

# Virus occurrence and transport in a school septic system and unconfined aquifer.

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**A study was conducted to analyze viral indicators of fecal contamination using a high school population as a community source. The study also examined the occurrence, distribution and transport rate of viruses in a productive sand and gravel aquifer. Concentrated septic tank effluent samples were tested for virucidal activity. Results indicated that recovery values for enteroviruses in the ground water ranged between 5 and 30%.**

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## Introduction

Domestic septic system effluent typically contains about  $3 \times 10^7$  coliform bacteria/100 mL and, following some types of human viral infections, up to  $1 \times 10^7$  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 Harides 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. 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 vital 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

## Virus occurrence and transport in a school septic system and unconfined aquifer.

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 [null set]X174 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.sup.2] drainfield [ILLUSTRATION FOR FIGURE 1 OMITTED]. 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 [ILLUSTRATION FOR FIGURE 2 OMITTED]. 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 [ILLUSTRATION FOR FIGURE 2 OMITTED]).

### 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 [ILLUSTRATION FOR FIGURES 1 AND 2 OMITTED]. 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 [ILLUSTRATION FOR FIGURES 1 AND 2 OMITTED]. Wells T1 to T7 were constructed of 3.2 cm diameter Schedule 80 PVC pipe with 0.61 m of hand-slotted perforations (20

## Virus occurrence and transport in a school septic system and unconfined aquifer.

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 [ILLUSTRATION FOR FIGURE 2 OMITTED]. 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 [ $\mu$ m] 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 [degrees] C while the coliphage sample was held at 4 [degrees] 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 [ $\mu$ m] 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

## Virus occurrence and transport in a school septic system and unconfined aquifer.

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 [null set]X174 coliphage were grown to high titers in broth cultures, and cell debris removed by low-speed centrifugation (4 [degrees] C, 15 minutes at 3500 x g) in a Beckman J6 centrifuge. The coliphage suspensions ([less than] 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  $1 \times 10^9$  PFU/mL for MS2 and  $1.12 \times 10^6$  PFU/mL for [null set]X174. 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 [degrees] C, while the other tube was sealed and suspended below the water table in well #19 (average temperature about 10 to 11 [degrees] 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.B. (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.

[TABULAR DATA FOR TABLE 2 OMITTED]

## Results

### Site Hydrogeologic Properties

The septic tank effluent discharges to the drain field 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

## Virus occurrence and transport in a school septic system and unconfined aquifer.

unit transmits the majority of the shallow ground water [ILLUSTRATION FOR FIGURE 2 OMITTED]. 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 [ILLUSTRATION FOR FIGURE 1 OMITTED]. 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, 940, #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 [TABULAR DATA FOR TABLE 3 OMITTED] 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.

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	[less than]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	[less than]1 in 3mL	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.

### 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

## Virus occurrence and transport in a school septic system and unconfined aquifer.

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)	[less than]1 in 33L(a)	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	[less than]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	[less than]1 in 3.33 L	0.31	0.31
31	[less than]1 in 3.33 L	17	17
37	0.31	[less than]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 reaching the ground water appears to have remained much the same as that measured in March 1995 (Table 4) and shows excellent

## Virus occurrence and transport in a school septic system and unconfined aquifer.

correlation with the chloride indicator plume [ILLUSTRATION FOR FIGURE 3 OMITTED]. 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 [ILLUSTRATION FOR FIGURE 4 OMITTED]. 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.sub.10] of concentration with every 5 m of transport.

### 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 [ILLUSTRATION FOR FIGURE 6 OMITTED].

The seeding of high concentrations of MS2 and (X174 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  $1 \times [10.\text{sup}.9]$  PFU/mL of MS2 and  $1.1 \times [10.\text{sup}.6]$  of [null set]X174. 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 [null set]X174 titers at any well were always at least tenfold higher than background concentrations [ILLUSTRATION FOR FIGURE 4 OMITTED].

