br_tADJ = the attachment transformed Br data.
This hypothesis is also supported by the asymmetric shape of the bacteriophages' breakthrough curves which have steep leading edges. However, they have not been affected to the same degree as has the polio results. Additional experiments are needed to test this hypothesis.

Breakthrough curves also contain long tails indicating a portion of the attached virus were being slowly re-released into the aqueous phase. This process is not well understood, but we have observed this slow release over a period of more than six months (data not shown). Further research that addresses the mechanisms and health-related significance of this process is needed.

CONCLUSIONS

The results of bromide and multi-virus seeding experiments in this hydrogeologic setting conducted under natural gradient conditions suggest: 1) the average rate of transport for a portion of seeded virus is the same as the average groundwater flow velocity defined by bromide; 2) virus attachment to aquifer sediments significantly reduces the aqueous virus concentration during transport (see Figure 7); 3) polio virus concentrations verses time and distance declined at a faster rate than the coliphage, and thus MS2, ØX174 or PRD1 could be used to conservatively estimate polio virus transport under similar hydrogeological conditions; 4) long tails on virus

breakthrough curves implies that once viruses enter the aquifer system, some of the attached virus release into the ground water over time; 5) attenuated polio virus breakthrough are most likely truncated by virus attachment along the flow path and thus, unattached polio virus are actually traveling at rates similar to bromide in this hydrogeologic setting. These findings imply that a planned or unplanned release of virus impacted water into a highly conductive aquifer creates two problems. The first is a high concentration of virus moving with the average velocity of the groundwater. Our work shows that measurable virus peaks reach and pass the commonly used 30.5 m setbacks between wells and septic systems in Montana and most States. The second is the presence of attached viruses that slowly release back into the aqueous phase and are transported downgradient.

Additional investigations are needed to resolve factors controlling virus transport in coarse-grained field settings. Investigations of the attachment-detachment process, virus mass balance determinations, and long term field scale survival studies are essential.

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Table 1: Aquifer Characteristics.

Hydrologic Properties	
Porosity	0.15
Gradient	0.0004
Average K(m/d)	900-13,800
Average GW Velocity (m/d)	27
Water Chemistry	
Water Type	Calcium Bicarbonate
Conductivity (uS/cm)	288
Dissolved Oxygen (mg/L)	3.5
pH	7.2
Temperature (°C)	10.3
Ca (mg/l)	53.7
Mg (mg/l)	16.7
Na (mg/l)	8.6
K (mg.l)	2.3
Fe (mg/l)	0.01
Br (mg/l)	<0.1
Cl (mg.l)	7.3
SO_4 (mg/l)	16.3
HCO_3 (mg/l)	249
NO3-N (mg/l)	0.66
Dissolved Organic Carbon (mg/l)	2.1

Table 2. Initial Concentration of Injected Tracers

Virus Tracer	PFU/ml
MS2	5.60E+10
PRD1	5.40E+09
ØX174	2.90E+07
Polio (Chat)	3.40E+06
Ionic Tracer	**mg/L**
Bromide	1143

Table 3. Apparent Transport Velocities (m/d) Calculated from Breakthrough Curve Peaks

Well Distance	M2 7.5m	M7 19.4m	M14 30m
Bromide	22.5-30	26-29.25	NC*
MS2	30	23.4-39	25.7-36
PRD1	30	26-39	36
ØX174	30	33.4-39	18-36
Attenuated Polio	45	33.4-58.5	NC

*Not calculated because peak concentrations were below detection limits.

Table 4. Relative Breakthrough (RB), Relative Attenuation (RA) and Collision Efficiency (α) of Viruses After 7.5 and 19.4 m of Transport.

	Well M2-9 (7.5 m from I4)			Well M7-9 (19.4 m from I4)		
Virus	**RB%**	**RA%**	**α**	**RB%**	**RA%**	**α**
MS2	51 (29-92)*	49 (8-71)	0.004-0.182**	15 (8-26)	85 (74-92)	0.004-0.202
PRD1	29 (16-51)	71 (49-84)	0.014-0.632	12 (7-21)	88 (79-93)	0.005-0.385
ØX174	35 (19-62)	65 (38-81)	0.006-0.311	6 (4-12)	94 (88-96)	0.007-0.319
Polio Type 1 (Chat Strain)	1 (0.6-1.9)	99 (98.1-99.4)	0.047-2.108	0.2 (0.1-0.5)	99.8 (99.5-99.9)	0.019-0.866

*Range values (given in parentheses) were calculated using 95% confidence limits of ±14% for bromide, ±15% for the bacteriophage and attenuated polio virus. ** Collision efficiency range calculated using a mean grain size of 0.00125 m (coarse sand) and 0.012 m (medium pebbles).

Figure Legends

Figure 1. Location map of the Erskine Study Area. The black rectangle is the location of the concentrated tracer network monitoring wells, while the gray irregular polygon represents the more broadly characterized study area.

Figure 2. Location of Injection Well I4, Multi-Level Monitoring Wells (M0-M19), and Production Wells W1and W2 in the tracer well network.

Figure 3. Bacteriophage concentrations over time at M2 (3A) , and M7 (3B) located 7.5 m and 19.4 m, respectively from I4. Error bars for bromide and viruses are represent the 95% CL at each measurement. For the viruses, these values vary from a lower limit of ±15%, based on total number of PFU counted. Bromide error bars represent a total error of ±14%.

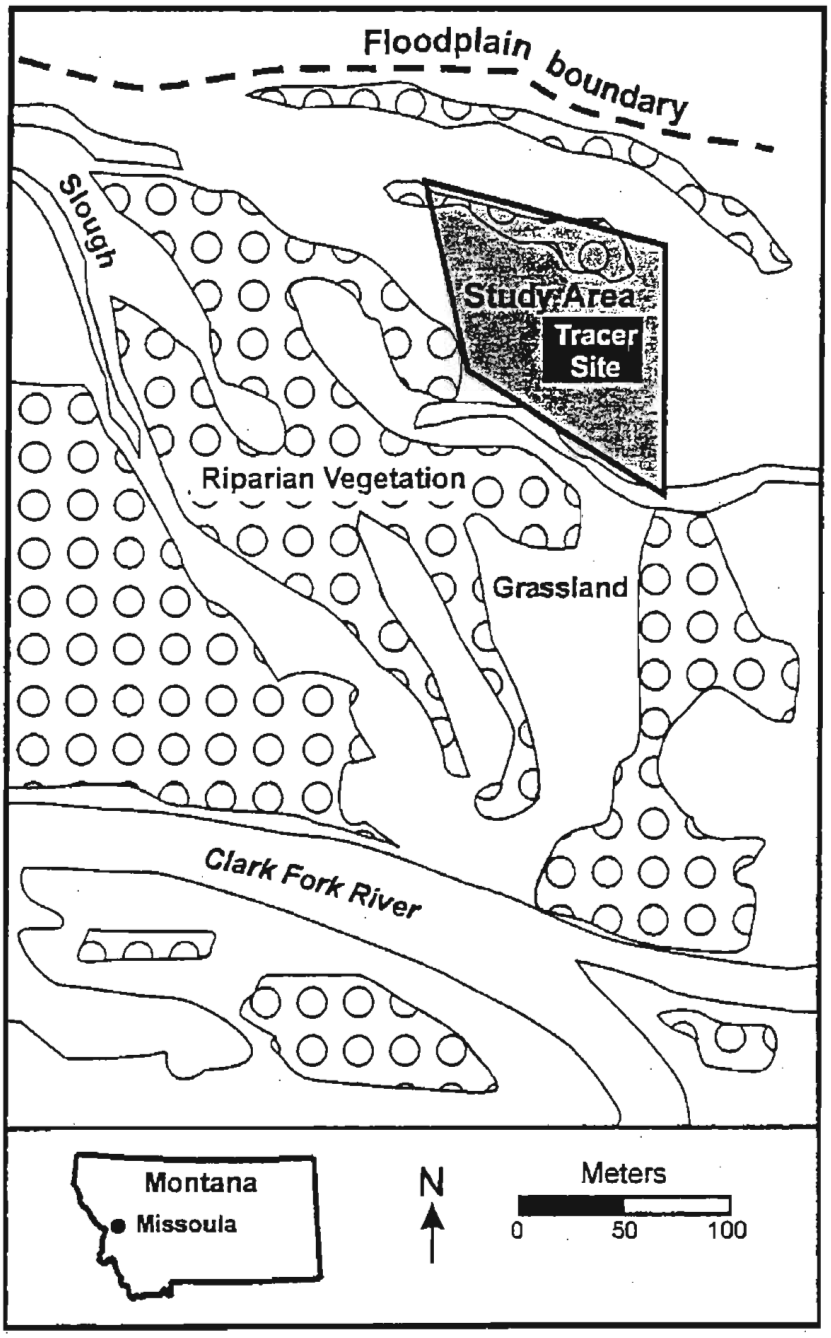
Figure 4. Bacteriophage concentrations over time at M14 (4A) and M17 (4B) located 30 m and 40 m, respectively, from I4. Error bars are as in Figure 3.

Figure 5. Concentrations (PFU/ml) of PRD-1 at 72 h. Figure 5A depicts an aerial map view of the bacteriophage plume at the 0.6 m sampling ports. Figure 5B depicts the vertical distribution of bacteriophage concentration for wells located near the centerline of the plume. Well locations are shown in Figure 2. The gray bar on the right vertical axis represents the perforated interval of I4.

Figure 6. Bromide and virus concentrations over time at the Injection Well, I4. Error bars are as in Figure 3.

Figure 7. Peak C/C_0 values for all four viruses and bromide plotted against distance from I4. Polio and bromide concentrations were below detection limits at 30.0 m and beyond.

Figure 8. C/C_0 of attenuated polio virus and bromide as they arrived at well M2, 7.5 m from I4. The adjusted bromide data curve was derived by transforming the Br data with Equation 5 to illustrate how attachment may have caused a shift in the polio virus breakthrough curve. In this example, the net attachment rate constant, K, equals 0.15/h.


