In cooperation with the
Maryland Department of the Environment

Occurrence and Distribution of Enteric Viruses in Shallow Ground Water and Factors Affecting Well Vulnerability to Microbiological Contamination in Worcester and Wicomico Counties, Maryland

Water-Resources Investigations Report 01-4147

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Conversion Factors and Vertical Datum

Multiply	By	To obtain
inch (in.)	2.54	centimeter
inch per year (in/yr)	2.54	centimeter per year
million gallons per day (Mgal/d)	3,785	cubic meters per day
gallon per day (gal/d)	3.785	liter per day
gallon (gal)	3.785	liter
mile (mi)	1.609	kilometer
square mile (mi^2)	2.590	square kilometer
acre	4,047	square meter

Temperature in degrees Fahrenheit (°F) can be converted to degrees Celsius (°C) by using the following equation:

$$°C = (°F - 32) / 1.8$$

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter (µg/L).

Concentrations of microbiological constituents in water are given either in plaque-forming units (pfu) or colony-forming units (cfu) per 100–1,000 milliliters (mL).

Vertical datum: In this report, “sea level” refers to the National Geodetic Vertical Datum of 1929—a geodetic datum derived from a general adjustment of the first-order level nets of the United States and Canada, formerly called Sea Level Datum of 1929.

Occurrence and Distribution of Enteric Viruses in Shallow Ground Water and Factors Affecting Well Vulnerability to Microbiological Contamination in Worcester and Wicomico Counties, Maryland

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Abstract

The U.S. Geological Survey, in cooperation with the Maryland Department of the Environment and the Wisconsin State Laboratory of Hygiene, conducted a study to characterize the occurrence and distribution of viral contamination in small (withdrawing less than 10,000 gallons per day) public water-supply wells screened in the water-table aquifer in the Coastal Plain in Worcester and Wicomico Counties, Maryland.

Two hundred seventy-eight well sites were evaluated with regard to simulated ground-water flow paths, land use, natural soils groups, and well characteristics, such as well depth and well age. Flow and transport simulations of the water-table aquifer indicated that wells screened less than about 50 feet below land surface (shallow wells) were most vulnerable to surface contamination, which in some cases could originate from as far as 2,000 feet upgradient of the well. Animal-feeding and agricultural-storage operations were considered among the most likely sources for viral contamination; therefore, sites close to these activities were considered most vulnerable. Soil groups were evaluated with regard to depth to water and moisture-holding capacity. Wells with shallow depths to water or in very sandy soils were considered more vulnerable to contamination than deep wells (greater than 50 feet) and those completed in finer-grained soils. Older wells and wells where coliform bacteria had been detected in the past were classified as highly vulnerable. On the basis of this evaluation, 27 sites considered to be susceptible were sampled.

Samples were collected by pumping up to 400 gallons of untreated well water through an electropositive filter. Water concentrates were subjected to cell-culture assay for the detection of culturable viruses and reverse-transcription polymerase chain reaction/gene probe assays to detect nonculturable viruses; grab samples were analyzed for somatic and male-specific coliphages, *Bacteroides fragilis*, *Clostridium perfringens*, enterococci, *Escherichia coli*, total coliforms, total oxidized nitrogen, dissolved organic carbon, organic nitrogen, total phosphate, orthophosphate, acid-neutralizing capacity, pH, specific conductance, temperature, and dissolved oxygen.

Eleven percent of the samples analyzed (3 of 27) tested positive for either culturable viruses or the presence of viral ribonucleic acid. Approximately 15 percent of the samples (4 of 27) tested positive for one or more bacterial contaminants.

Introduction

In response to the 1996 Amendments to the Safe Drinking Water Act, the U.S. Environmental Protection Agency (USEPA) is developing the Ground-Water Rule (GWR) to protect users of public ground-water supplies from viral contamination (U.S. Environmental Protection Agency, 2001). Because total coliform bacteria is often used as an indicator of the presence of pathogenic contamination from microbial pathogens, many ground-water suppliers use the absence of coliform as justification for not disinfecting source water. In addition, because of the high cost and complex analytical methods involved, direct monitoring for viruses is seldom done in public-water supplies (U.S. Environmental Protection Agency, 1986).

In 1998, the U.S. Geological Survey (USGS), in cooperation with the Maryland Department of the Environment (MDE) and the Wisconsin State Laboratory of Hygiene (WSLH), began a study to characterize the occurrence and distribution of enteric viruses in small (less than 10,000 gallons per day, or gal/d) public water-supply wells in the Coastal Plain Physiographic Province in Worcester and Wicomico Counties, Maryland. Because of difficulties associated with direct monitoring for viral contamination (such as cost and turn-around time), it has not been feasible to routinely document the presence or absence of viruses in public-water supplies. Therefore, studies are needed to characterize the occurrence and distribution of viral contamination in ground water used for drinking water throughout the United States.

Background

Viruses are among the smallest of the disease-causing microorganisms found in the aquatic environment. In 1996, the USEPA amended the Safe Drinking Water Act to require that all States develop methods for assessing the vulnerability of drinking-water supplies to various contaminants including enteric viruses. More than 120 different types of potentially harmful enteric viruses are excreted in human feces, and are widely distributed in type and number in domestic sewage, agricultural wastes, and septic drainage systems (Gerba, 1988). Many of these viruses are stable in water and have long survival times with half-lives ranging from weeks to months. Because they may cause disease even when just a few virus particles are ingested, low levels of environmental contamination may affect water consumers.

From 1971 to 1979, approximately 57,974 people in the United States were affected by outbreaks of waterborne pathogens (Craun, 1986). Outbreaks of waterborne disease attributed to enteric viruses are poorly documented, even though viruses are commonplace in natural waters contaminated with human feces. Illnesses in humans caused by waterborne viruses range from severe infections such as myocarditis, hepatitis, diabetes, and paralysis to relatively mild conditions such as self-limiting gastroenteritis. It has not been possible to identify the etiologic agent or agents responsible for community illness in approximately half of the reported waterborne outbreaks because the isolation and identification of the causative agent was either unsuccessful or not attempted (Craun and McCabe, 1973; Craun, 1986; Sobsey, 1989). Additional analyses indicate that caliciviruses such as the Norwalk virus and other enteric viruses may be responsible for as much as 60 percent of the reported waterborne outbreaks of gastroenteritis since the clinical features of the cases in many of these epidemics are consistent with viral infections and bacterial pathogens were ruled out as disease agents (Keswick and Gerba, 1980; Kaplan and others, 1982; U.S. Environmental Protection Agency, 1988; Herwaldt and others, 1992). Despite the inherent difficulties associated with the identification of viruses in water, disease outbreaks have been attributed to specific episodes of viral contamination in ground water (Craun and others, 1976; Hejkal and others, 1982; Herwaldt and others, 1992; Divizia and others, 1993; Beller and others, 1997). Because approximately half of the reported outbreaks of waterborne disease in the United States from 1970 through 1990 had undefined etiologies, establishing causality between specific viral agents and illness caused by contaminated water supplies remains difficult. Nevertheless, enteric viruses such as the Norwalk and Norwalk-like viruses have been established as the major cause of viral gastroenteritis among adults worldwide (Beller and others, 1997).

The USEPA has identified numerous potential sources of viral contamination in ground water, including wastewater in commercial and industrial settings, septic systems in residential and municipal settings, and condensed animal-feeding operations in rural or agricultural areas. Currently, the Total Coliform Rule is used to screen for fecal contaminants, and is the only Federal drinking-water regulation in effect for determining the presence of microbes in public ground-water systems.

Other nonpathogenic microorganisms have also been suggested as viral indicators. Coliphages are bacterial viruses that infect the coliform bacterial group. Some coliphages are superficially similar to the enteric viruses in that they share symmetrical structures, morphologies, and sizes, with similar half-lives in natural waters. Some coliphages, particularly those that infect "male" strains of *Escherichia coli* (*E. coli*), or "male-specific" coliphages, can be found in human feces and have been identified in large numbers in human wastewater (Havelaar, 1986). Male-specific coliphages and other bacteriophages have been proposed as viral indicator microorganisms because (1) outbreaks of viral etiology have been documented in waters that met coliform criteria for drinking purposes (Kukkula and others, 1999); (2) viruses may be considerably more resilient in the environment than coliforms; and (3) the infectious dose of many viral diseases is considerably lower than that observed for enteric bacterial disease (Hejkal and others, 1982).

Other microorganisms under consideration as viral indicators include the fecal streptococci and enterococci,

certain anaerobic bacteria such as *Clostridium perfringens*, *Bacteroides fragilis* and the Bifidobacteria. Low recovery rates have been reported for some of them (*Bacteroides fragilis* and Bifidobacteria); however, they are relatively sensitive to inactivation by chlorine (Sartory, 1980) and their reported presence in water has sometimes been inconsistent (Allsop and Stickler, 1985).

Purpose and Scope

The purpose of this report is to describe microbiological occurrence in ground water in small public water-supply systems, and possible factors affecting well vulnerability to microbiological contamination in the Coastal Plain Physiographic Province in Worcester and Wicomico Counties, Maryland. This report relates the occurrence of microorganisms to recharge area, ground-water flow, site characteristics, and selected chemical constituents.

Thirty samples were collected from March 1999 through October 1999. Twenty-seven of these samples were selected based on suspected vulnerability to viral contamination. Three samples were collected as negative controls. An uncalibrated steady-state ground-water flow model was constructed to aid in understanding how hydrogeology affects small public water-supplies in the study area.

Location and Description of Study Area

Worcester and Wicomico Counties cover 985 mi^2 (square miles), and are bordered to the east by the Atlantic Ocean, to the west by Dorchester and Somerset Counties, Maryland, to the north by Sussex County, Delaware, and to the south by Accomack County, Virginia (fig. 1).

The study area is located on the southeastern Delmarva Peninsula, a coastal lowland drained by a series of short tidal streams. The area is characterized by low topographic relief with altitudes ranging from sea level to about 80 ft (feet) above sea level. The coastal areas of Worcester County are fringed by barrier beaches, tidal lagoons, and tidal wetlands. The wetlands extend inland along the banks of the major rivers, the Nanticoke, Wicomico, and Pocomoke.

