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Reconnaissance for Selected Pathogens, and Review of Pertinent Literature, for the New River Gorge National River, West Virginia, 2000

Open-File Report 02-65

U.S. Department of the Interior
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By Terence Messinger

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Charleston, West Virginia
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U. S. Department of the Interior
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Multiply	by	to obtain
cubic feet per second (ft^3/s)	0.02832	cubic meter per second
mile (mi)	1.609	kilometer
inch	2.54	centimeter
centimeter	10,000,000	nanometer (nm)
fluid ounce	29.57	milliliter (mL)

Temperature in degrees Celsius (°C) can be converted to degrees Fahrenheit (°F), and conversely, by the following equations:

$°F = (1.8 \times °C) + 32$ $°C = (°F - 32) \times 0.5555$

WATER-QUALITY UNITS

Abbreviated water-quality units: Chemical concentrations and water temperature are given in metric units. Chemical concentration in water is given in milligrams per liter (mg/L) or micrograms per liter (µg/L). Milligrams per liter are a unit expressing the concentration of chemical constituents in solution as weight (milligrams) per unit volume (liter) of water. One thousand micrograms per liter is equivalent to one milligram per liter. For concentrations less than 7,000 mg/L, the numerical value is the same as for concentrations in parts per million.

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ABSTRACT

Giardia and enteric viruses were detected in a reconnaissance study of Madam Creek and Dunloup Creek, two tributaries of the New River Gorge National River, in 2000. *Cryptosporidium* and pathogenic bacteria were not detected in these tributaries. The two streams were identified in previous studies as consistently having some of the highest indicator-bacteria concentrations among New River Gorge tributaries. This study used the best available commercial methods for identifying and enumerating pathogens. However, these methods were developed for regular monitoring at water-treatment facilities or documenting the causes of disease outbreaks, and provided ambiguous results when used in this occurrence study. The World Health Organization suggests a study design for monitoring recreational waters. Frequent sampling for multiple fecal indicator organisms is the recommended first step. Regression modeling that uses environmental characteristics measurable in real time to predict bacteria concentrations and make operational decisions is recommended for contaminated waters.

INTRODUCTION

Fecal-indicator-bacteria concentrations in several New River Gorge tributaries frequently exceed West Virginia guidelines for waters used for contact recreation (Sullivan, 1993*a*, 1993*b*, Purvis and Wilson, 1999; Wilson and Purvis, 2000). Based on indicator-bacteria concentrations and observation of upstream land uses and waste-disposal practices, the National Park Service has identified contamination of tributaries with sewage as the greatest water-resources problem in the New River Gorge National River (Jesse Purvis, National Park Service, oral commun., September 2001). Some contaminated tributaries, including Dunloup Creek and Marr Branch, are used by Park visitors. Others, including Madam Creek and Wolf Creek, enter the New River a short enough distance upstream from paddler put-ins, take-outs, or other areas heavily used by Park visitors that these tributaries do not mix with cleaner waters of the mainstem before flowing past the river put-ins (Mott and others, 1996). The National Park Service (NPS) has expressed concern that contaminated tributary waters pose a health risk to Park visitors (National Park Service, 1998).

West Virginia guidelines for indicator-bacteria concentrations in waters used for contact recreation are expressed in terms of fecal coliform bacteria (West Virginia Environmental Quality Board, 2001). Epidemiologic studies supported by the U.S. Environmental Protection Agency (USEPA) in the 1970s found no relation between fecal-coliform-bacteria concentrations and the incidence of gastroenteritis among swimmers who had visited a lake in Ohio, but found a significant positive correlation among the same group of swimmers between incidence of gastroenteritis and concentrations of both *Escherischia coli* (*E. coli*) and enterococcus (Cabelli and others, 1979). Accordingly, the USEPA recommended that *E. coli* and enterococcus replace fecal coliform and fecal streptococcus bacteria as indicators of fecal contamination in surface waters (U.S. Environmental Protection Agency, 1986). This recommendation has not been implemented by West Virginia and 36 other States as of 1997 (U.S. Environmental Protection Agency, 1998). Subsequent studies have confirmed that *E. coli* and enterococcus are mean-

ingful indicators of risk of diarrhea to swimmers, but fecal coliform are not (Francy and others, 1993). The USEPA has developed a goal of getting States to adopt *E. coli* and enterococcus as indicator bacteria for water-contact recreation standards by 2002 (U.S. Environmental Protection Agency, 1999*a*).