Floodplain boundary
Slough
Study Area
Tracer Site
Riparian Vegetation
Grassland
Clark Fork River
Montana
Missoula
N
Meters
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50
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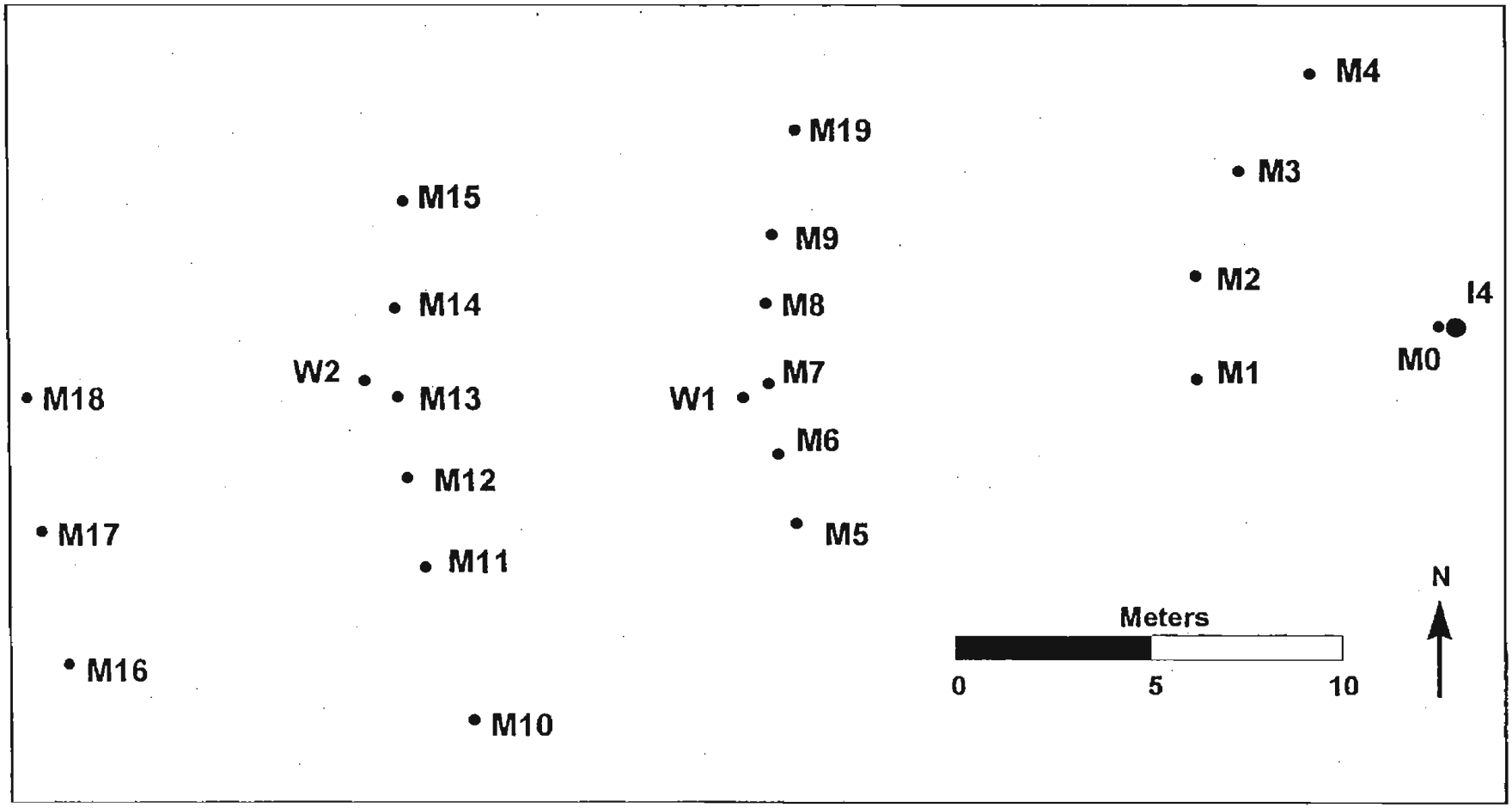
M4
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M3
M15
M9
M2
I4
M14
M8
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W2
M7
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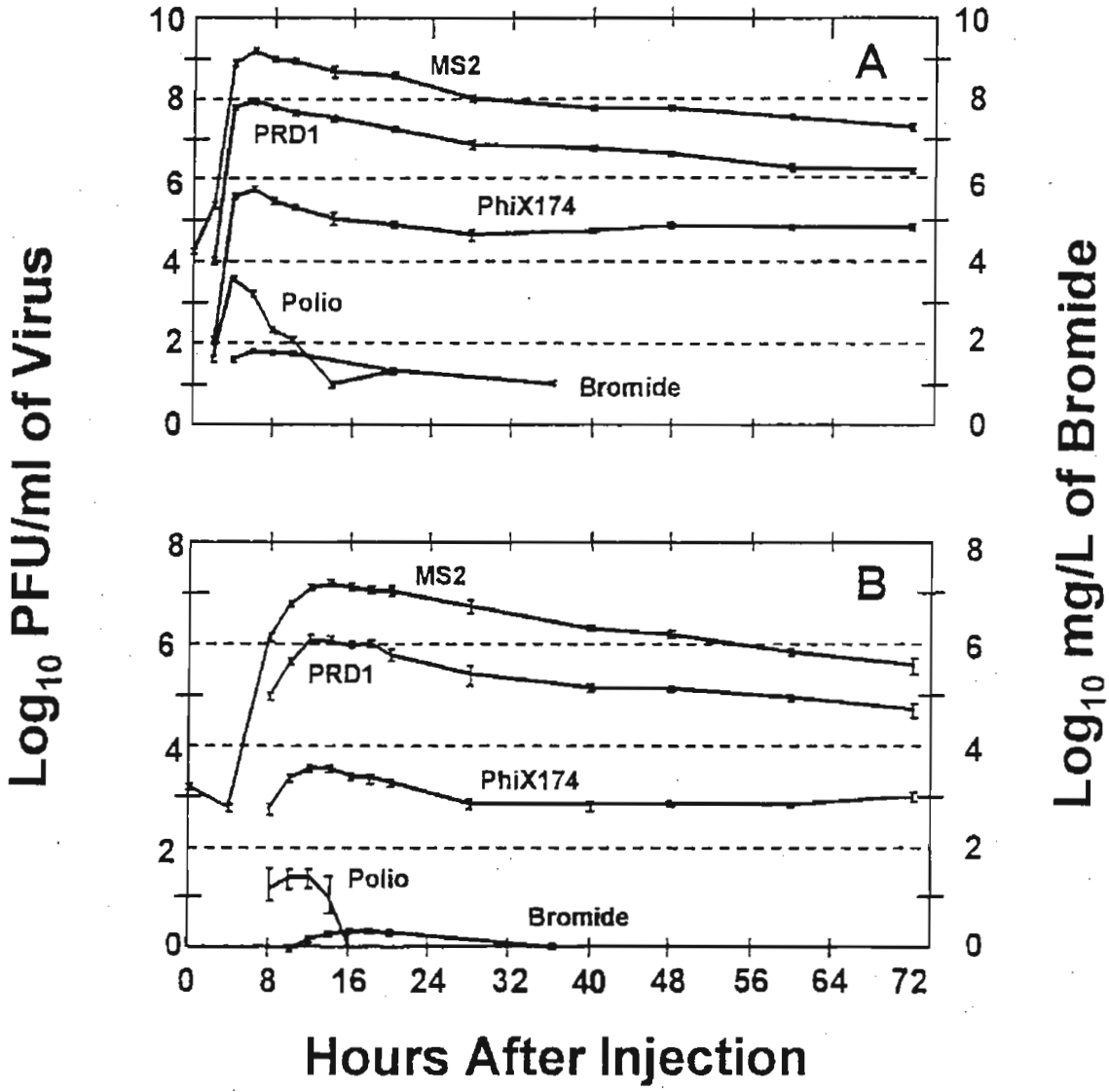
A
MS2
PRD1
PhiX174
Polio
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B
MS2
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2
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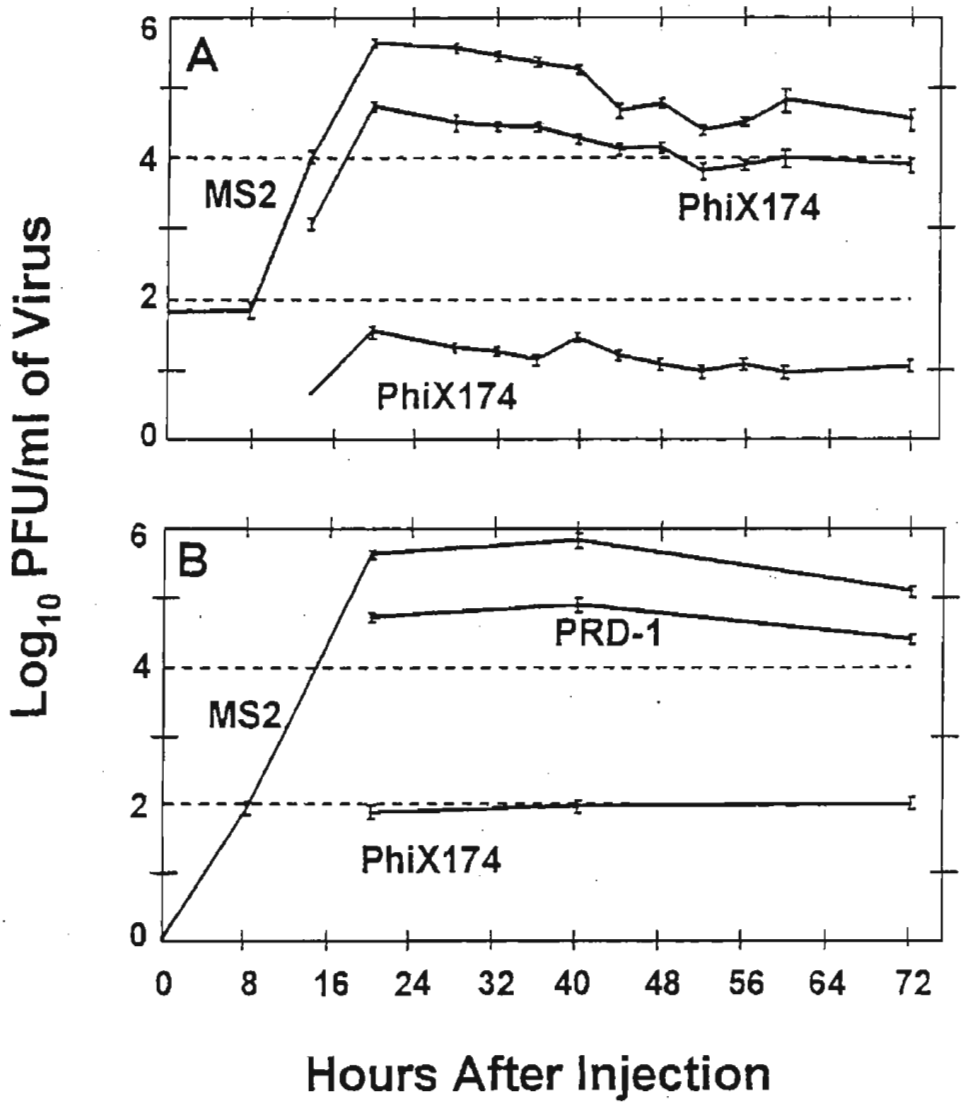
Log10 PFU/ml of Virus
Log10 mg/L of Bromide
Hours After Injection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B
MS2
PhiX174
PhiX174
PRD-1
MS2
PhiX174
0
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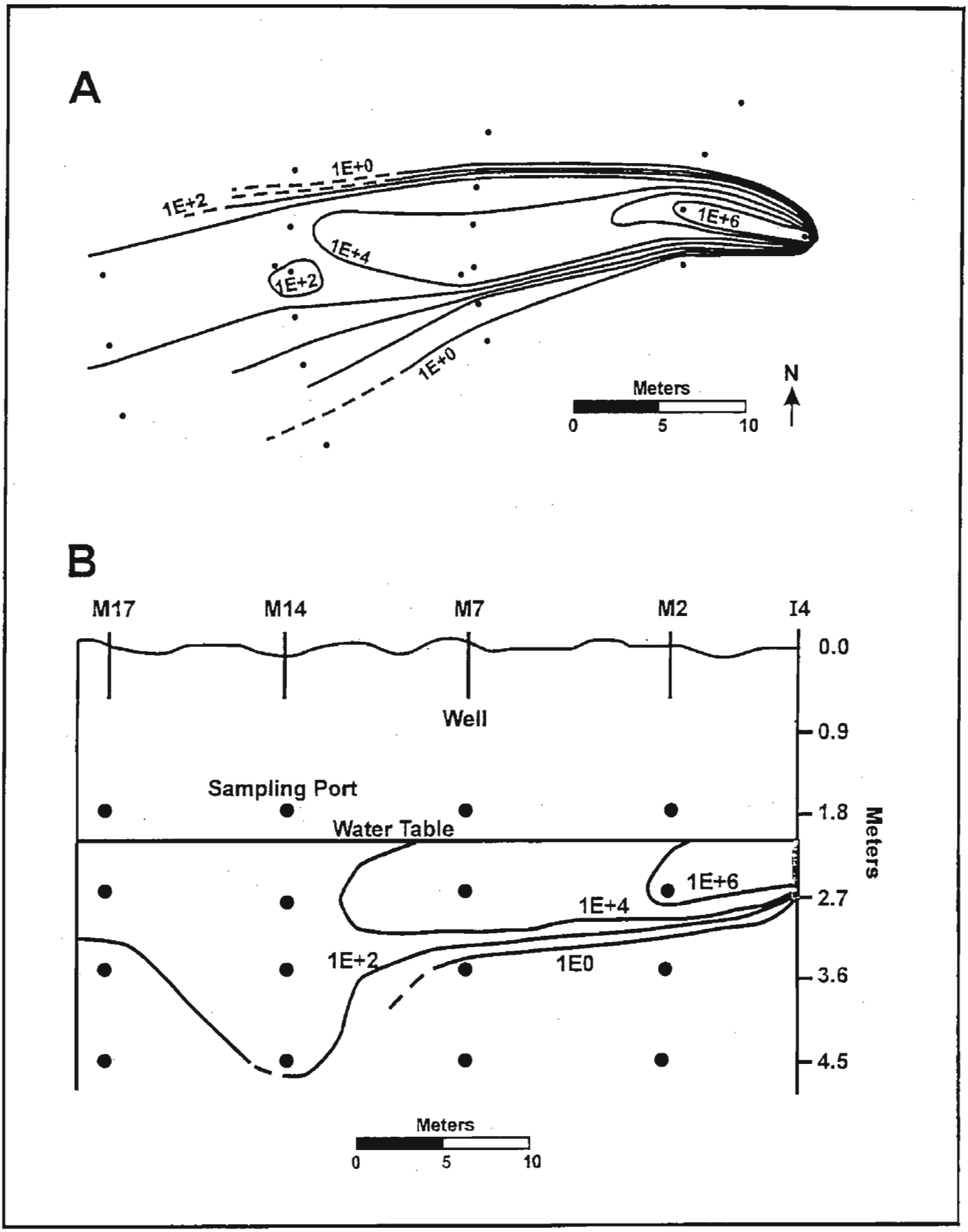
56
64
72
Log10 PFU/ml of Virus
Hours After Injection