The study area is mostly rural and has a total population of 131,187 (U.S. Bureau of the Census, 2001). Salisbury, Maryland, is the Wicomico County seat, and is the largest municipality in the study area, with a population of 23,743 and an additional 6,400 part-time residents attending Salisbury State University. During the summer months, the population in Ocean City, Maryland, a beach resort in Worcester County, can increase to several hundred thousand people (U.S. Bureau of the Census, 2001). All public water in the study area is provided by ground water, and all areas of the study area outside the incorporated boundaries of Salisbury and Ocean City are dependent on septic systems for sewage disposal.

The climate of the study area is temperate. Average temperatures range from about 36 °F (degrees Fahrenheit) in the winter to about 78 °F in the summer. Rain and snowfall vary seasonally and average about 45 in/yr (inches per year) (National Oceanic and Atmospheric Administration, 1977).

Hydrogeologic Setting The study area is located on the unconsolidated sediments of the Coastal Plain Physiographic Province. The sediments were deposited in a southeastwardly thickening wedge that extends from the Fall Line to the Continental Shelf. Most of this wedge is composed of Cretaceous stratified sands, silts, and clays deposited in nonmarine and marine marginal environments. Overlying the Cretaceous sediments are marine, estuarine, and fluvial clays, silts, sands, and gravels of Tertiary to Quaternary age (Owens and Denny, 1979). A locally important paleochannel system was identified west of the Wicomico River near Salisbury. Weigle (1972) describes the paleochannel system as trending southeastward from the northwestern corner of Wicomico County to just north of Salisbury. Andreasen and Smith (1997) characterize the paleochannel system between 0.6 to 1.8 mi (miles) wide, and from about 90 to about 200 ft below sea level. They also discuss the use of this ground-water resource as a major part of the city of Salisbury's municipal water supply. Where the water-table aquifer does not overlie buried paleochannels, its saturated thickness tends to vary in relation to the presence or absence of relatively impermeable materials, changes in depth to the water table, and the presence of subcropping aquifers (Bachman and Wilson, 1984).

The western and central part of the study area is underlain by the Pleistocene-age Parsonsburg Sand, the Pliocene-age Omar Formation, Walston Silt, and Beaverdam Sand (Phillips and Bachman, 1996). The Beaverdam Sand is the main water-bearing unit pumped for commercial supply, and is generally confined by the Walston Silt and the Omar Formation. The Walston Silt and the Omar Formation consist of clayey silts and poorly sorted sands. Stream channels frequently cut through the Walston Silt and Omar Formation and both are discontinuous throughout the study area (Phillips and others, 1993). The Parsonsburg Sand overlies the Walston Silt where present. In areas where the Walston Silt is absent, the Parsonsburg Sand is in direct contact with the Beaverdam Sand (Andreasen and Smith, 1997). In the easternmost part of the study area, the Beaverdam Sand is overlain by the Pleistocene-age Sinepuxent and Ironshire Formations. The Sinepuxent Formation is a silty sand, and the Ironshire Formation is a gravelly sand (Owens and Denny, 1979) (fig. 2).

Water levels in the water-table aquifer tend to be shallow, frequently less than 2 ft below the land surface. As a result, drainage ditches have been constructed in the central and southern parts of the study area. In areas where row crops are cultivated, extensive networks of ditches have been dug. These ditches tend to be less than 3 ft deep and drain to larger, deeper ditches that in turn connect to natural drainageways. Many of the natural drainages have been straightened and deepened. Further discussions of the Delmarva Peninsula's stratigraphy and hydrogeology can be found in Rasmussen and Slaughter (1955), Owens and Denny (1979), and Hansen (1981).

Land Use In a recent study of five different hydrologic systems, Francy and others (2000) found that water collected

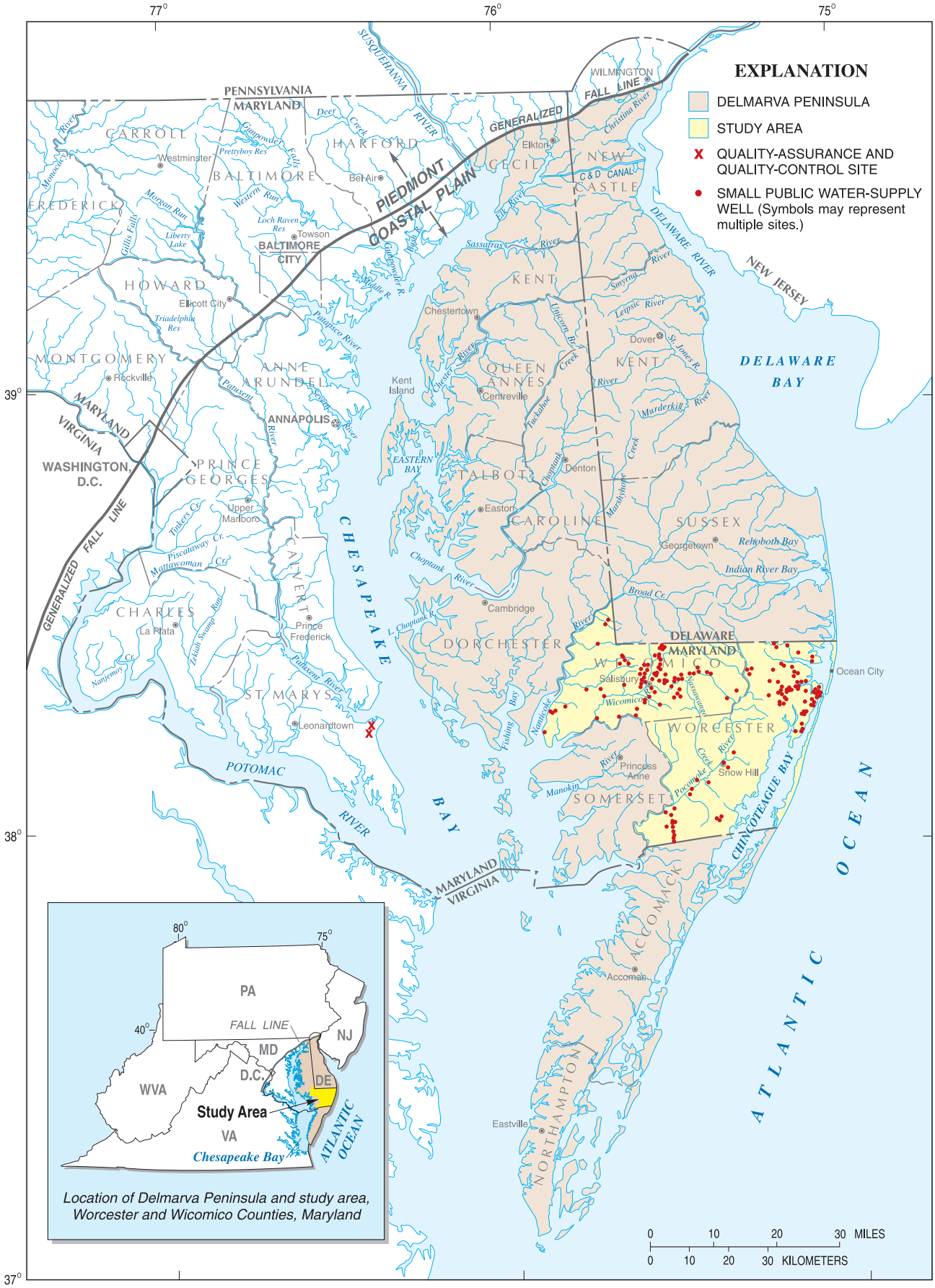


Figure 1. Location of study area, Worcester and Wicomico Counties, Maryland, and distribution of small public water-supply wells in 1998.

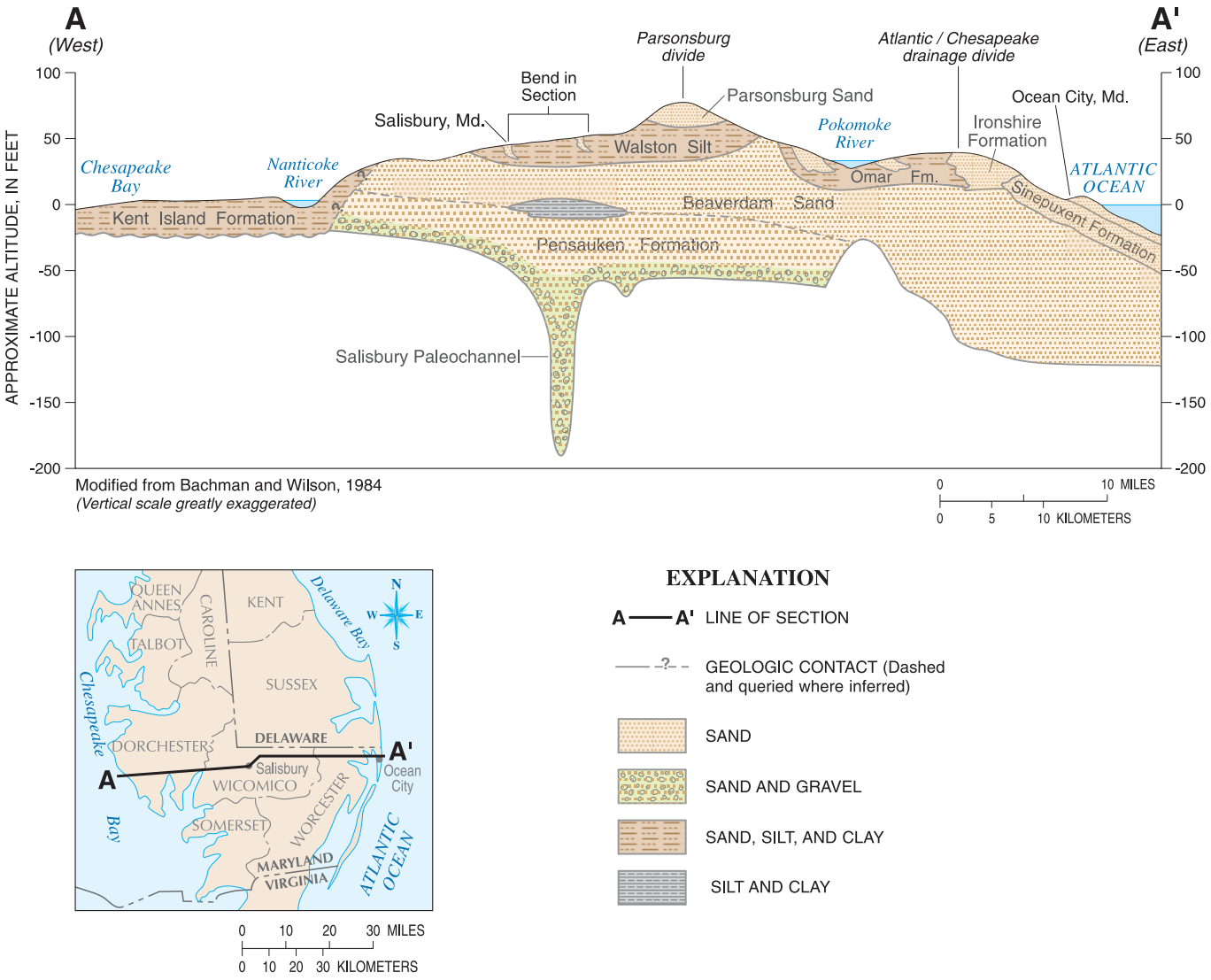


Figure 2. Generalized geologic cross section from Chesapeake Bay through Salisbury to Ocean City, Maryland.