Breakthrough curves for bromide, MS2, and [null set]X174 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 [null set]X174 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 [ILLUSTRATION FOR FIGURE 6A OMITTED], its concentrations decreased more slowly than the corresponding bromide concentration. This same relationship was also observed at the injection well [ILLUSTRATION

## Virus occurrence and transport in a school septic system and unconfined aquifer.

FOR FIGURE 7 OMITTED]. 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 [null set]X174 are plotted against transport distance, their titers declined at the same rate, approximately  $-1 [\log.\text{sub}.10]/2.5 \text{ m}$  [ILLUSTRATION FOR FIGURE 4 OMITTED]. 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 [degrees] C: MS2 decreased less than threefold, while [null set] x 174 had no discernible loss (data not shown). MS2 and [null set] x 174 survival over time in the sealed tube suspended in the ground water (10 to 11 [degrees] 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 [ILLUSTRATION FOR FIGURE 7B OMITTED]. 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 ([ILLUSTRATION FOR FIGURE 1 OMITTED]; 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 vital 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



## Virus occurrence and transport in a school septic system and unconfined aquifer.

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 septic effluent at high concentrations. These concentrations varied in the septic tank effluent ranging between  $7 \times [10.\text{sup.}3]$  PFU/L and  $5 \times [10.\text{sup.}6]$  PFU/L. Both types of coliphage had similar time-weighted averages during our study period of 674,000 phage/L for male-specific coliphage and 466,000 phage/L for somatic coliphage (DeBorde et al. 1997).

Coliphage were also detected in the unconfined aquifer. As the effluent leaves the drainfield at this site, it rapidly percolates through the uniform, thin unsaturated zone and enters the underlying ground water. Inorganic chemistry of the ground water indicates a detectable "plume" of mixed effluent and ground water extends about 90 m downgradient from the edge of the drainfield (well #19 to well #36; [ILLUSTRATION FOR FIGURE 3 OMITTED]). As shown by our general surveys of septic waste-associated coliphage in ground water, these viral indicators tracked well with the chemical indicators of septic waste (Tables 4 and 5; [ILLUSTRATION FOR FIGURE 3 OMITTED]). The highest concentrations of coliphage were coincident with the maximum concentrations of inorganic septic waste constituents. Coliphage could be consistently found in samples of 4 L or less along 17.4 m of ground water flowpath beyond the drainfield. Coliphage were sporadically detected out to 38 m, e.g., the peak of somatic coliphage seen in well #31 (Table 5). Whether these sporadic occurrences are the end result of high transient inputs of coliphage to the septic effluent or coliphage that were mobilized by some change in water chemistry (Bales et al. 1995) is unknown. based on the concentrations found in the ground water at 17.4 m from the drain field, if larger sample volumes were taken from wells beyond this distance, we would most likely have found ever-decreasing concentrations of coliphage.

Sampling of wells #19, #40, #41, and #26 prior to the coliphage seeding experiment provided us with information as to the average change in septic waste-associated coliphage concentrations as a function of transport distance (Berg et al. 1984). Both classes of coliphage behaved similarly. At sites where coliphage were consistently found (out to 17.4 m), their concentrations were reduced at a rate of approximately  $-1 [\log.\text{sub.}10]/5$  m of transport in this ground water system. While these septic waste-associated coliphage concentrations can provide an overview of virus occurrence and distribution, they cannot be used to determine transport rates or virus survival rates.

### Coliphage Seeding

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

### Virus Transport

Even though highly adsorptive, the two seeded and cloned coliphage strains had some individuals that move through the

## Virus occurrence and transport in a school septic system and unconfined aquifer.

ground water at least as fast as conservative bromide tracer [ILLUSTRATION FOR FIGURE 6 OMITTED]. These fast-moving virus particles have not yet adsorbed to the aquifer sediment, which would have slowed their movement. This small fraction of unbound viruses may represent a portion of the source that by chance has not yet encountered the aquifer matrix, or it may represent a subset of the main population that has different surface characteristics. This fraction of fast-moving infectious viruses migrating with or ahead of the conservative tracer constitutes the highest density of virus/mL to impact downgradient wells. In a high-velocity 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 [null set]X174 coliphage can be compared with similar background coliphage data. The concentrations of MS2 decreased at twice the rate ( $-1 [\log.\text{sub}.10]/2.5 \text{ m}$ ) determined for the background male-specific coliphage ( $-1 [\log.\text{sub}.10]/5 \text{ m}$ ). 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 [null set]174 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 [ILLUSTRATION FOR FIGURE 6 OMITTED], 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

## Virus occurrence and transport in a school septic system and unconfined aquifer.

under the septic system drainfield [ILLUSTRATION FOR FIGURE 4 OMITTED], 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.

### Acknowledgments

This work was supported by grant MSU-G&C#291413 from the Montana University Systems Water Research Center, and by grant WQ1 699-520-95 from the National Water Research Institute jointly funded by the U.S. EPA.

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