A
1E+0
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5
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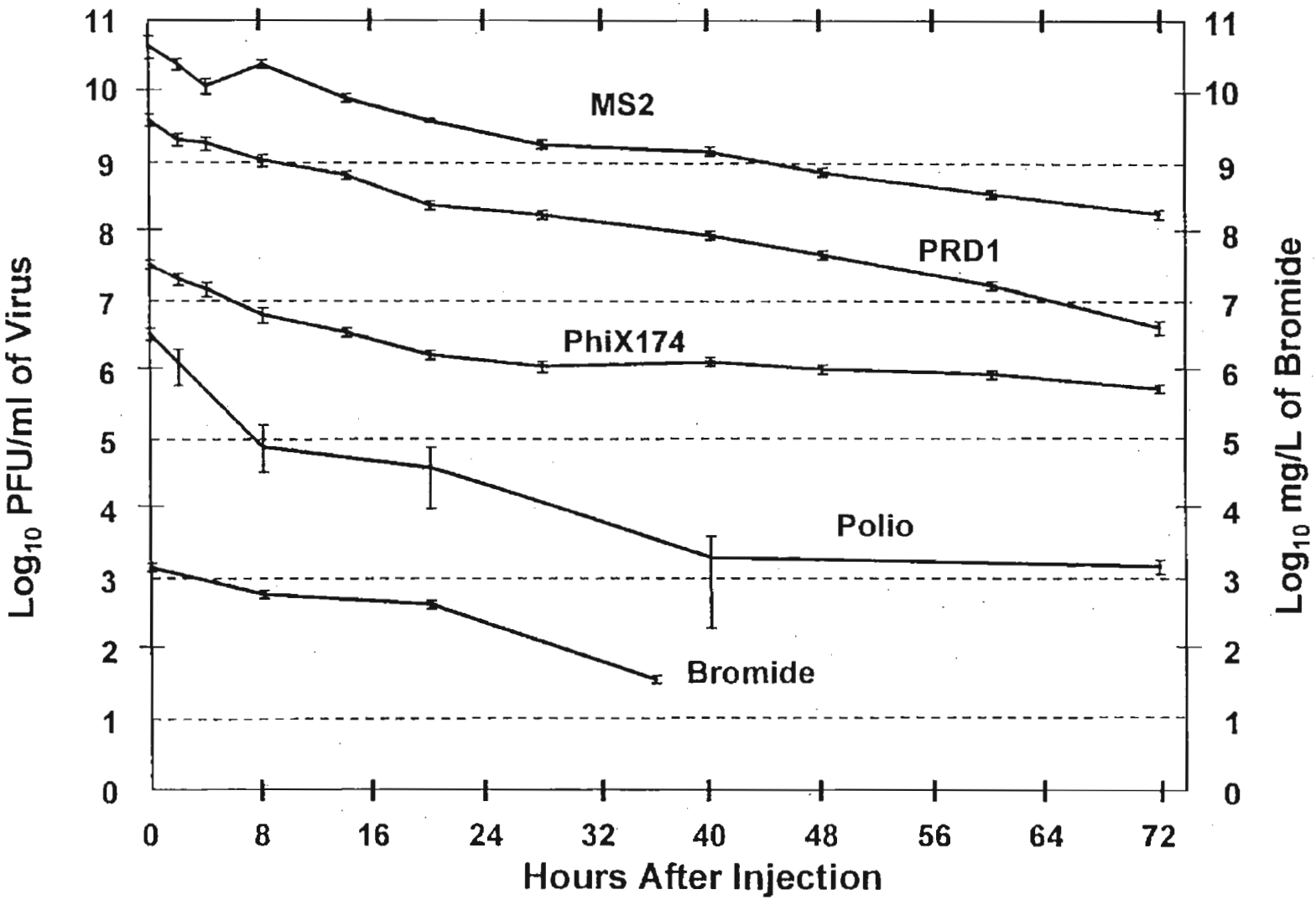
MS2
PRD1
PhiX174
Polio
Bromide
Log10 PFU/ml of Virus
Log10 mg/L of Bromide
Hours After Injection
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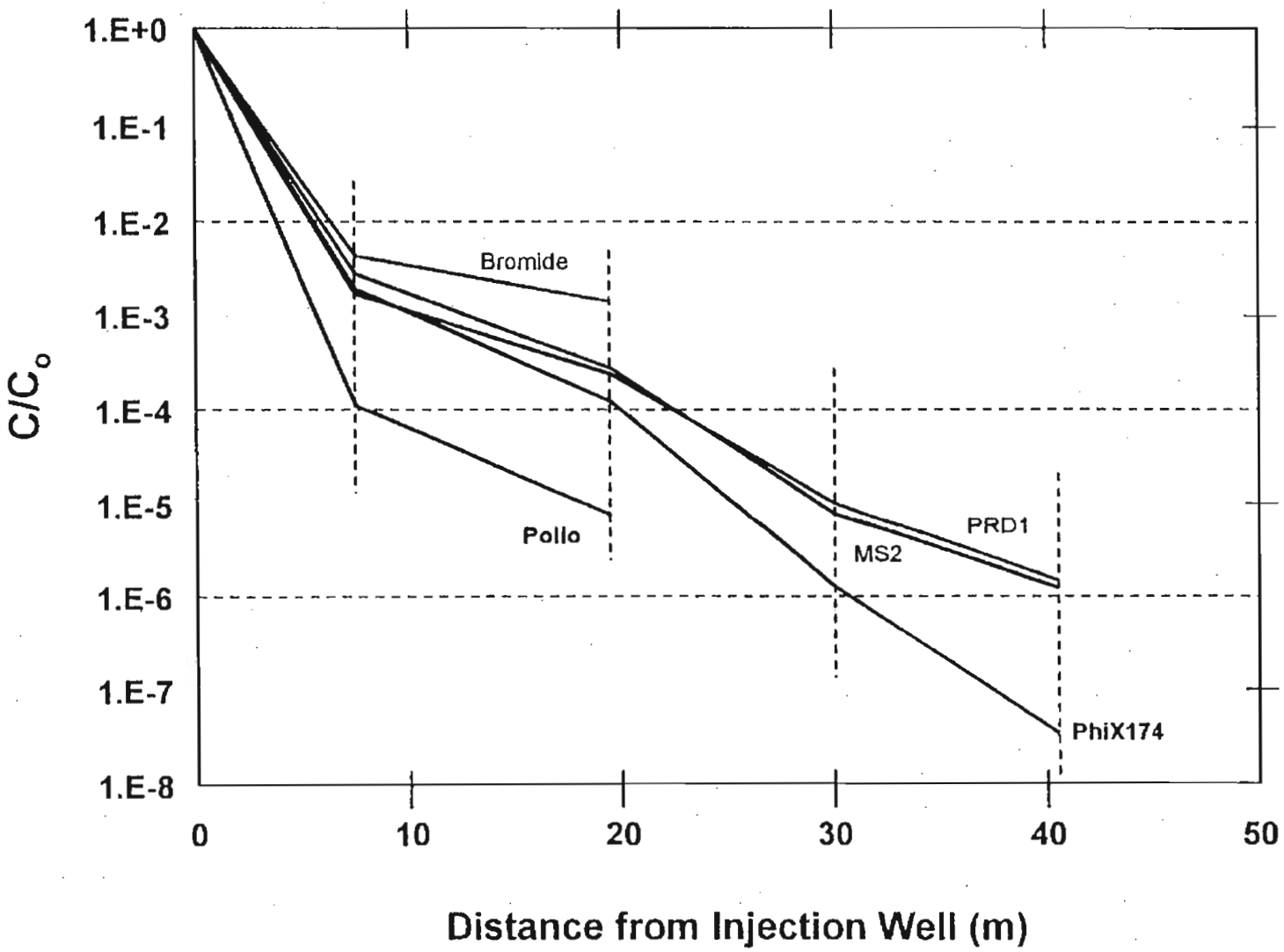
1.E+0
1.E-1
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C/C$_o$
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10
20
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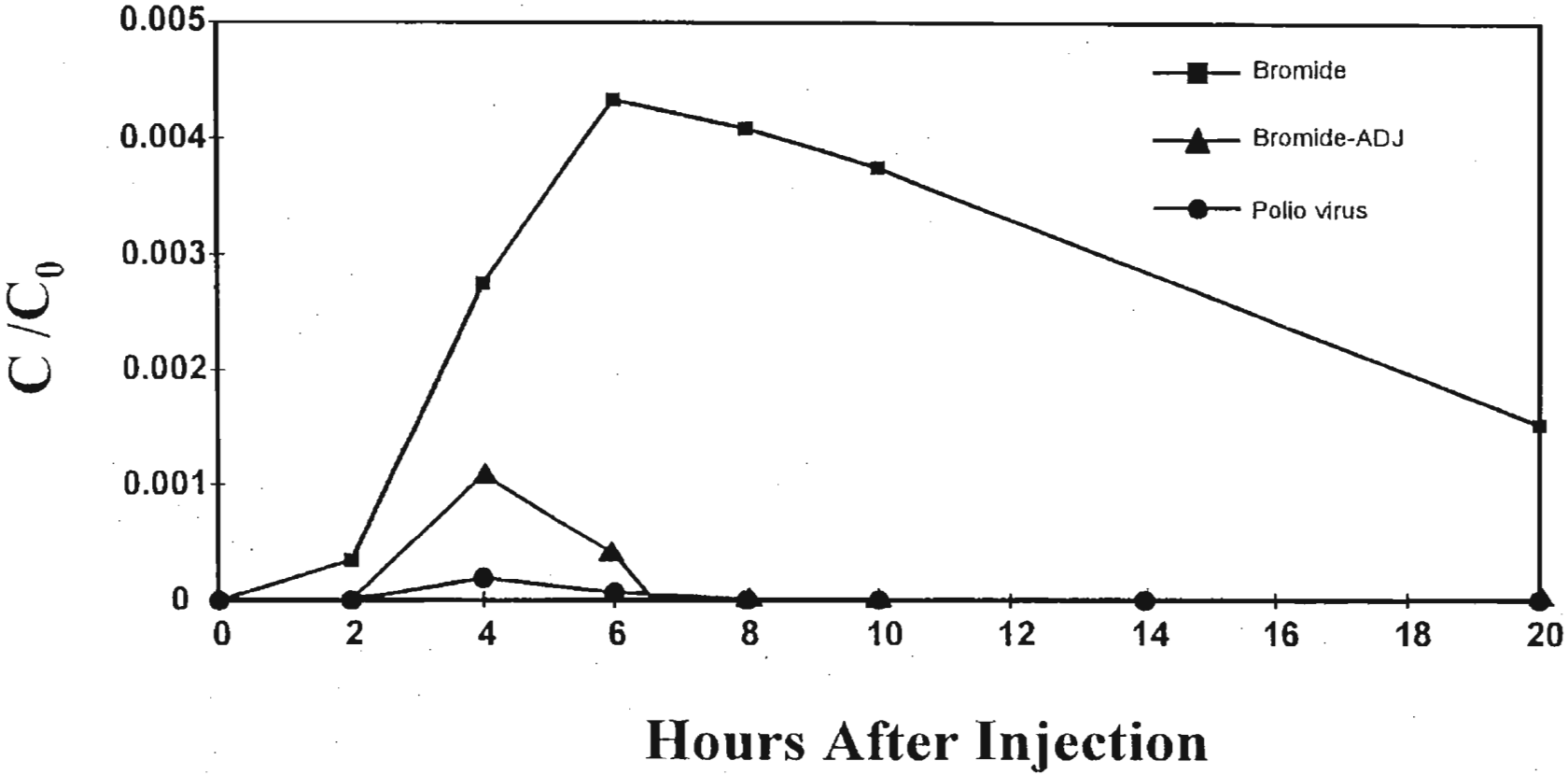
50
Distance from Injection Well (m)
Bromide
Pollo
PRD1
MS2
PhiX174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16
18
20
Bromide
Bromide-ADJ
Polio virus
Hours After Injection