from wells associated with agricultural and commercial land uses had higher (and nearly statistically significant) total coliform concentrations than samples collected from wells in other land-use categories ($\alpha = 0.05$, $p = 0.07$). Land use in Worcester and Wicomico Counties was characterized based on MDE 1:63,360-scale maps (Maryland Department of the Environment, 1994). The predominant land uses are forest and agriculture. Nearly 40 percent of the study area is covered by deciduous, evergreen, and mixed forests. In both counties, poultry production provides the majority of agricultural revenue. Poultry farms account for about 1 percent of the land use and are scattered throughout the study area; however, the manure generated by these operations is used for fertilizer throughout the area. Wicomico County alone produced over 76 million broiler chickens in 1997, making it the number one producer of poultry in the State and the tenth largest in the Nation (U.S. Department of Agriculture, 1999). Twenty-six percent of the study area is used as cropland (primarily for corn and soybeans to support the poultry industry). In 1994, 3.5 percent of the study area was classified as residential. Most of the residential development is centered in Worcester County near Ocean City, Maryland. Residential land use has increased in this area because of the rising value of vacation property. In addition, an increasing number of people have chosen to live year round near the Atlantic Coast.

Ground-Water Use Shallow ground water in Worcester and Wicomico Counties is the major water-supply source for industry, agriculture, and domestic self-supplied drinking water (Hamilton and others, 1991). Approximately 23 Mgal/d (million gallons per day) of ground water is pumped from the shallow water-table aquifer in Wicomico and Worcester Counties (Judith Wheeler, U.S. Geological Survey, oral commun., 2000). The majority of shallow ground water used in the two-county area is for public-water

supply (11 Mgal/d) and domestic self-supply (4 Mgal/d), while the remaining 8 Mgal/d is used for agriculture and irrigation. Depths to the water table generally are shallow, ranging from 0 to 12 ft, but depths can be up to 30 ft in well-drained areas (Hamilton and others, 1993). Although there has been no direct correlation in the study area between the consumption of fecally contaminated water from a public water-supply system and waterborne illnesses, the relatively shallow depth to the water table and the common use of septic systems as a means of sewage disposal, increase the potential for enteric viruses and other pathogens to be transported to the water table.

Acknowledgments

The authors would like to thank John Grace and Lyn Poorman of the Maryland Department of the Environment for logistical support in this study. Travis Sterner and Andrea Korsak, also of the Maryland Department of the Environment, assisted in providing information on water system owners and operators. Funding for this study was provided through a cooperative effort between the Maryland Department of the Environment and the U.S. Geological Survey.

Study Design and Methods

Viruses are not capable of reproduction outside of a suitable host or host cell; therefore, the occurrence of viruses in a water supply is directly related to the supply's proximity to fecal contamination. Various studies have indicated that pathogen occurrence in ground-water supplies and pathogen transport in porous media may be affected by hydrogeology, soil type, and well-construction characteristics (Bales and others, 1995; DeBorde and others, 1998; Abbaszadegan and others, 1998; Gerba, 1999). Numerous studies on the traveltimes and inactivation rates of viruses in ground water have been conducted, and the results do not compare well. In experiments with live attenuated viruses, Noonan and McNabb (1979) documented that viruses could travel more than 2,953 ft, whereas Vaughn and Landry (1977) showed a maximum travel distance of 150 ft. Wellings and others (1975) suggested that virus survival time in ground water may be as little as 28 days, whereas Gerba and others (1975) suggested that at very low temperatures (below 4 °C), viruses can survive in ground water for months to years. In laboratory studies, Gerba (1999) related virus survival to soil moisture and depth to the water table stating that although many viruses are resistant to inactivation by desiccation, viruses that must pass through an unsaturated soil zone may be permanently adsorbed to soil particles, thus rendering them either inactive or unavailable for transport.

In this study, a geographic information system (GIS) was used to cross-reference areal and point-source data that were either considered to be surrogates for potential sources of fecal contamination, or factors that could affect the susceptibility of a small (less than 10,000 gal/d) public-water supply well in the study area to viral or microbiological contamination.

Land-use data (Maryland Department of the Environment, 1994) and the historical presence or absence of coliform bacteria in the public water-supply system were used as surrogates for potential sources of enteric viruses. Data on well depth, well age, and soil type (Daft-McCune-Walker, Inc., 1990) were used to evaluate a small public water-supply well's susceptibility to contamination. To better understand ground-water flow between potential microbiological sources of contamination and extraction wells, a steady-state ground-water flow model of a hypothetical part of the study area was constructed using MODFLOW, the USGS finite-difference ground-water flow model (McDonald and Harbaugh, 1988). Particle tracking using MODPATH (Pollock, 1994) was used to determine recharge area and to estimate the minimum depth necessary for a public water-supply system well to avoid intercepting potentially contaminated water from the water-table aquifer. Areal and point-source data for each site were then analyzed and combined to create an overall rank-score for each site. The overall rank-score reflected a site's likelihood of vulnerability to viral or microbiological contamination.

Target Population

A public ground-water supply system in the State of Maryland includes any system that provides piped water for human consumption and has at least 15 service connections, or regularly serves at least 25 individuals daily at least 60 days out of the year. Small public water supplies in Worcester and Wicomico Counties can be classified this way and provide less than 10,000 gal/d for public use, draw water from shallow wells (typically less than 300 ft), and are located in rural and low-density suburban areas. Currently (2001), many small public water supplies do not have the regulatory need or the financial resources necessary to disinfect their finished water. As a result, they are among the most susceptible to contamination from viral pathogens.

Small public supplies are divided into two categories, community water systems and non-community water systems. Community systems serve at least 15 connections used by year-round residents, or serve at least 25 residents throughout the year. Examples of community systems include mobile-home parks and small apartment buildings. Non-community systems are further divided into transient and non-transient systems. A non-transient, non-community system serves at least 25 of the same people for more than 6 months per year. Schools and day-care facilities are typical examples of non-transient, non-community systems. A transient non-community system serves less than 25 of the same people for more than 6 months per year. Examples include offices, churches, and markets (State of Maryland [n.d.]).

Ground-Water Flow

A steady-state ground-water-flow model of a hypothetical part of the study area was developed using MODFLOW, a three-dimensional finite-difference flow

model (McDonald and Harbaugh, 1988) to understand how hydrogeology affects small public water supplies in the study area. The flow model simulated flow paths in the shallow water-table aquifer for a range of hydrogeologic conditions and heterogeneities present in the study area. Simulated recharge areas and traveltimes, combined with land use, helped determine the likelihood of contaminated recharge reaching a typically constructed public-supply well. In conjunction with the flow model, advective particle transport was simulated using MODPATH (Pollock, 1994), the USGS particle tracker, to help visualize flow paths areally and vertically. A particle in this context is "an infinitely small imaginary particle" (Anderson and Woessner, 1992) that is tracked using the velocity vectors from the flow model. Particle flow-path results were then used to determine generic well-recharge areas and traveltimes for hydrophilic, advectively transported contaminants. Biological contaminants are not necessarily hydrophilic, or advectively transported, so the results from the model are a conservative estimate of contaminant movement.

The model simulates ground-water flow over 556 acres and has 100,000 active cells (100 rows by 100 columns by 10 layers). Each cell is 49 ft on a side (fig. 3). The model incorporates major surface-water and geologic features typically found throughout the study area. The stratigraphy and thickness of modeled units are based on studies by Bachman and Wilson (1984), and Shedlock and others (1993). Model input parameters for horizontal and vertical hydraulic conductivity, and recharge were derived from a review of the pertinent literature (table 1).

A vertical representation of the subsurface extends from 66 ft above sea level to 39 ft below sea level. The upper 20 ft are divided equally into two layers designated as the water-table aquifer, and represent the Parsonsburg Sand. The Walston Silt and the Omar Formation, where present, are represented as layers 3 and 4, and represent a semi-confining unit between 0 and 20 ft thick. The bottom 60 ft are designated as the semiconfined aquifer and represent the Beaverdam Sand. The semiconfined aquifer is divided into six layers of variable thickness. The semi-confining unit between the water-table aquifer and the semiconfined aquifer is not continuous throughout the study area. This discontinuity is represented in two locations where the semi-confining unit is absent and the water-table aquifer is in direct contact with the upper semiconfined aquifer. These areas are irregularly shaped and are simulated only in layers 3 and 4. Their locations and approximate sizes and shapes are projected onto layer 1 and are shown in figure 3.

The southern and northern model boundaries are no-flow boundaries—areas where there is no flow into or out of the model. These boundaries are coincidental with ground-water flow that is parallel to the boundary. The western boundary is designated as a constant-head boundary. Layer 1 of the eastern boundary simulates a regional, north-south trending ground-water sink and is modeled using river cells that simulate the effect between a surface-water feature and the ground-water system. The remaining layers of the eastern boundary are simulated as no-flow boundaries. Water movement between the river and the aquifer is proportional to the product of the hydraulic conductivity between the streambed and the aquifer, and the head difference between the stream level and the streambed. The model encompasses a sufficiently large domain so that the model boundaries do not affect simulated flow near the center of the model domain. Initial heads for the model were set 3.3 ft below land surface throughout the model domain.