The NPS bacteria-monitoring program in the New River Gorge uses fecal coliform as its indicator organism, because results of fecal-coliform tests can be expressed in terms of an enforceable standard (Jesse Purvis, National Park Service, oral commun., September 2001). This monitoring, however, provides little information concerning possible health risks to Park visitors, because fecal-coliform concentrations have little demonstrated relation to health risks. Critics of the NPS monitoring approach have pointed out that fecal coliforms are poor indicators of human-health risks, and have challenged the significance of the monitoring results on the grounds that indicator-bacteria concentrations provide no information about sources or potential danger (Jesse Purvis, National Park Service, oral commun., September 2001).

Study Objectives

To further explore the potential human-health risk implied by high concentrations of fecal coliform bacteria, the U.S. Geological Survey sampled selected pathogens from two tributaries of the New River—Madam Creek near Brooklin, and Dunloup Creek near Thurmond (fig. 1). The purpose of this report is to present the results of these samples, and to provide information from scientific literature that may help readers interpret these results.

Pathogens were selected on the basis of the availability of methods from production laboratories. The pathogens collected included protozoans, *Cryptosporidium* sp. and *Giardia* sp.; bacteria, *Salmonella* sp.; and enteroviruses detectable by the Total Culturable Virus (TCV) assay, primarily coxsackieviruses, polioviruses, and echoviruses. Tests were also made for the presence of selected indicator organisms, including fecal coliform bacteria, *E. coli*, *Clostridium perfringens*, and male-specific coliphage. Some supporting flow and water-chemistry data were also collected. The project was conceived as a pilot study to explore the value and feasibility of a more extensive study of pathogen occurrence in the New River Gorge.

Description of Study Area

The New River Gorge is a scenic canyon in the Appalachian Plateaus in south-central West Virginia (fig. 1). The New River forms in North Carolina, and flows north to Gauley Bridge, West Virginia, where it joins the Gauley River to form the Kanawha River. Most New River Gorge tributaries form in uplands and flow across a plateau until they reach the lip of the Gorge, then plunge sharply into the Gorge. New River Gorge tributaries, near their mouths, have steep gradients, coarse substrates, and often, a deceptively pristine appearance.

The economy of the study area relies heavily on extractive industries such as coal mining and timbering, and historically, relied more heavily on these industries (Messinger and Hughes, 2000). Most of the population of New River Gorge tributary basins is rural, although towns including Beckley, Mount Hope, Oak Hill, and Fayetteville are within basins draining to the New River Gorge.

Humans, pets, and wild animals are likely to be the major sources of fecal bacteria in New River Gorge tributaries. Park employees have documented a high incidence of household waste draining directly to streams (Jesse Purvis, National Park Service, oral commun., September 2001). Agriculture in the basins draining to the New River Gorge is generally scattered and not intense (Messinger and Hughes, 2000). Livestock is unlikely to be an important source of fecal bacteria in New River Gorge tributaries, although it may be a more important source in basins draining to the nearby Bluestone River park unit.

The two streams sampled in this study were selected because they were among the most contaminated in the New River Gorge. Among 301 stream sites sampled once each in the West Virginia Division of Environmental Protection's assessments of the New and Gauley River watersheds in 1998-1999, Dunloup Creek had the fourth highest and Madam Creek had the forty-ninth highest fecal coliform concentrations (John Wirts, West Virginia Division of Environmental Protection, written commun., 2001). Dunloup Creek drains a greater area (48.5 mi^2) than does Madam Creek (12.3 mi^2). Dunloup Creek was sampled downstream from a municipal wastewater treatment plant, and both streams receive direct discharges of household waste.