18

Virus Transport in the Floodplain Groundwater of a Headwater Stream, Western Montana, USA

William W. Woessner and Dan C. DeBorde

ABSTRACT

High gradient headwater streams often have coarse-grained floodplain sediments that, when saturated, allow rapid transport of groundwater. The world wide use of these aquifers for water supply has focused attention on the need to determine if the introduction of human wastes, via surface water recharge or land sewage disposal poses a threat to users. We attempt to answer these questions in a cold (10°C), high hydraulic conductivity (900 to 13,800 m/d), Clark Fork River floodplain aquifer by instrumenting a site with 109 monitoring wells and conducting multiple virus seeding experiments. As part of these experiments, bromide and the bacteriophage MS2 were seeded into the groundwater and their behavior monitored under natural gradient conditions and within the capture zone of a well pumping at 378 l/min. Results showed a portion of the virus traveled at rates similar to the bromide tracer. Most of the viruses readily adsorbed and desorbed over time during both pumping and non-pumping transport. Virus travel over a distance of 19.8 m under a natural gradient reduced the aqueous concentrations by 3 to 4 $\log_{10}$. Transport in the capture zone of a pumping well was enhanced by 2 to 3 times when compared to rates under natural gradient conditions. Groundwater contaminated by sewage would remain a health risk after transport over the typical recommended setback distance of 30.4 m in the studied aquifer. However, in highly productive headwater aquifers, adsorption and die-off should provide sufficient natural disinfection when appropriate setback distances are determined.

Keywords: Groundwater contamination, virus transport, tracers, water supply.

INTRODUCTION

Headwaters are important components of river systems and to the health of nearby inhabitants. Their development often leads to resource management conflicts (Woessner and Potts 1989). The floodplains of headwater streams not only provide relatively flat land for dwellings, they form

the direct links between the stream and the associated valley groundwater system. In mountainous regions, high stream gradients and associated bedrock geology often result in floodplain deposits that are coarse grained, and limited in vertical and horizontal extent. The stream and riparian ecology, and often the residents of associated communities, rely on these aquifers to sustain them. Unfortunately, the same properties that make these aquifers prolific also allow for easy contamination and the rapid transport of degraded groundwater to riparian zones, stream channels and water supply wells. Induced infiltration of polluted surface water can also occur when well withdrawals lower the water table beneath the stream channels and in zones in which the stream is influent. Viral contamination of groundwater supplies by sewage originating from land based disposal systems (sewage lagoons, waste land, farms or septic systems) or sewage impacted surface water sources, is a public heath concern (Alhajjar *et al.* 1987, Wellings *et al.* 1975; Mathess and Pekdeger 1981, Pekdeger and Mathess 1983, Bitton *et al.* 1984, Gerba *et al.* 1991, Gilbert *et al.* 1976, Yates *et al.* 1985, Yates and Yates 1989, Yates and Jury 1995, Jansons *et al.* 1989a, b, Bales *et al.* 1995). Seventy percent of viral and bacterial disease outbreaks, occurring in populations relying on groundwater for their potable supply, have been tied to consumption of untreated sewage impacted groundwater (Keswick and Gerba 1980). When assessing risk, regulators wish to determine if separation distances between sewage sources and wells can be defined such that natural disinfection will occur by physical dispersion, adsorption and viral inactivation before viruses reach wells (Macler 1995).