Bachman and Wilson (1984) suggested that shallow ground-water-flow systems (less than 100 ft) on the Delmarva Peninsula commonly extend for less than 1 mi, and that the water table is generally a subdued reflection of the topography with relative ground-water elevation maximums located on topographic highs and ground-water elevation minimums located in valleys. An analysis of topographic data from USGS 7.5-minute quadrangle maps of the study area (U.S. Geological Survey, 1942, 1967, 1982, and 1992) indicated topographic gradients between 1 and 6 percent. A slope of less than 1 percent was most representative of the study area; therefore, the land surface of the model has a west-to-east slope of 0.67 percent. Drainage ditches, which are common in the study area, were represented by drain cells arranged in a dendritic pattern so that a single primary ditch and multiple secondary ditches drain toward the eastern river boundary. Drain cells remove water from the water-table aquifer at a rate proportional to the difference between the head in the aquifer and the elevation of the bottom of the drain. The elevations of the bottom of the ditches are between 17.5 and 7.3 ft above sea level. The ditches extend below land surface from 0.75 to 3 ft.

A series of 20 model scenarios was performed, each with a single pumping well at one of five different locations labeled A through E on figure 3. Well placement was designed to determine whether a simulated conservative pathogen originating at the water table could reach a public water-supply well by flow where the confining unit is absent; by flow through the confining unit as a result of pumping; or by induced flow from either the river or the drainage ditches. Four scenarios were investigated for each of the five well locations (the four possible combinations of two depths of the well screens, and two pumping rates). For the shallow scenario, the well was screened in layer 2; for the deep scenario, the well was screened in layer 6. Simulated wells had a single screened interval between 5 and 10 ft—typical of public water-supply wells in the study area. Each depth scenario was tested at two pumping rates, 1,000 gal/d to reflect typical use in the study area, and 10,000 gal/d to reflect extreme use in the study area.

It is apparent from the discussion presented earlier that values for virus survival times (in ground water) vary substantially. For this reason, a conservative, high-end estimate of 3 years was used during analysis of particle-transport data. The reverse particle-tracking capabilities of MODPATH were used graphically to illustrate how far and in what direction potentially contaminated ground water

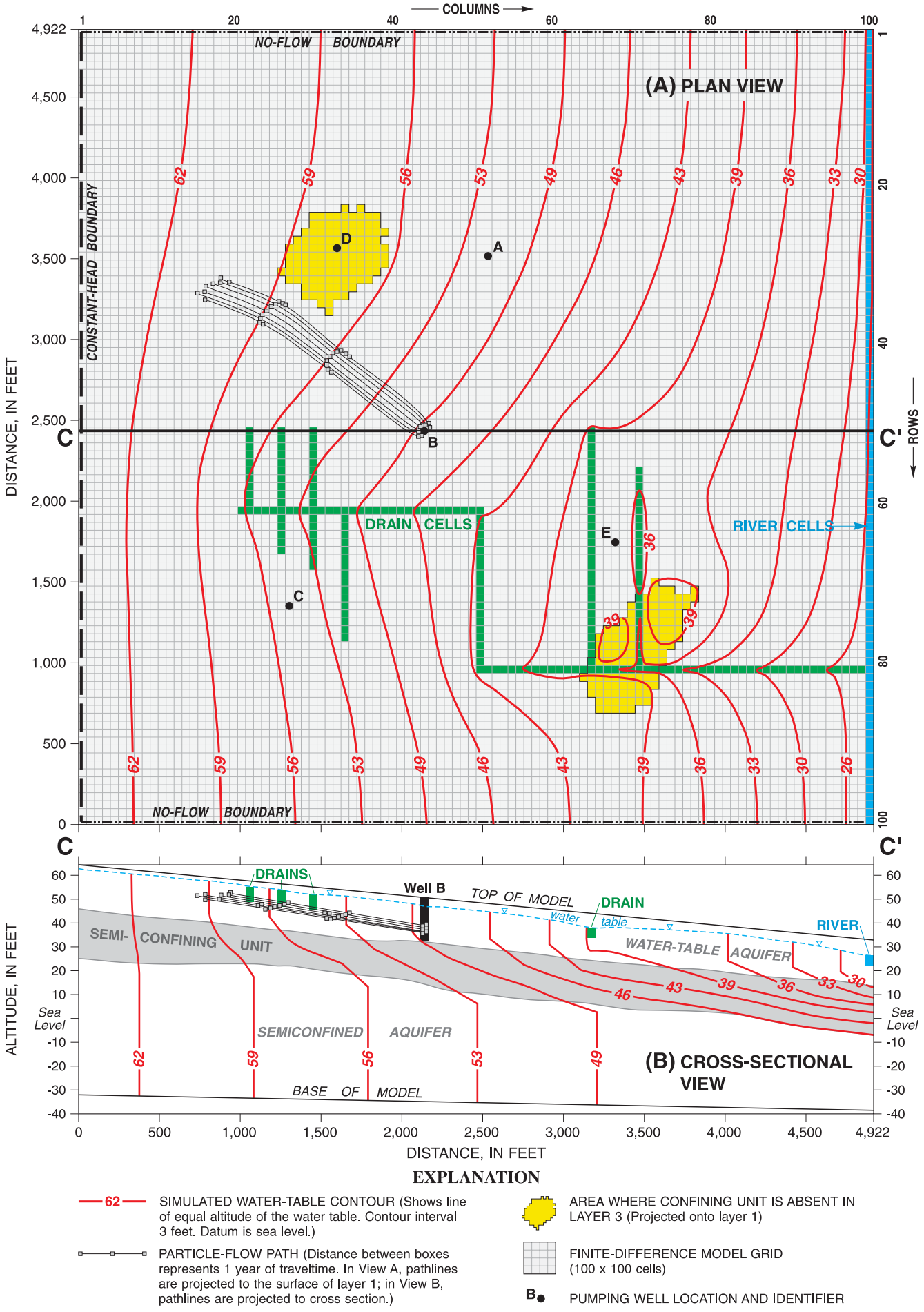


Figure 3. Finite-difference grid for the ground-water-flow model of a hypothetical part of the study area, and 3-year backwards particle-flow path from well B in **(A)** plan view and **(B)** cross-sectional view.

Table 1. *Values for horizontal and vertical hydraulic conductivity, layer thickness, and recharge for input to the ground-water-flow model*

[ft/d, feet per day; in/yr, inches per year; ft, feet; N/A, not applicable; A, Freeze and Cherry, 1979; B, Cushing and others, 1973; C, Fetter, 1994; D, Andreasen and Smith, 1997; E, Shedlock and others, 1993; F, Phillips and others, 1993; G, Owens and Denny, 1979; H, Rasmussen and Slaughter, 1955]

Model layer	Hydrologic unit	Horizontal hydraulic conductivity (ft/d)	Vertical hydraulic conductivity (ft/d)	Hydraulic conductivity reference (s)	Total thickness (ft)	Total thickness reference	Recharge (in/yr)
1	SURFICIAL AQUIFER	50	5	A, B	20	E	18.02 H
2		50	5	A, B			N/A
3	CONFINING UNIT	0.1	0.001	C	0– 20	F	N/A
4		0.1	0.001	C			N/A
5	CONFINED AQUIFER	100	10	A, D	30–50	G	N/A
6		100	10	A, D			N/A
7		100	10	A, D			N/A
8		100	10	A, D			N/A
9		100	10	A, D			N/A
10		100	10	A, D			N/A

could travel in 3 years. Particles were arranged around the center of the well screen for each of the 20 scenarios. Particle tracks were projected backward in time to the point where they intersected the water table. Very few particles (less than 5 percent) intersected the water table within 3 years. In other words, it would take longer than 3 years for approximately 95 percent of the particles to move from the top of the water table to the well. The maximum distance a particle traveled in any scenario for the 3-year period was 1,969 ft. Particle tracks for all scenarios indicate that almost all water extracted by the public water-supply well is recharged upgradient of the well. A plan view and a cross-sectional view of a 3-year particle track for a well pumped at 1,000 gal/d are shown in figure 3a and 3b, respectively. The well is screened in layer 2 and is typical of other modeled scenarios. An example of the following conditions is shown in figure 4—the well is screened in the semiconfined aquifer in an area where the semi-confining unit is absent, and pumps 10,000 gal/d. In this scenario, although the water-supply well is screened in the semiconfined aquifer, it could intercept potentially contaminated water from the water-table aquifer. From this hypothetical analysis, wells screened at depths less than 49 ft below land surface where the confining unit was absent were most vulnerable to contamination (location D, fig. 3). Comparison of particle tracks for wells pumping 1,000 and 10,000 gal/d indicates that there is little difference in particle pathways or travel-times between the simulations of the two pumping rates.

The usefulness of the model output is dependent on how well the ground-water flow model represents the flow system in the study area, and on the appropriateness of the application of hydrologic and geologic parameters as stated in the literature. It is important to note that because the ground-water flow model is based on a hypothetical part of the study area, it is not calibrated and is used only as a conceptual tool to aid in understanding the flow system. A site-by-site application of the model was not performed. Rather, the generic well-recharge zone based on scenarios described above was applied to each public water-supply well.

Site Selection

Sites were selected from the 278 small public water-supply systems that were active in Worcester and Wicomico Counties in the winter of 1998 (fig. 1). The majority of these systems are located along major thoroughfares or clustered around population centers (Salisbury and Ocean City, Maryland). Site selection was based on a non-weighted ranking system. Quantitative ranks were assigned based on suspected vulnerability. A rank of 0 was assigned to a criterion if there was a greater potential for microbial contamination. A rank of 1 was assigned if the criterion was determined to be less vulnerable. The sum of the ranks for each site was divided by the total number of ranked criteria; thus, no site was penalized for an absence of data. Sites with the lowest total scores were considered most vulnerable to microbial contamination. Ranking criteria included areal data such as land use and soil type. Site-specific data such as total well depth, age of the well, and the historical presence or absence of fecal coliform bacteria also were used to

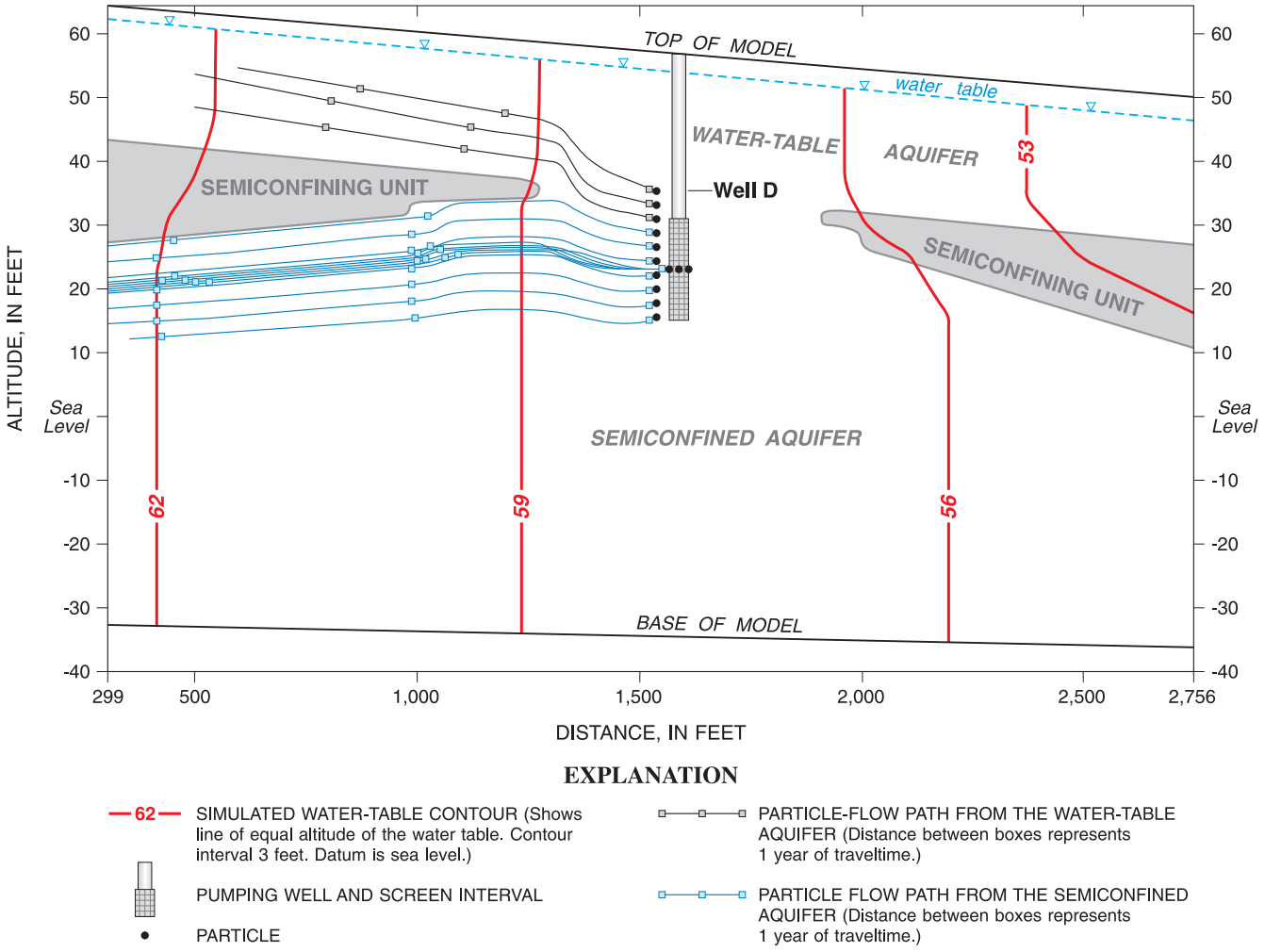


Figure 4. Cross-sectional view of 3-year backwards particle-flow path from well D, screened in the semiconfined aquifer.

assess vulnerability. Sites were ranked from most to least vulnerable. Sites that tied were randomly ordered.

A GIS was used to plot land-use and soils data for each public water-supply well inside a 1,969-ft-diameter circular buffer. The USEPA has published a list of potential sources of viral contamination (U.S. Environmental Protection Agency, 1999) that can be related to land use. Land-use data (Maryland Department of the Environment, 1994) were ranked in order of their likelihood as pathogen sources. Potential sources from agricultural activity included animal-feeding operations and agricultural-storage facilities. Potential sources from non-agricultural land uses included residential land use between 0.2 and 2 dwellings per acre, schools, churches, industrial and manufacturing facilities, golf courses, and parks.

The physical nature of a soil can be important in determining whether or not viruses applied at or near the surface can ultimately be transported to the water table. Thus, relatively permeable soils with a consistently high or fluctuating water table may promote transport of viruses and prevent viral inactivation. Worcester and Wicomico County soils data were ranked for vulnerability based on depth to water and moisture-holding capacity (Daft-McCune-Walker, Inc., 1990). Well-drained soils (natural soils groups B1 and E1) were ranked as having a greater potential to pass viruses to the water table and assigned a rank of 0. All other natural soils groupings in the study area were assessed as being less vulnerable and assigned a rank of 1.

Well permits, where available, provided data on the depth of each well and the year of construction. Based on a particle-tracking analysis from MODPATH (Pollock, 1994), total well depths less than 49 ft were ranked as vulnerable to contamination. In a nationwide study of public water-supply system wells, Abbaszadegan and others (1998) suggested that faulty or missing surface seals could provide a means of well contamination. In 1960, construction techniques for public water-supply wells in the State of Maryland changed, and concrete or bentonite clay surface seals were required

(John Grace, Maryland Department of the Environment, oral commun., 2000). Wells with construction dates prior to 1960 were ranked as vulnerable and assigned a value of 0.

One hundred of the 278 sites had historical or current data for the presence or absence of fecal coliform bacteria. These data, when present, were ranked so that if a public water-supply system had ever had a positive test for fecal coliform bacteria, the site was ranked as vulnerable and given a value of 0. Sites that had been tested and found free of coliform bacteria were assigned a rank of 1. Sites without bacterial data were not ranked on this criterion.

Site canvassing began in February 1999. The owner or operator of each public water-supply system was contacted and given a general description of the purpose of the study and asked to voluntarily participate. Initial response rates were low. Rates of participation declined with the onset of a hydrologic drought that affected the study area in the summer and fall of 1999. Eleven of the samples collected were from sites that ranked in the top 12 percent of the most vulnerable sites. Thirteen other samples were from sites that ranked in the next 35 percent of the most vulnerable sites. Three of the samples were collected from sites that ranked among the 5 percent considered to be least vulnerable sites. The 27 sampled sites are shown in figure 5.

Sample Collection

Thirty samples were collected from March 1999 through October 1999. Twenty-seven of these sampling sites were selected based on their vulnerability rank. Two samples were collected from deep (575 to 600 ft below sea level) Coastal Plain wells outside the study area to be used as negative controls. One double-filtered sample was also collected as a negative control. All samples were analyzed for a suite of enteric pathogens, nutrients, and field parameters (table 2). Samples for microbiological analyses were submitted to the WSLH in Madison, Wisconsin. Nutrient samples were analyzed at the USGS National Water-Quality Laboratory (NWQL) in Denver, Colorado. Specific conductance, acid-neutralizing capacity, dissolved oxygen, and pH were measured in the field by employees of the Baltimore, Maryland, office of the USGS using methods from Wilde and Radtke (1998).

Samples for microbiological analysis were collected based on protocols established under the Information Collection Rule (U.S. Environmental Protection Agency, 1996). All equipment and sampling containers used to collect microbiological samples were sanitized and sealed at WSLH. Prior to sampling, public water-supply system wells were purged of standing water. Purging was done in accordance with protocols for pH, water temperature, and specific conductance established for the USGS National Water-Quality Assessment Program and described in Koterba and others (1995). USGS personnel collected samples by passing 400 gal (gallons) through a Virosorb 1 MDS [a] positively charged cartridge filter. Samples were collected from the hose connection closest to the well head. Systems using chlorination, filtration, or water-softening equipment were evaluated and sampled before treatment. Sites with treatment systems that could not be bypassed were not sampled. Samples with a pH of greater than 8.0 were neutralized by continuously injecting a 1.0 Normal solution of hydrochloric acid to the sample stream through a one-way vacuum valve prior to filtration. Samples were shipped on ice by overnight courier to NWQL in Denver, Colorado, and WSLH in Madison, Wisconsin. Sample holding times were within USEPA standards for routine monitoring of ground water (U.S. Environmental Protection Agency, 1996). Sample holding times did not exceed 24 hours.

Sample Analysis Viral analysis was performed at WSLH by cell culture and a modification to the reverse-transcriptase, polymerase chain reaction (RT-PCR) method developed by Abbaszadegan and others (1999). Viruses were eluted from the 1 MDS filter using beef extract / glycine solution. The extract was concentrated and eluted again with sodium phosphate. Gene probe assays were conducted by subjecting a portion of the water concentrate (650 microliters, or μL) to guanidinium isothiocyanate-phenol /chloroform extraction under acid conditions to extract viral ribonucleic acid (RNA). Samples were then purified through molecular exclusion drip columns composed of sephadex G-100 and RNA was ethanol-precipitated in a vacuum evaporator. Viral RNA was then reverse-transcribed and subjected to PCR in a thermal cycler [b] according to a thermal profile specific to each virus group. For hepatitis A viruses, enteroviruses and rotaviruses, the thermal profile included a 4-minute pre-incubation at 95 °C (degrees Celsius) to denature viral RNA, followed by 35 cycles of denaturation (75 seconds), annealing (75 seconds), and polymerization (75 seconds) at 95 °C, 55 °C, and 72 °C, respectively. For the caliciviruses, following the initial 95 °C denaturation step, the thermal profile included 40 cycles of 94 °C (75 seconds), 50 °C (75 seconds), and 60 °C (120 seconds). Following PCR, all virus samples were incubated at 72 °C for 10 minutes to extend incompletely polymerized deoxyribonucleic acid (DNA) strands. Following nucleic acid amplification, samples were subjected to agarose gel electrophoresis to identify presumptive viral-positive samples. DNA was then transferred to nylon membranes under vacuum [c], membranes were crosslinked by ultraviolet irradiation and were then probed using 3′-digoxigenin end-labeled oligoprimers specific to each virus group to confirm identity. Only the samples that were confirmed by oligo-probing were considered positive for viral nucleic acid.

a. Cuno Corporation, Meriden, Connecticut.
b. Stratagene, LaJolla, California.
c. Bio-Rad Corporation, Hercules, California.

Figure 5. Location of sampled public water-supply wells in Worcester and Wicomico Counties, Maryland.