Case Study: This river, a headwater stream of the Columbia River, is located in the Rocky Mountains of western Montana. The aquifer is the sole source of water supply for the community of Missoula (population 50,000) and the 20,000 rural residents occupying the 35 by 8 km valley floor. In a remote portion of the active floodplain, viruses were seeded under natural gradient conditions and in the capture zone of a well pumping at 378 l/min. The rates of transport, plume areas, and concentrations were compared with a conservative ion tracer.

METHODS

Site Conditions

The study site is located at the Erskine Fishing Access, 24 km west of the City of Missoula, Montana, in a grassland portion of the active floodplain of the gravel-cobble bedded Clark Fork River (Figure 1). Experiments were conducted in the upper 6 m of the unconfined aquifer. The water table occurs 2.1 to 2.5 m below land surface. The geologic matrix is

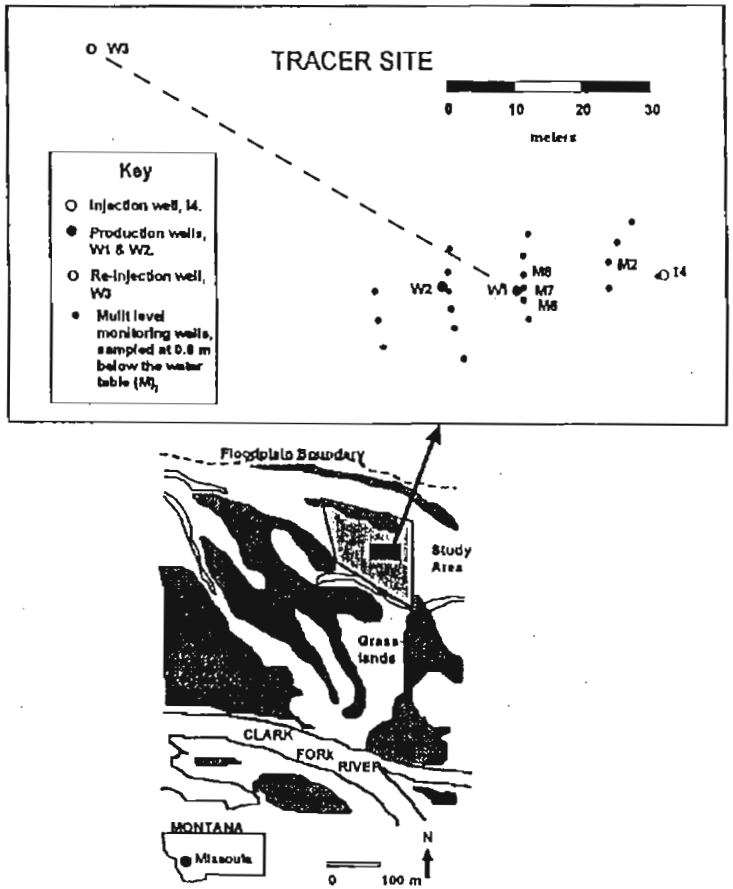

Fig. 1: Location map showing the floodplain of the Clark Fork River, the study area and details of the tracer well network. The dashed line shown in the tracer site insert represents the W1 discharge hose.

composed of clast supported cobbles and gravel with a medium to coarse-grained sand matrix. Groundwater transport velocities range between 22 to 79 m/d. Aquifer hydrologic properties are presented in Table 1 (DeBorde *et al.* 1997).

Table 1: Aquifer Characteristics (DeBorde *et al.* 1997)

Hydrologic Properties	
Porosity	0.15
Gradient	0.00043
Avg. K (m/d)	900-13,800
GW Velocity (m/d)	27
Water Chemistry	
Water Type	Calcium, Bicarbonate
Spec. Conductivity	288 µS/cm
DO	3.5 mg/l
pH	7.2
Temp. (C)	10.3

Field Methods

The general study area was instrumented with 89 single level (1.3 to 5.08 cm diameter) piezometers that were used to define groundwater flow, hydraulic properties and to conduct preliminary tracer tests. Once groundwater flow paths had been identified, a tracer site was instrumented with 20 bundle piezometers. Each multilevel well was constructed by using a 4.5 m long, 1.3 cm diameter, PVC tube perforated at the end over 5 cm and wrapped with nylon mesh screen. Attached to the outside of this tube at 1.8 m, 2.7 m, and 3.6 m below ground surface were 0.5 cm diameter polyethylene tubing with 5 cm of perforated ends wrapped with nylon screen. These instruments were installed with a direct driving GEOPROBE that pushed and vibrated a 3.8 cm diameter dual tube setup. After removal of the center rod, the multi-level sampler was inserted and the outer tube removed. The formation was allowed to collapse around the instrument. In addition to the multi-level samplers, three 10.2 cm diameter steel cased production wells (W1, W2, and W3) with 3 m of 0.12 cm wide slots were installed by driving the casing and screen to 4.6 m (Figure 1).

This work compares and contrasts the behavior of MS2 and bromide under both natural and forced gradient transport. Initial concentrations of tracers and test durations are presented in Table 2. Natural gradient experiments (September and October, 1996) involved seeding the ground water with 18.9 L of sodium bromide, and 37.8 L of the bacteriophages MS2, PhiX174 and PRD1 and the attenuated polio virus type-1 (CHAT strain) as slug inputs (DeBorde *et al.* 1997). The forced gradient experiments (March and April, 1997) were conducted by injecting 37.8 L of bromide and MS2 tracers into ground water at 14 during the pumping of W1 at 378 l/min.

Bromide tracer tests were conducted 3 to 10 days prior to virus seedings in an attempt to avoid ionic strength effects. A natural gradient bromide test was conducted in March, prior to the forced gradient test. It was established that flow fields and velocities were similar to those measured during the September and October natural gradient experiment.

Table 2: Initial Concentration of Injected Tracers and Test Duration

Natural Gradient Test	Initial Concentration	Forced Gradient Test	Initial Concentration
Virus Tracer (72 h)*	PFU/ml	Virus Tracer (10 h)	PFU/ml
MS2	5.61E + 10	MS2	5.83E + 10
Ionic Tracer (36 h)	mg/l	Ionic Tracer (8 and 20 h)	mg/l
Bromide	1443	Bromide	1250 (20 h) 2749 (8h)

* Test duration

Sampling of the multilevel samplers was accomplished using peristaltic pumps with dedicated pump tubing attached to each sampling port. Samples for bromide analysis were collected in 50 ml polyethylene bottles and virus samples were placed in sterile 50 ml polypropylene tubes. Samples from W1 were collected from the pump discharge line at the well head. Samples were transported from the field in ice filled coolers and held in the lab at 4°C until analysis.

Analytical Methods

Bromide was determined using standard ion chromatography methods (Pfaff 1993). Analytical detection limits were 0.1 mg/l for the natural gradient experiment and 0.01 mg/l for the pumping test. Virus analyses were performed as described by DeBorde *et al.* (1997). MS2 concentrations for both experiments were determined using appropriate host bacteria in single layer agar plaquing assays at a detection limit of 0.1 PFU/ml (Plaque Forming Unit) (DeBorde *et al.* 1997). Sampling and analytical errors were determined from analytical controls and field duplicates. (Table 3).

Table 3: Estimated Analytical and Sampling Error

Natural Gradient Test	% Error as 95% CL*	Forced Gradient Test	% Error as 95% CL
Virus Tracer	PFU/ml	VirusTracer	PFU/ml
MS2	15	MS2	15
Ionic Tracer	mg/l	Ionic Tracer	mg/l
Bromide	14	Bromide	14

* CL = Confidence Limit

RESULTS

Results of the natural gradient multi-virus seeding experiment have been described by DeBorde *et al.* (1997). Here we observed: 1) the average transport rate for a portion of the seeded virus ranged from 22 to 39 m/d; 2) virus adsorbed readily to the aquifer matrix with the attenuated polio virus attaching at higher rates than the bacteriophage; 3) some of the adsorbed virus desorbed and reentered the groundwater system as evidenced by tailing of the breakthrough curves; 4) polio virus peak concentrations appeared to break through at observation points ahead of the bromide peaks, which was suggested to be a result of pore exclusion or a truncation of the breakthrough by a high adsorption rate.

Bromide Transport under Natural Gradient Conditions

Concentration vs. time data for bromide and MS2, obtained at monitoring wells located at distances of 7.5 and 19.4 (M2 and M7, respectively), are presented in Figure 2. Multi-level ports were sampled at each monitoring well. However, the lack of significant vertical mixing allowed for the results obtained by sampling the 2.7 m port (0.6 m below the water table) to be used to represent virus and bromide concentrations. Sampling intervals over the 72 h experiment varied from 2 h intervals during periods of anticipated peak breakthrough to longer periods during non peak times (DeBorde *et al.* 1997). The concentrations of tracers were also measured at well W1 via a 1.3 cm diameter polyethylene tube lowered in the well to 0.6 m below the water table (Figure 2). Within the sampling frequency and the portion of the production well being sampled, it appeared MS2 breakthrough occurred at about the same time and with similar peak concentrations at both M7 and W1, between 12 and 14 h. Based on breakthrough curve analysis, virus peaks appear to arrive earlier than bromide peaks. However, error analysis (Table 3) makes peak separation for bromide unclear, thus transport rates of MS2 and bromide are interpreted to be similar (22 to 39 m/d).

Figure 3 presents the relative concentrations (C/C_0) for bromide and MS2 peaks as a function of distance. Cold groundwater and the short duration of the sample collection period (72 h) eliminated die-off (DeBorde *et al.* 1997) as a significant contributor to virus loss. Clearly, adsorption of MS2 occurred, reducing the relative concentration at well M2 (7.5 m) and well M7 (19.4 m) (DeBorde *et al.* 1997). Breakthrough curves at all locations had long tails, indicating a portion of the adsorbed virus were being release during the sampling period (Figure 2).

MS2 and Bromide Transport under Pumping Conditions

For each forced gradient seeding experiment, W1 was pumped for 1h 20 min at 378 l/min to establish a steady state flow field, then either

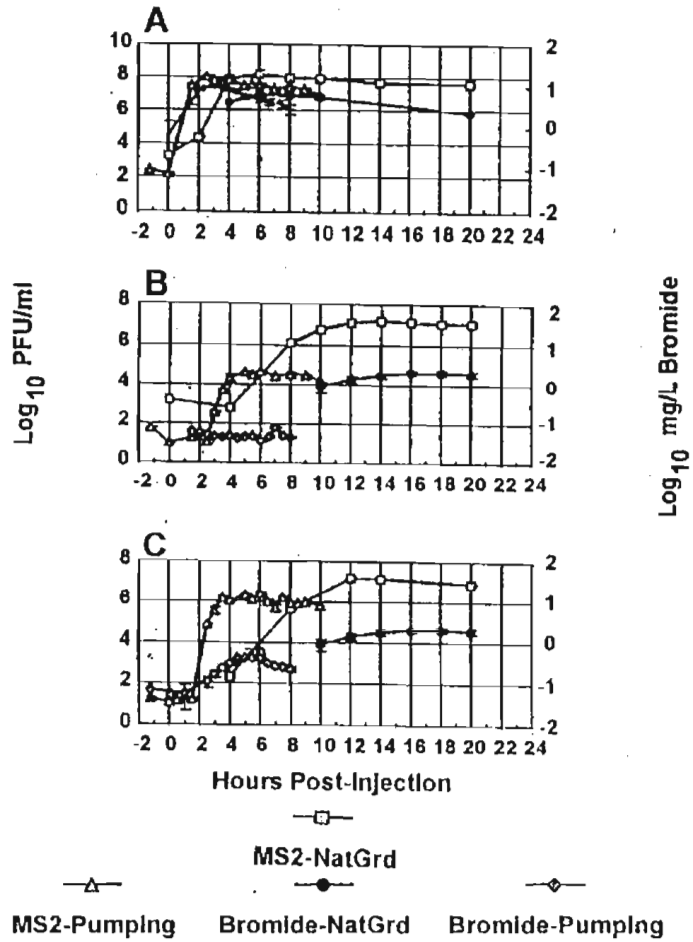

Fig. 2: Comparison of breakthrough curves for bromide and MS2 at monitoring wells M2(A) and M7(B), and production well W1(C) under natural gradient (NatGrd) and pumping conditions. Tracers were injected at 14 at the water table (Figure 1). Samples were taken from the monitoring well ports located 0.6 m below the water table, and 0.6 m below the water table in W1, during the natural gradient experiment and directly from the well discharge during the forced gradient experiment. Error bars are shown though they are often smaller than the symbols representing sampling data.

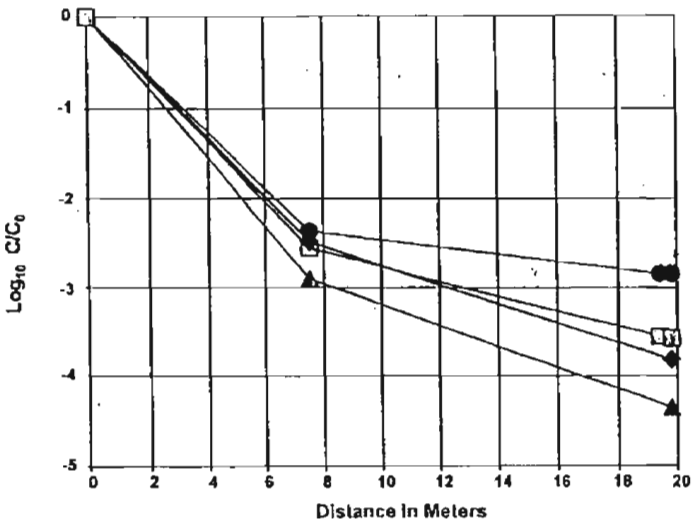

Fig. 3: Comparison of relative peak concentrations, C/Co, at 7.4, 19.4 and 19.8 m from I4 during natural gradient (NatGrad) and pumping experiments.

bromide or MS2 were seeded at I4 as the well maintained its discharge over the next 10 to 20 h (Table 2). Sampling of the multi-level ports at one half hour intervals during the pumping experiment found the tracers concentrated at 2.7 m below land surface (0.6 m below the water table). Bromide and MS2 breakthrough under forced gradient conditions are shown in Figure 2. It appears that bromide and virus peaks arrived at monitoring well M2 and W1 at similar times (2.5 and 5.5 h, respectively) within resolution limits of likely analytical and field errors. Interestingly, the MS2 concentration remained high after peak arrival at W1. Well M7, immediately in front of W1 (within 0.4 m), contained a lower concentration than the pumping well at its port located 0.6 m below the water table. It appears the preferential flow path taken by the virus under enhanced gradient conditions resulted in the virus peak by-passing M7.

Analysis of the peak relative-concentration data showed lower relative concentrations of both bromide and MS2 arrived at monitoring well M2 and pumping well W1 than were measured during the natural gradient experiment (Figure 3). This reflects the increased dispersion occurring along the flow path and the additional dilution at W1 caused by radial flow to the well. The difference between the relative bromide and MS2

concentration was greater at M2 (7.5 m) than under natural gradient conditions. Interestingly, the reverse relationship occurred at W1. Though the duration of the pumping experiment was short, breakthrough curves appeared to tail indicating the processes of both adsorption and desorption were occurring continuously. Mass balance calculations show about 6% of the seeded MS2 and 41% of the bromide tracer were recovered at W1 during 8 hours of pumping.

DISCUSSION

The behavior of MS2 under natural gradient conditions showed a portion of the virus adsorbed to sediment while a second portion of the virus acted conservatively, breaking through at monitoring wells at rates similar to bromide. Evaluating the peak-concentration data it appears that MS2 concentration decreases at approximately 1 $\log_{10}$ every 6.2 m under natural gradient conditions in this heterogeneous floodplain aquifer. Assuming the virus in a source of contamination impacting this aquifer entered at a concentration of 1×10^4 PFU/l (an average value for virus in a household septic tank) and behaved as MS2, the dispersion and adsorption processes would reduce the concentration in the groundwater by about five logs over a standard 30.4 m setback distance. Clearly this reduction is unacceptable for health safety in these coarse-grained headwater aquifers. If one arbitrarily chose 1 virus/1000 l as the maximum acceptable virus concentration in drinking water as once suggested by WHO, then an additional 12.4 m (total of 43 m) would be required to achieve this standard. Such a setback distance would be most applicable in aquifers influenced by low yielding domestic wells that would not significantly alter the ground water velocity within the well capture zone.

The operation of a well that is continuously pumping at 378 l/min in the heterogeneous floodplain aquifer at the Erskine Tracer Site enhanced the transport of the virus. Virus seeded 19.8 m from the pumping well arrived 2 to 3 times sooner than under natural gradient conditions. Virus concentrations increased rapidly at the pumping well and then remained high during the 10 hr of pumping. While the virus arrived sooner at the pumping well, the concentrations were somewhat lower due to the dilution that occurs by the combining of virus impacted water with virus free water within the well and the increased longitudinal dispersion during transport. At our site, under pumping conditions of 378 l/min, a reduction of 1 $\log_{10}$ every 5 m was observed over a transport port distance of 19.8 m. Thus, assuming the same source concentration as above, the maximum permitted concentration of 1 virus/1000l would be achieved at 35 m, just slightly more than the standard setback distance. Even though the virus concentration in the well discharge is lower than the concentration

of virus under natural gradient conditions, under some situations the more rapid transport of virus within the capture zone of some wells may be significant. If waste sources contain higher than suggested concentrations of virus, set back distances will need to be increased. In aquifer settings where die-off rates are higher due to warmer ground water conditions, rapid transport may minimize the importance of die-off in controlling virus concentrations at the well head.

CONCLUSION

This work examined the behavior of virus seeded into a cold water, coarse grained, high hydraulic conductivity floodplain aquifer of the Clark Fork River. In sum, under natural gradient conditions, most virus was adsorbed, yet concentration peaks traveled at rates similar to bromide. Dispersion and adsorption reduced concentrations by 6 to 7 $\log s_{10}$ over 40.5 m of transport. Pumping conditions enhanced virus transport rates along a 19.8 m path by 2 to 3 times. Preferential flow within the aquifer resulted in higher than predicted virus concentrations in the well discharge. This potential for rapid virus transport over large distances in headwater aquifers implies a need for the careful management of headwater systems to prevent pathogenic contamination of water supplies.

Regulations, attempting to protect headwater groundwater supplies from viral contamination, will need to consider the processes affecting the virus concentrations along the flow path during both natural and forced gradient transport. At our site, typical 30.4 m set back distances between viral sources and a 378 l/min production wells appear to be inadequate to protect public health and safety.

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Virus Transport in the Capture Zone of a Well Penetrating a High Hydraulic Conductivity Aquifer Containing a Preferential Flow Zone: Challenges to Natural Disinfection

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Abstract

Regulators are formulating criteria to protect groundwater supplies from pathogenic contamination. One of their goals is to establish the set of conditions that would allow for natural disinfection in aquifer systems. We investigated the transport of the bacteriophage MS2 in the unconfined gravel rich floodplain of the Clark Fork River in Western Montana. Field tracer experiments revealed the presence of a narrow zone of preferential flow within the aquifer. By placing a well pumping at 408 L/min in this zone and seeding bromide and MS2 into the capture zone, and numerically simulating aquifer conditions with and without the presence of a high hydraulic conductivity zone, the influence of preferential flow on contaminant transport was examined. Analysis showed that the presence of a preferential flow zone in the capture zone of the pumping well resulted in a 12 to 18% wider capture zone, an increase in transport rates to the well of 56% and a 2.6 times higher concentration at the well head. Natural disinfection criteria will need to account for such variations in travel times and concentrations in cold-water, high-conductivity, fluvially derived aquifers if well head water quality is to be protected..