Table 2. *Microbiological, nutrient, and field constituents sampled in the study area*

[WSLH, Wisconsin State Laboratory of Hygiene; USEPA, U.S. Environmental Protection Agency; USGS, U.S. Geological Survey; NWQL, National Water-Quality Laboratory; mL, milliliter; mg/L, milligrams per liter; °C, degrees Celsius; do, ditto; MPN, most probable number]

Constituent	Method	Units	Analytical Laboratory	Reference
Enteric virus	Cell-culture	Presence / absence	WSLH, Madison, Wi.	USEPA, 1996
Enteric virus	Reverse-transcriptase polymerase chain reaction (RT-PCR)	Electrophoresis (presumptive) and Membrane Hybridization (confirmatory)	WSLH, Madison Wi.	Abbaszadegan and others, 1999
Somatic coliphage	quantitative, 1 MDS filter/elution and enrichment	plaque-forming units per 500 mL	WSLH, Madison, Wi.	Sinton and others, 1996
Male-specific coliphage	quantitative, 1 MDS filter/elution and enrichment	plaque-forming units per 500 mL	WSLH, Madison, Wi.	do.
Bacteroides fragilis phages	quantitative, 1 MDS filter/elution and enrichment	plaque-forming units per 500 mL	WSLH, Madison, Wi.	USEPA, 1996
Total coliforms	Colilert	colonies per 100 mL	WSLH, Madison, Wi.	do.
Clostridium perfringens	quantitative membrane filtration	colonies per 200 mL	WSLH, Madison, Wi.	Bisson and others, 1979
Enterococci	Enterolert	MPN / 100 mL	WSLH, Madison, Wi.	USEPA, 1996
Escherichia coli	Colilert	colonies per 100 mL	WSLH, Madison, Wi.	do.
Total organic carbon	infrared analyzer	mg/L	USGS, NWQL, Denver, Co.	Wershaw and others, 1987
Nitrogen (nitrite plus nitrate, ammonium and organic nitrogen)	colorimetry	mg/L	USGS, NWQL, Denver, Co.	Fishman and Friedman, 1989
Phosphorous (total ortho)	colorimetry	mg/L	USGS, NWQL, Denver Co.	do.
pH	electrode	pH units	USGS, Field	Wilde and Radtke, 1998
Specific conductance	contact-type electrode	microsiemens per centimeter at 25 °C	USGS, Field	do.
Acid-neutralizing capacity	titration	mg/L as bicarbonate	USGS, Field	do.
Temperature	thermistor	°C	USGS, Field	do.
Dissolved oxygen	idometric	mg/L	USGS, Field	do.

A portion of the water concentrate was also reserved for inoculation into cell cultures in order to determine if culturable viruses were present. An inoculum equivalent to 100 liters of the original sample was divided into 10 aliquots and introduced onto confluent monolayers of Buffalo green monkey kidney (BGMK) cells in 25-cm^2 (square centimeter) tissue culture flasks according to the Information Collection Rule (ICR) method (U.S. Environmental Protection Agency, 1996). Following a 60-minute adsorption period, flasks were supplemented with maintenance medium containing 2 percent fetal calf serum [d] and flasks were incubated for 2 weeks at 37 °C. Flasks were examined on days 1, 2, 3, 7, and 14 following infection to identify whether viral cytopathic effects (CPE) were evident. Samples negative for viral CPE were reinoculated onto a second series of flasks that were examined using the same method for 2 weeks. Samples confirmed as positive for culturable viruses were serotyped to identify viral species.

Samples for total coliforms, *E. coli*, and enterococci were collected in two 100-mL (milliliter) sterile containers prior to the installation of the virus filtration apparatus at the sampling sites. The Enterolert Quanti-tray system was used to enumerate enterococci. *E. coli* and total coliforms were analyzed using the Colilert Quanti-tray system. Both kits are commonly used and are available from Idexx Laboratories in Westbrook, Maine. Analysis for *Clostridium perfringens (C. perfringens)* was performed according to methods previously described (U.S. Environmental Protection Agency, 1996). These methods require that a 200-mL sample be anaerobically incubated after membrane-filtration onto mCP medium.

Samples were analyzed for male-specific and somatic coliphage using the single-agar layer (SAL) direct-plating method (Sinton and others, 1996). This method requires that a 100-mL sample be inoculated with a mixture of yeast-extract agar, antibiotics, and an *E. coli* host culture. *Bacteroides fragilis (B. fragilis)*, a class of bacteriophage that infect anaerobic bacteria, were extracted from a 100-mL sample and plated under anaerobic conditions using a *B. fragilis* HSP40 host cell. Inoculated plates were incubated for 24 hours. Coliphage viruses on an incubation plate are identified by counting plaques. Plaques are areas where infected bacteria have died, forming a clearing in a lawn of bacterial growth.

Quality Control Quality-assurance and quality-control (QA/QC) samples were collected to ensure that equipment cleaning and sterilization techniques were adequate, and to assess the possible field contamination of samples. Two samples that served as negative controls were collected from public water-supply wells with properties not vulnerable to contamination and located outside of the study area. Negative controls are used to identify the reliability of contamination at or near a censoring level, and to ensure that sampling equipment is sterilized and that no contamination of the sample occurs in the field. Ground water from the study area was not used as a negative control because no single source could be guaranteed to be free of pathogens. Additionally, it was considered impractical to sterilize a sufficient volume of source water to create a secure supply of microbial-free water. Therefore, wells that were deep (greater than 500 feet) and distant from the study area were used as a probable virus-free source of negative control water. The two wells selected were located in St. Marys County, Maryland (fig. 1), and were a part of a network of wells being monitored in an ongoing water-resource study (Klohe and Feehley, 2001). The two wells sampled were finished 575 ft and 600 ft below sea level. Neither negative-control sample contained detectable microbiological material.

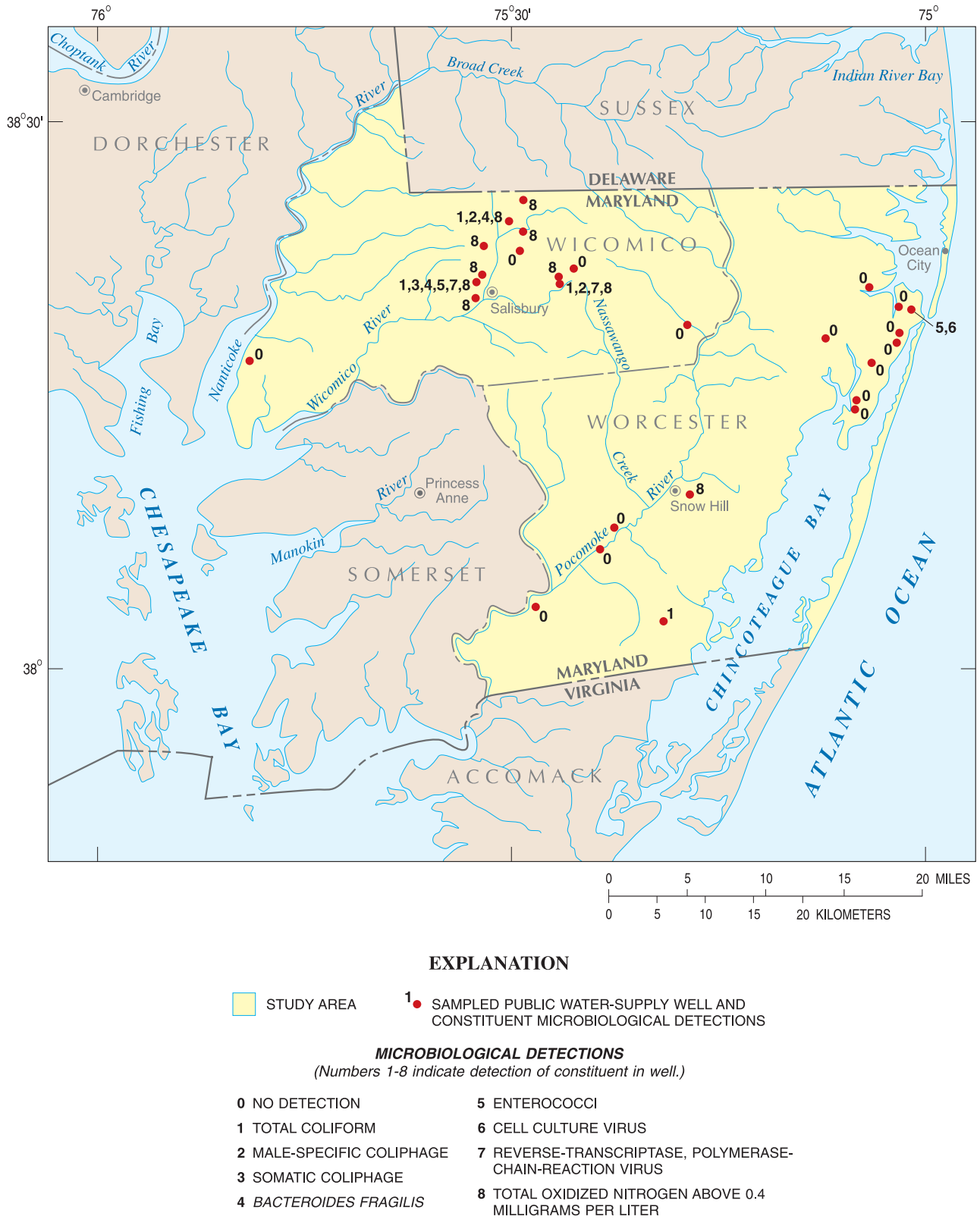
Field spikes provide information on sampling and analytical bias. Because the use of live attenuated poliovirus near a public water supply was considered an unacceptable health risk, field spikes were not collected for this study.

Occurrence and Distribution of Viral Contamination

Results from enteric virus, coliphage, fecal-indicator bacteria, and nutrient contamination are shown in figure 6. All other data, including microbiological data, are presented in Appendix A. Enteric viruses, as analyzed by cell-culture or RT-PCR (or both) were found in 11 percent of the samples. In 15 percent of the environmental samples, fecal-indicator bacteria (total coliforms, enterococci, or both) were detected, and in 37 percent of the samples, total oxidized nitrogen (nitrite plus nitrate as nitrogen in mg/L, or milligrams per liter) was greater than 0.4 mg/L. Hamilton and others (1993), in a survey of 296 wells completed in the water-table aquifer of the Delmarva Peninsula, suggested this value as indicative of anthropogenic effects. Nitrate was chosen as an indicator of human activity because it is introduced to the ground-water system in both agricultural and residential areas. Two of these samples had nitrate levels that were above the USEPA maximum contaminant level (MCL) for drinking water of 10 mg/L.

There were no detections of *C. perfringens* or *E. coli* at any of the sites; however, four sites had total coliform values between 9 and 2,400 cfu/100 mL (colony-forming units per 100 mL). Two sites tested positive for enterococci. There were two positive cultures for male-specific coliphage, with 3 and 6 pfu/500 mL (plaque-forming units per 500 mL). Somatic coliphage was detected in one sample at 53 pfu/500 mL. The anaerobic bacteriophage, *B. fragilis,* was detected in two samples at concentrations of 2 and 7 pfu/500 mL. One sample produced a positive virus cell culture that was later identified as rotavirus. In addition,

[d.] Hyclone Laboratories, Logan, Utah.



EXPLANATION

STUDY AREA

1 SAMPLED PUBLIC WATER-SUPPLY WELL AND CONSTITUENT MICROBIOLOGICAL DETECTIONS

MICROBIOLOGICAL DETECTIONS

(Numbers 1-8 indicate detection of constituent in well.)

0 NO DETECTION
1 TOTAL COLIFORM
2 MALE-SPECIFIC COLIPHAGE
3 SOMATIC COLIPHAGE
4 *BACTEROIDES FRAGILIS*
5 ENTEROCOCCI
6 CELL CULTURE VIRUS
7 REVERSE-TRANSCRIPTASE, POLYMERASE-CHAIN-REACTION VIRUS
8 TOTAL OXIDIZED NITROGEN ABOVE 0.4 MILLIGRAMS PER LITER

Figure 6. Location of microbiological contamination and total oxidized nitrogen detection above 0.4 milligrams per liter in sampled public water-supply wells in Worcester and Wicomico Counties, Maryland.

two other samples were identified by use of RT-PCR as containing viral RNA, and were later serotyped as rotavirus.

For statistical analysis, bacteria, viral, and coliphage results were recoded to a nominal scale of presence or absence. Any sample showing positive results for total coliforms or enterococci was identified as having a bacterial positive. A similar procedure was used for coliphage positives and virus positives. These nominal data were compared among variables such as overall vulnerability-rank score, components of the vulnerability-rank score (land use, soil type, well depth, well age, and historical coliform contamination data), and all nutrient and field data using a rank-transformed analysis-of-variance (ANOVA). A general linear model (GLM) procedure was used for an unbalanced test design with continuous explanatory variables (SAS Institute, 1994). Kruskal-Wallis contingency tables for ordinal response variables were used to compare the categorical components of the overall vulnerability-rank score with nominal pathogen data. Null hypotheses, that the distribution of data in each response category are the same, were rejected at the 95-percent confidence level ($\alpha = 0.05$).

A GLM analysis found that there were no significant relations between the presence of viruses and coliphage and the overall vulnerability-rank score. The GLM did indicate a significant difference between the mean overall vulnerability-rank score for a public-supply well as a function of bacterial contamination ($p = 0.045$). No significant relations were found among bacterial, coliphage, and virus contamination and land use, soil type, well depth, well age, and historical coliform contamination.

Nutrient and field data for each sample were compared to nominal pathogen data. Although not statistically significant, because of the limited number of positives for coliphage, mean total oxidized nitrogen concentrations were higher in samples contaminated with either male-specific, somatic, or *B. fragilis* coliphage than in samples not contaminated with coliphage (8.1 mg/L and 2.19 mg/L nitrite plus nitrate as nitrogen, respectively). Similar results were noted for dissolved oxygen in the presence of coliphage contamination (8.4 mg/L and 2.3 mg/L dissolved oxygen, respectively). No other correlations were found among nominal microbiological groups and nutrient or field data.

Three sites sampled in 1999 with detections of viral RNA or culturable viruses were resampled in the summer of 2000 for enteric viruses, fecal-indicator bacteria, and coliphage. A fourth site with detections of total coliforms, male-specific coliphage, and *B. fragilis* was also resampled. In addition to microorganisms, a suite of 46 compounds associated with domestic and industrial wastewater and 20 pharmaceutical compounds frequently found in the human waste stream were sampled. All four sites tested negative for viral microorganisms. Three of the four sites had no measurable concentration of any analyzed compound. The fourth site contained 0.040 µg/L (micrograms per liter) of tri (2-chloroethyl) phosphate, a fire retardant, and 0.217 µg /L of ethanol,2-butoxy-,phosphate, a plasticizer. Both compounds could be associated with materials used in well construction. The presence of these compounds does not indicate a sewage contamination source.

Enterococci are a gram positive bacteria that normally inhabit the mammalian intestinal tract, and are a common bacterial component of sewage. Although enterococci bacteria have been recommended as indicators for determining the microbiological quality of marine recreational waters, there is inadequate documentation validating the effectiveness of this group of microorganisms as viral indicators in ground water. The only site that tested positive for culturable viruses in the summer of 1999 also tested positive for enterococci. This site is currently served by a municipal sewer, but the owner may have practiced on-site sewage disposal in the past. Samples collected during 1999 were negative for any other fecal-indicator bacteria or coliphage other than enterococci. To verify enterococci results during the summer 2000 resampling, three enterococci samples were collected from the contaminated well. The first sample was collected after well purging and prior to virus filtration. The second sample was collected after approximately 1 hour of pumping. The third was collected after 400 gal had been passed through the virus filter—approximately 2 hours after pumping began. Sample results were 18, 31, and 46 cfu/100 mL of enterococci, respectively. The sample collected prior to filtration (18 cfu/100 mL) had an associated replicate that when analyzed produced 6 cfu/100 mL of enterococci. These data indicate a persistent source of enterococci contamination at the site, and could be the result of an unidentified source of contaminated water being drawn into the well. The data also could indicate a bacterial source in the well casing, well annulus, or plumbing prior to the sampled hose bib.

Summary and Conclusions

In 1998, the Maryland Department of the Environment asked the U.S. Geological Survey, in cooperation with the Wisconsin State Laboratory of Hygiene, to assess the occurrence and distribution of viral contamination in small (less than 10,000 gallons per day) public water-supply wells in Worcester and Wicomico Counties, Maryland. The purpose of the study was to describe the occurrence of microbiological contamination in ground water from small public water-supply systems in the Coastal Plain Physiographic Province in Worcester and Wicomico Counties, Maryland.

A hypothetical steady-state ground-water flow and transport model was used to guide the investigation in determining likely flow paths, recharge areas, and travel-times for hydrogeologic characteristics known to exist in the study area. A non-weighted ranking system that evaluated land use, soil type, well depth and age, and historical site data on bacterial detection was used to rank the 278 available small public-water supply wells. Based on this ranking and owner participation, 27 wells were sampled for enteric viruses, fecal-indicator bacteria, and coliphage along with a

suite of nutrient and field data.

Results indicate that 11 percent of the sites showed enteric virus contamination. Fifteen percent of the samples had detections of fecal-indicator bacteria and 37 percent of the sites showed total oxidized nitrogen levels above a natural background level of 0.4 mg/L. There were no detections of *Clostridium perfringens* or *Escherichia coli*. Three sites had detections of total coliforms and two sites tested positive for enterococci bacteria. There were two positive cultures for male-specific coliphage and *Bacteroides fragilis*. Somatic coliphage was detected in one sample, and another sample produced a positive cell culture and was later serotyped as rotavirus.

A rank-transformed analysis-of-variance test determined that a significant difference existed between the mean overall vulnerability-rank score of sites with bacterial contamination and those without ($p = 0.045$). In addition, public water-supply wells contaminated with coliphage had higher mean concentrations of total oxidized nitrogen and dissolved oxygen (8.1 milligrams per liter compared to 2.19 milligrams per liter nitrite plus nitrate as nitrogen, respectively, and 8.4 mg/L compared to 2.3 mg/L dissolved oxygen, respectively) than the public supply wells not contaminated with coliphage.

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Appendix A. *Microbiological and water-quality data for 30 public water-supply wells in Worcester and Wicomico Counties, Maryland, March through September 1999*

[cfu/mL, colony-forming units per milliliter; pfu/mL, plaque-forming units per milliliter, which is a measure of the concentration of infectious phage; MPN/100 mL, most probable number per 100 mL; °C, degrees Celsius; µS/cm, microsiemens per centimeter; mg/L, milligrams per liter; NGVD, National Geodetic Vertical Datum of 1929,also referred to as sea level; $CaCO_3$, calcium bicarbonate; **NC**, negative-control sample; <, less than; >, greater than; n/a, not applicable; –, no data collected; **E**, estimated value (not quantitative); **R**, quality-control replicate sample, which is only for the microbiological value; no field or metals data collected]

Local well no.	Date sampled	Rank	Total coliforms, membrane filtered, (cfu/100 mL)	*Escherichia coli*, filtered (cfu/100 mL)	Male-specific coliphage (pfu/500 mL)	Somatic coliphage (pfu/500 mL)	*Bacteroides fragilis*, bacteriophage (pfu/500 mL)	*Clostridium perfringens* (cfu/200 mL)
SM Df 61	08/03/1999	**NC**	<1	<1	<1	<1	<1	<1
SM Df 98	08/04/1999	**NC**	<1	<1	<1	<1	<1	<1
WI Bf 80	03/09/1999	190	<1	<1	<1	<1	<1	<1 [a]
WI Bf 81	06/01/1999	11	14	<1	6	<1	2	<1
WI Bf 82	06/30/1999	52	<1	<1	<1	<1	<1	<1
WI Ce 292	06/22/1999	41	<1	<1	<1	<1	<1	<1
WI Ce 293	06/29/1999	46	<1	<1	<1	<1	<1	<1
WI Ce 294	06/29/1999	47	>2,419	<1	<1	53	17	<1
WI Ce 296	08/03/1999	58	<1	<1	<1	<1	<1	<1
WI Cf 208	05/25/1999	32	<1	<1	<1	<1	<1	<1
WI Cf 209	06/14/1999	6	<1	<1	<1	<1	<1	<1
WI Cf 210	06/14/1999	5	11	<1	3	<1	<1	<2 [b]
WI Cf 211	06/15/1999	45	<1	<1	<1	<1	<1	<1
WI Db 64	06/28/1999	54	<1	<1	<1	<1	<1	<1
WI Dh 15	09/22/1999	73	<1	<1	<1	<1	<1	<1
WO Bg 59	06/15/1999	35	<1	<1	<1	<1	<1	<1
WO Bg 60	06/22/1999	9	<1	<1	<1	<1	<1	<1
WO Bg 61	08/17/1999	270	<1	<1	<1	<1	<1	<1
WO Cf 62	08/03/1999	43	<1	<1	<1	<1	<1	<1
WO Cg 81	05/03/1999	90	<1	<1	<1	<1	<1	<1
WO Cg 82	08/02/1999	275	<1	<1	<1	<1	<1	<1
WO Cg 83	09/22/1999	268	<1	<1	<1	<1	<1	<1
WO Cg 84	09/22/1999	**R**	<1	<1	<1	<1	<1	<1
WO Dd 61	05/25/1999	23	<1	<1	<1	<1	<1	<1
WO Df 8	05/12/1999	20	<1	<1	<1	<1	<1	<1
WO Df 9	05/12/1999	19	<1	<1	<1	<1	<1	<1
WO Ec 41	05/04/1999	18	<1	<1	<1	<1	<1	<1
WO Ec 42	07/26/1999	64	<1	<1	<1	<1	<1	<1
WO Fb 103	05/17/1999	22	<1	<1	<1	<1	<1	<1
WO Fc 49	06/07/1999	17	9	<1	<1	<1	<1	<1