Introduction

Considerable strides have been made in the last decade to protect groundwater users from consuming contaminated groundwater. Programs such as the Well Head Protection Program (WHPP) Source Water Assessment Programs (SWAP) and comprehensive state groundwater protection programs under the Federal Safe Drinking Water Act (SDWA) designate time or distance related zones where the presence of potential water contaminants are prohibited or limited. Recently, attention has focused on developing protection criteria not only for dissolved contaminants, but also for pathogens including virus [1]. Federal and state regulators are attempting to establish natural disinfection standards for viruses. Such criteria would establish source-set back distances and groundwater travel times that would result in acceptable risks to public health.

Designing natural disinfection criteria is hampered by the complex movement of groundwater in the vicinity of a pumping well [2-6]. The presence of heterogeneous geologic material makes exact prediction of contaminant concentrations difficult. When a detailed characterization of the aquifer heterogeneity is known, numerical and stochastic techniques can be used to assess capture zones and travel times with less uncertainty [7,8]. However, in most settings, standard aquifer testing is relied upon to develop average hydraulic properties for regions surrounding wells, and using either analytical methods or numerical methods to calculate well head protection zones. To date, this approach also has been used when

attempting to establish virus well head protection criteria. In fracture-controlled groundwater systems and in coarse-grained floodplain aquifers, preferential flow zones may enhance transport of solutes and virus by 10's to 100's of times over rates predicted from average aquifer hydraulic properties [9,10]. The affect of such a zone on the transport dynamics and well head concentrations of virus and bromide tracers injected in the capture zone of a pumping well is the focus of this paper. These results are used to illustrate the importance of considering the presence such preferential flow zones when developing natural disinfection criteria.

Methods

A production well and pump system capable of yielding 408 L/min were installed at the Erskine Tracer Site located in the coarse-grained unconfined floodplain aquifer of the Clark Fork River in western Montana (Figure 1). During the development of this site bromide and rhodamine-WT were used to establish preliminary groundwater flow directions and rates. Standard aquifer testing using a production well (W1), and single level and nested monitoring wells were used to derive average hydraulic conductivities of this 4.3 m thick aquifer (Table 1). Natural gradient transport experiments were conducted using bromide,

Table 1. Aquifer Characteristics

Hydrologic Properties		Water Quality	
Porosity	0.15	Water Type	Calcium Bicarbonate
Gradient	0.00043	Conductance (μS/cm)	288
Average K(m/d)	2,000	DO (mg/l)	3.5
High K Zone (m/d)	13,000	pH	7.2
Long. Dispersivity (m)	1.60	Temp (°C)	10.3
Trans. Horizontal Dispersivity (m)	0.24		
Trans. Vertical Dispersivity (m)	0.024**		

** 0.1 of Trans. Horizontal Dispersivity

free bacteriophage and the attenuated polio virus [11]. The results of these efforts showed a preferential zone of high hydraulic conductivity parallel to the flow direction (Figure 1), about 3 meters wide and extending 2 to 3 meters below the water table. The natural gradient groundwater velocity in this zone was estimated at 29 m/d based on bromide tracer breakthrough curve analysis. Aquifer dispersivities were derived using methods described by Sauty [12] and by fitting using MT3D96 [13] (Table 1).

Production well W1 is a 10 centimeter diameter sandpoint with 3 meters of 60 slot screen that extends from the water table to a depth of 2.8 meters (Figure 1). The aquifer base is located 4.3 meters below the water table at which point the cobble dominated system becomes sand rich. All but the bottom 0.5 meter of the production well is screened within the preferential flow zone. A bromide and virus tracer test was conducted by pumping W1 at 408 L/min and seeding the tracers in the preferential flow zone 20 meters up-gradient from W1. Samples from multi level wells were collected at 1/2 h intervals using peristaltic pumps and from the pumping well discharge. Standard ion chromatography techniques were used to analyze for bromide and EPA techniques were utilized for MS2 analysis [11].

The influence of preferential flow on bromide and virus transport within the well capture zone was evaluated by comparing results of the field experiment with the outcome of numerical simulations. The modeling effort simulated groundwater flow and bromide transport in an aquifer with both a preferential flow zone (3 m wide and 34 m long) and without such a zone (average hydraulic properties used throughout) (Figure 2).

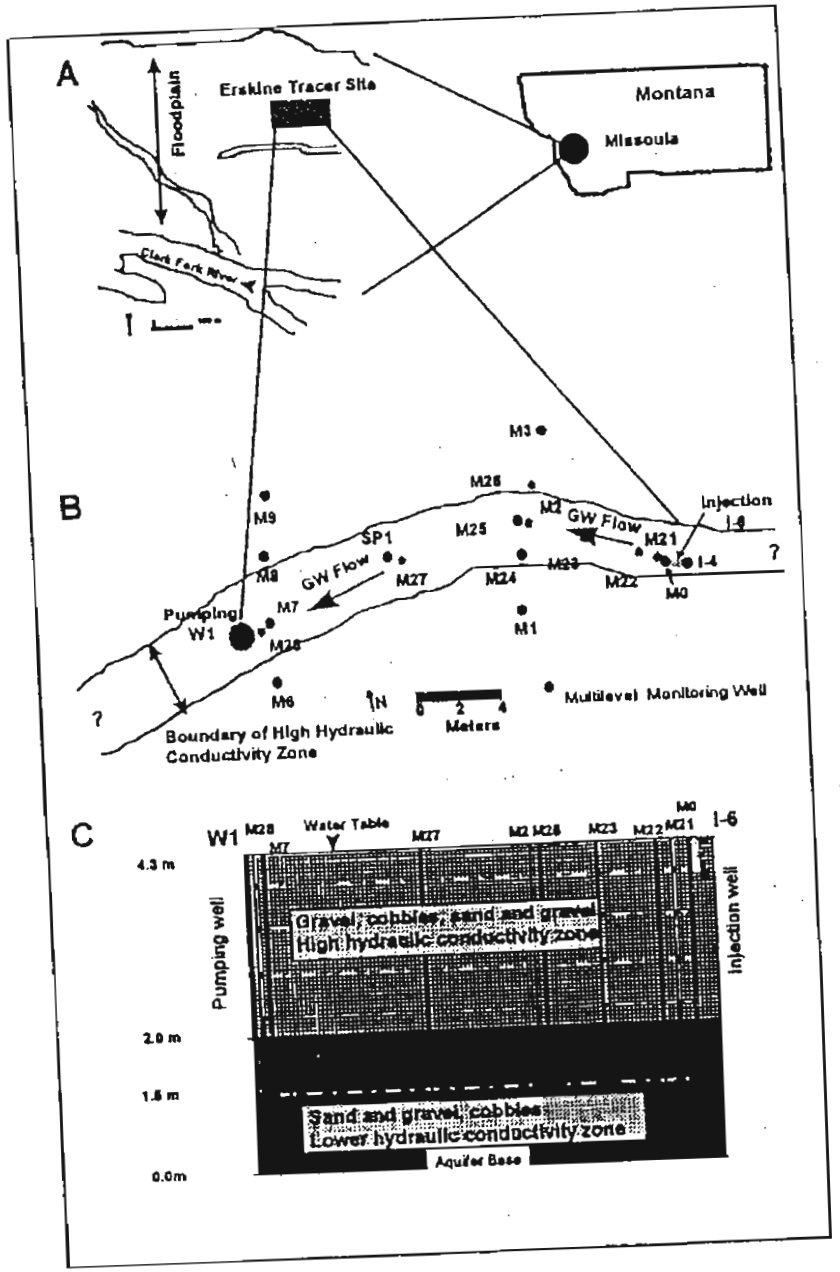

Figure 1: Location map of the (A) Erskine Tracer Site, (B) production well (W1), observations wells(M), injection wells(I) and preferential flow zone, and (C) well design and a general geologic cross section.

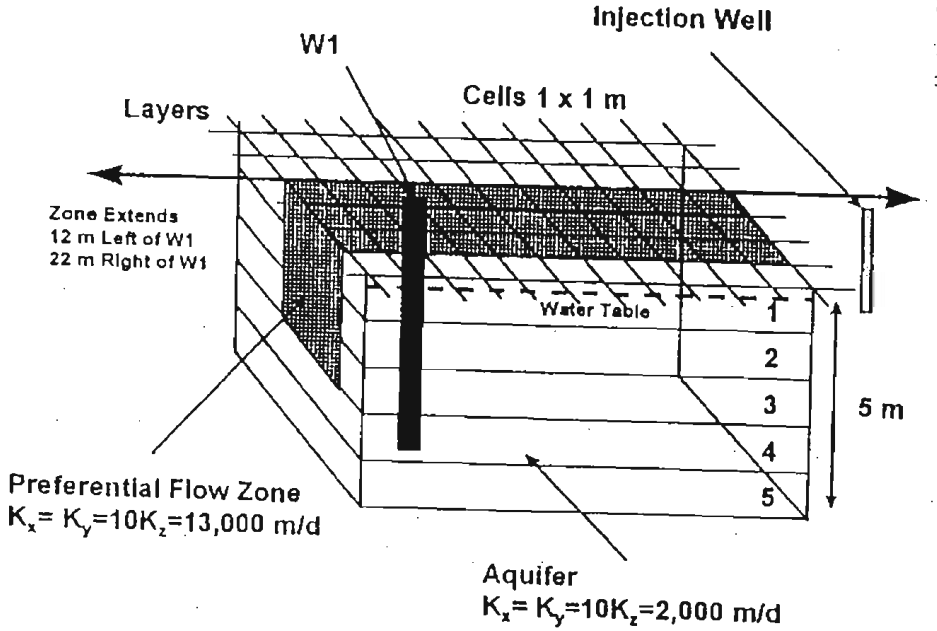

Figure 2: Numeral flow model grid, layers, properties and assignment of a preferential flow zone in the vicinity of production well W1. The five layer model is 400 m x 400 m with cell sizes expanding to 4 x 4 m away from the area surrounding W1.