Enterococci (MPN/100 mL)	Cell culture	Cytopathic effects	Resolved	Hepatitis A virus	Enterovirus	Rotavirus	Calicivirus genotypes I, II	Local well no.
negative	negative	n/a	n/a	negative	negative	negative	negative	SM Df 61
negative	negative	n/a	n/a	negative	negative	negative	negative	SM Df 98
negative	negative	n/a	n/a	negative	negative	negative	negative	WI Bf 80
negative	negative	n/a	n/a	negative	negative	negative	negative	WI Bf 81
negative	negative	n/a	n/a	negative	negative	negative	negative	WI Bf 82
negative	negative	n/a	n/a	negative	negative	negative	negative	WI Ce 292
negative	negative	n/a	n/a	negative	negative	negative	negative	WI Ce 293
positive	negative	n/a	n/a	negative	**positive**	**positive**	negative	WI Ce 294
negative	negative	n/a	n/a	negative	negative	negative	negative	WI Ce 296
negative	negative	n/a	n/a	negative	negative	negative	negative	WI Cf 208
negative	negative	n/a	n/a	negative	negative	negative	negative	WI Cf 209
negative	negative	**yes**	**yes**	negative	**positive**	**positive**	negative	WI Cf 210
negative	negative	n/a	n/a	negative	negative	negative	negative	WI Cf 211
negative	negative	n/a	n/a	negative	negative	negative	negative	WI Db 64
negative	negative	n/a	n/a	negative	negative	negative	negative	WI Dh 15
negative	negative	n/a	n/a	negative	negative	negative	negative	WO Bg 59
positive	**positive**	**yes**	**yes**	negative	negative	**positive**	negative	WO Bg 60
negative	negative	n/a	n/a	negative	negative	negative	negative	WO Bg 61
negative	negative	n/a	n/a	negative	negative	negative	negative	WO Cf 62
negative	negative	n/a	n/a	negative	negative	negative	negative	WO Cg 81
negative	negative	n/a	n/a	negative	negative	negative	negative	WO Cg 82
negative	negative	n/a	n/a	negative	negative	negative	negative	WO Cg 83
negative	negative	n/a	n/a	negative	negative	negative	negative	WO Cg 84
negative	negative	n/a	n/a	negative	negative	negative	negative	WO Dd 61
negative	negative	n/a	n/a	negative	negative	negative	negative	WO Df 8
negative	negative	n/a	n/a	negative	negative	negative	negative	WO Df 9
negative	negative	n/a	n/a	negative	negative	negative	negative	WO Ec 41
negative	negative	n/a	n/a	negative	negative	negative	negative	WO Ec 42
negative	negative	n/a	n/a	negative	negative	negative	negative	WO Fb 103
negative	negative	n/a	n/a	negative	negative	negative	negative	WO Fc 49

Appendix A. *Microbiological and water-quality data for 30 public water-supply wells in Worcester and Wicomico Counties, Maryland, March through September 1999–Continued*

Local well no.	Date sampled	Viral [c] presence	Bacterial [d] presence	Bacteriophage [e] presence	Water temperature (°C)	Air temperature (°C)	Specific conductance, laboratory (μS/cm)	Oxygen, dissolved (mg/L)
SM Df 61	08/03/1999	no	no	no	17	30.5	273	2.50
SM Df 98	08/04/1999	no	no	no	19	28.5	301	2.90
WI Bf 80	03/09/1999	no	no	no	12.6	6	170	6.67
WI Bf 81	06/01/1999	no	**yes**	**yes**	17	28.5	190	8.40
WI Bf 82	06/30/1999	no	no	no	16	22	82	9.53
WI Ce 292	06/22/1999	no	no	no	15	21.5	129	9.20
WI Ce 293	06/29/1999	no	no	no	14.5	27	119	6.35
WI Ce 294	06/29/1999	**yes**	**yes**	**yes**	15	32	153	9.53
WI Ce 296	08/03/1999	no	no	no	17	26	99	6.91
WI Cf 208	05/25/1999	no	no	no	16	19	62	.20
WI Cf 209	06/14/1999	no	no	no	16	30.5	113	4.41
WI Cf 210	06/14/1999	**yes**	**yes**	**yes**	16	30.5	172	7.11
WI Cf 211	06/15/1999	no	no	no	16	22	68	3.15
WI Db 64	06/28/1999	no	no	no	15	26	301	–
WI Dh 15	09/22/1999	no	no	no	16	17	171	.28
WO Bg 59	06/15/1999	no	no	no	16	20.5	370	.17
WO Bg 60	06/22/1999	**yes**	no	no	15.5	21.5	453	.27
WO Bg 61	08/17/1999	no	no	no	14.5	27	172	.21
WO Cf 62	08/03/1999	no	no	no	15	27	174	.16
WO Cg 81	05/03/1999	no	no	no	14.5	11	372	.25
WO Cg 82	08/02/1999	no	no	no	16.5	28.5	278	.33
WO Cg 83	09/22/1999	no	no	no	16	11.5	419	.30
WO Cg 84	09/22/1999	no	no	no	–	–	–	–
WO Dd 61	05/25/1999	no	no	no	15	20	100	3.0
WO Df 8	05/12/1999	no	no	no	16	21.5	431	.27
WO Df 9	05/12/1999	no	no	no	17	21.5	355	.38
WO Ec 41	05/04/1999	no	no	no	16	16	1,110	.53
WO Ec 42	07/26/1999	no	no	no	17	30	–	.17
WO Fb 103	05/17/1999	no	no	no	17	20	167	.26
WO Fc 49	06/07/1999	no	**yes**	no	16	27.5	468	.24

a. Forty-three colonies observed in clostridia plates failed to conform as *Clostridium perfringens*.

b. Growth observed in clostridia plates failed to conform as *Clostridium perfringens*.

c. Viral presence; positive cell culture or the positive presence of Hepatitis A, Enterovirus, Rotavirus, or Calicivirus Ribonucleic acid.

pH, whole water, field (standard units)	Acid-neutralizing capacity, field (mg/L as $CaCO_3$)	Nitrogen, ammonia plus organic, dissolved (mg/L as N)	Nitrite plus nitrate, dissolved (mg/L as N)	Phosphorus, ortho (mg/L as P)	Carbon, organic, total (mg/L as C)	Elevation of land surface (feet above NGVD)	Depth of well (feet)	Local well no.
7.94	146	0.20	<0.05	<0.02	0.90	120	600	SM Df 61
8.90	155	.20	< .05	.06	.30	75	575	SM Df 98
5.77	9.5	< .10	11	< .01	–	46	100	WI Bf 80
5.70	12	.10	5.9	.02	4.50	46	60	WI Bf 81
5.8	7.5	**E** .09	4.2	.03	.20	50	70	WI Bf 82
5.46	7.5	**E** .10	8.4	.01	.70	40	80	WI Ce 292
5.81	6	.10	9.7	< .01	.70	35	78	WI Ce 293
5.14	1.5	.10	7.4	.01	.30	40	72	WI Ce 294
5.70	6	**E** .09	6.4	< .01	.20	24.6	70	WI Ce 296
6.0	12	**E** .06	< .05	.07	.70	50	101	WI Cf 208
5.76	19	.10	5.9	.02	2.10	15	78	WI Cf 209
5.88	8	**E** .08	11	.01	.20	15	83	WI Cf 210
5.72	15	**E** .09	< .05	.01	.10	40	70	WI Cf 211
6.61	120	.30	< .05	.89	7.0	10	60	WI Db 64
6.41	35	**E** .05	**E** < .05	.19	1.40	29	62	WI Dh 15
7.45	179	.30	< .05	.01	.80	5	240	WO Bg 59
7.41	202	.50	< .05	.17	4.3	10	250	WO Bg 60
6.83	64	.60	< .05	.48	1.1	5	80	WO Bg 61
6.51	69	.20	< .05	.25	–	35	270	WO Cf 62
7.14	172	.30	< .05	.28	_	11	280	WO Cg 81
7.09	109	.80	< .05	.33	2.2	10	217	WO Cg 82
7.79	195	.34	< .05	.26	1.7	5	280	WO Cg 83
–	–	–	–	–	–	–	–	WO Cg 84
6.0	17	**E** .06	2.3	.02	2	20	80	WO Dd 61
7.72	190	.40	< .05	.10	–	5	290	WO Df 8
7.35	164	.50	< .05	.03	–	5	79	WO Df 9
8.15	270	.70	< .05	.48	–	20	300	WO Ec 41
7.94	303	.90	< .05	.38	2.6	15	345	WO Ec 42
6.71	69.5	.50	< .05	.35	–	15	135	WO Fb 103
7.87	230	.40	< .05	.17	4.8	35	230	WO Fc 49

d. Bacterial presence; positive presence of either Total coliforms, *Escherichia coli*, or Enterococci.

e. Bacteriophage presence; positive presence of either Male-specific coliphage, Somatic coliphage, or *Bacteroides fragilis*.