Simulated capture zone size, transport rates and concentrations at the pumping well were compared with observed values. MODFLOW, MODPATH and MT3D96 as formulated within VISUAL MODFLOW [13] were used for simulation. A steady state, five layer model used a 400 x 400 grid with square four meter cells. The grid became variable in the area around the pumping well were cell sizes of one meter by one meter were assigned (Figure 2). Specified head boundaries were used on the right and left sides of each layer to generate the 0.00043 gradient observed in the field. Pumping rates for each layer were assigned by weighting the discharge by the screen length and layer hydraulic conductivity as described by Guiguer [13]. The flow model was calibrated to head and groundwater velocities under natural gradient and transient conditions (results of aquifer testing). The solute transport model was calibrated to bromide concentrations, velocity and concentrations during pumping experiments. Once a representative model of the field setting was derived, the non-preferential flow simulation was obtained by resetting the high hydraulic conductivity zone to the general aquifer properties (Figure 2). Comparison of virus behavior and well head concentrations during both scenarios then were estimated by correlating the behavior of bromide and virus concentrations in the preferential flow setting with that of the bromide behavior under average conditions.

Results

Field Pumping Experiment and Simulations

W1 was pumped at 408 L/min until steady state was accomplished (1.3 hr) at which time a slug of 37.8 L of tracer solution containing 1898 mg/L bromide and 3.36 x 1010 PFU/ml (plaque forming units/ml) of MS2 were injected in the preferential flow zone at 16 (Figure 1B). Breakthrough curves for bromide and MS2 at W1 are presented in Figure 4. Breakthrough curve plots show a portion of the MS2 bacteriophage travels at the same rate as the bromide. However, when a mass balance is computed at W1 for the 47 h of pumping, 78% of the bromide has been captured, while only 16% of the MS2 had arrived at the pumping well. A portion of the MS2 is attaching to the aquifer matrix as virus inactivation in this cold groundwater system is insignificant over the short duration of the experiment (DeBorde, et. al, 1998). The rapid dilution during transport and the use of multi-level wells showed the bromide tracer results were not significantly affected by solution density [14].

The capture zone of W1 was simulated using the calibrated flow model. Table 2 presents the capture zone width by layer under preferential flow conditions and average conditions.

Table 2: Capture Zone Widths Measured 200m Up-gradient of W1.

Layer number	Preferential FlowZone**	Average FlowConditions**
1	144	118
2	144	124
3	138	122
4	74	118
5	46	85

**Width in Meters

The transport time verses distance for the flowpath in layer 2, located 0.5 to 1.5 meters below the water table, beginning 30.5 meters from the pumping well and passing though the entire length of the preferential flow zone to the right of W1 is present in Figure 3. Concentrations at the well head (Figure 4) also were predicted from injection of bromide and virus at 16 by using MT3D and dispersion values presented in Table 1.

Simulation of Average Conditions

The calibrated numerical model used to simulate the preferential flow zone and the results of field experiments was modified by replacing the high hydraulic conductivity zone with the average hydraulic conductivity (2,000 m/d). Particle tracking was used to establish capture zones. Capture zone widths measuring 200 meters up-gradient of the pumping well were determined for each layer (Table 2). The relationship between calculated transport time and distance from W1 is presented in Figure 3. MT3D was used to predict the bromide concentration at W1 (Figure 4). The results of each of these analyses were compared with similar data derived for the preferential flow setting.

Discussion

The hydraulic conductivity values used to represent both the general aquifer characteristics and the preferential flow zone at the Erskine Site are large. They are, however, not unprecedented. Aquifers associated with the high gradient Clark Fork River have similar values [15] as do some gravel floodplain aquifers of Switzerland [10] and alluvial gravel aquifers of New Zealand [16]. The floodplains of high energy streams would be expected to be heterogeneous and contain large contrasts in hydraulic conductivities.

Field observations of transport over a distance of 20 meters revealed a four log decrease in peak bromide and MS2 concentrations in the discharge of the pumping well. However, mass balance analysis showed 80% less MS2 arrived at W1 than bromide over the 47 hours of pumping. As reported by other researchers and for earlier experiments at the Erskine Site [11], MS2 attach and sorb to the aquifer matrix leaving the majority of the seeded virus in

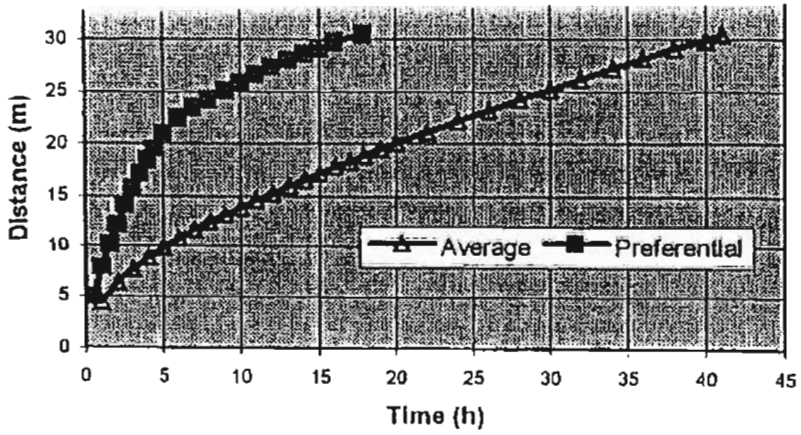

Figure 3: Travel times from 30 m up-gradient of W1 (0 m distance) during pumping at 408 l/min under preferential and average flow conditions.

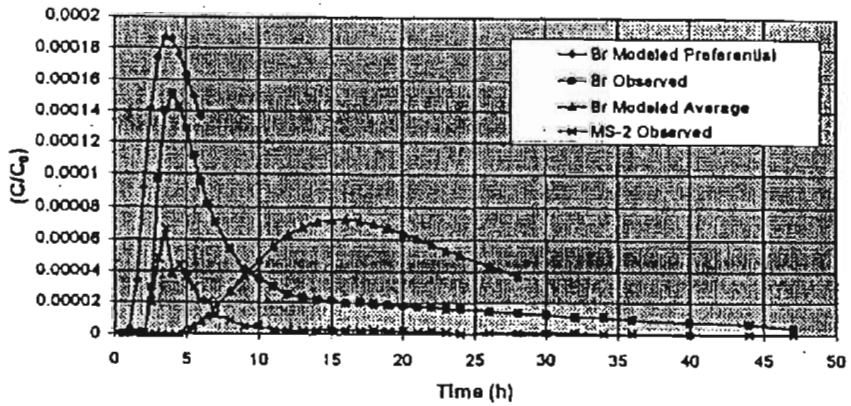

Figure 4: Breakthrough curves at W1 showing observed MS2 and bromide C/C_0, and modeled preferential and average flow bromide C/C_0.

The comparison of the capture zone dimensions presented in Table 2 shows that for the preferential flow scenario, the widest zones occur in the fully screened upper three layers. Layer 4 is only screened over half its thickness and the presence of the water table in layer one leaves only half the layer screened. Interestingly, the presence of the high hydraulic conductivity zone in the upper three layers collects additional flow lines expanding the size of the capture zone in these layers as compared to the average hydraulic conductivity results. However, in layers four and five, the average conditions create a wider capture zone. The preferential flow conditions observed at the site create a 12% to 18% wider capture zone in the upper most layers with the zones in layers four and five being 36% to 45% smaller. Thus, the preferential flow conditions cause additional groundwater to be captured near the water table and less near the aquifer base.

shows the influence of a preferential flow zone on the travel time of a contaminant from a potential source to the well. The total transport time is 17.9 hours compared to 41 hours under average aquifer conditions; a 56% reduction in transport time.

In addition to the more rapid transport of the contaminants under preferential flow conditions, the concentration at the pumping well is higher than would be predicted under average conditions. Figure 4 shows measured and predicted bromide concentrations at W1. The predicted peak concentration under average conditions is 0.138 mg/l and under preferential flow conditions is 0.355 mg/l. This indicates that under the Erskine field conditions, using average hydraulic properties to predict the well head concentration underestimates the field observed concentration by 2.6 times.

The transport of virus in the capture zone of W1 was observed during our field experiment. If it is assumed that the change in velocity and travel time that would result under assumed average conditions would not affect well head concentrations at this site, then the predicted virus concentrations under average conditions would most likely be 2.6 times lower than observed.

Analyses of the field experiment and simulations suggest that creating well head protection zones in coarse-grained, high hydraulic conductivity, fluvially-derived aquifers requires consideration of the probable presence of preferential flow zones with hydraulic conductivities over six times those estimated from standard aquifer tests. The consequences of the presence of such preferential flow zones are a wider capture zone, more rapid transport, and higher concentrations of contaminants at the well head. Observations of virus transport in these high hydraulic conductivity systems show virus loss by attachment to aquifer materials, however, a portion of the virus will arrive at the well head at travel times similar to more conservative tracers. Natural disinfection criteria rely on sufficient transport time in the aquifer to allow for attachment and die-off that achieves acceptable well head virus concentrations. However, accelerated transport and the arrival of higher than predicted total mass at the well head require natural disinfection criteria to include methods to adjust travel times (expand set back distances) in aquifers where preferential flow has been identified or is probable.

Acknowledgments

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The *State Source Water Assessment and Protection Programs Guidance* (US EPA August 1997) clearly outlines the role of the states as leaders of the assessment and protection process. The methodologies to be used by states and local governments for area delineation, monitoring, data management and measuring program effectiveness will be singularly important if the successful implementation of the *Safe Drinking Water Act* (SDWA) as amended in 1996 and the forthcoming *Ground Water Distinction Rule* (GWDR) are to be of maximum benefit.

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