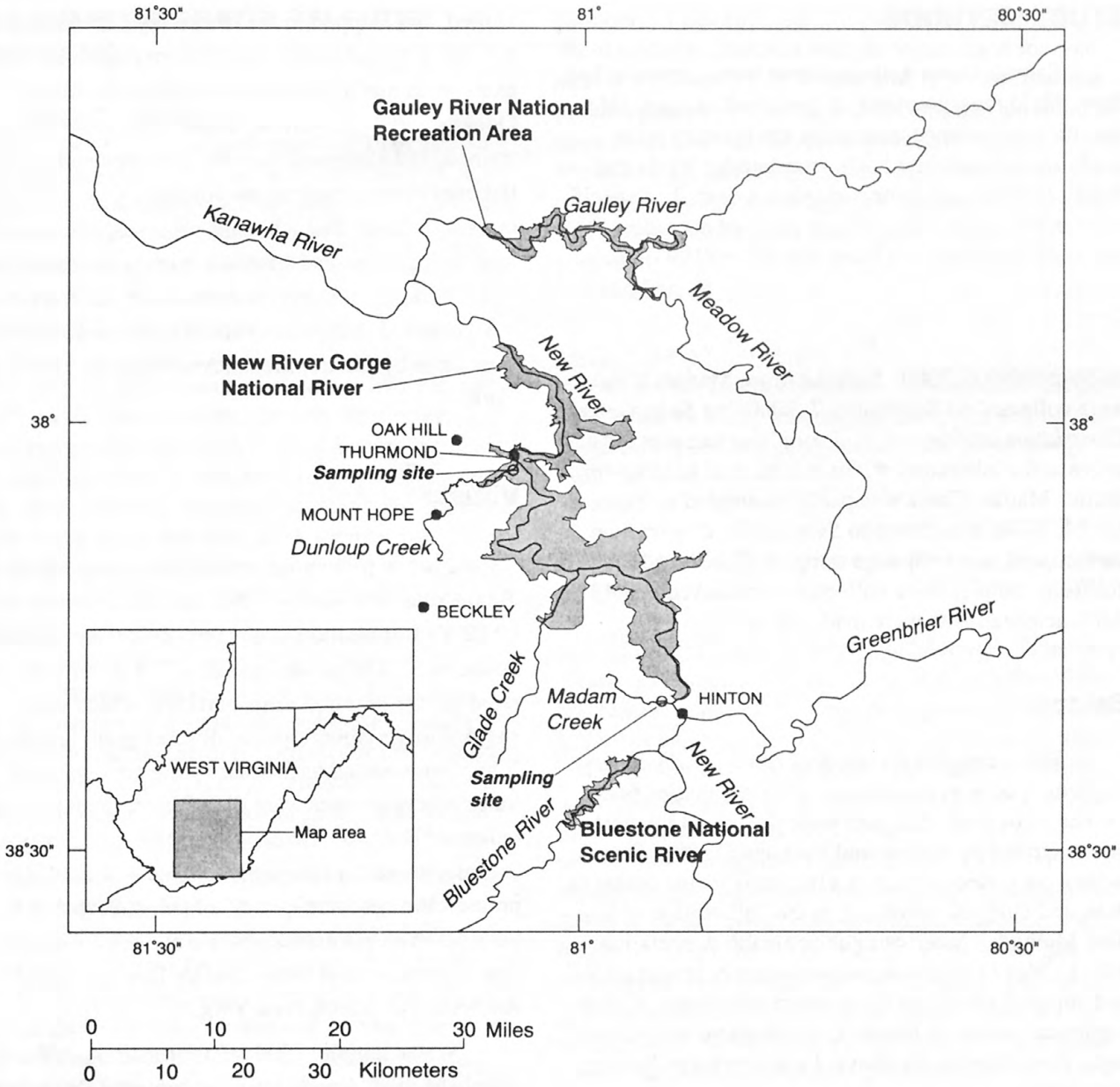

Figure 1. The New River Gorge National River, nearby National Park lands, and selected streams and towns.

STUDY METHODS

Samples were collected from both streams at low flow. Field measurements of dissolved oxygen, pH, specific conductance, and water temperature were made according to methods described by Wilde and Radke (1998). Indicator bacteria not described specifically below were collected and cultured according to methods described by Myers and Wilde (1997). Flow was measured in each stream according to methods described by Rantz (1982).

All samples from Dunloup Creek were collected on September 6, 2000. Samples from Madam Creek were collected on September 7, 2000, but *Salmonella*, *Clostridium perfringens*, and coliphage samples did not arrive at the laboratory within the method holding-time limits. Madam Creek was partly resampled on September 14, 2000; in addition to *Salmonella*, *Clostridium perfringens*, and coliphage samples, *E. coli* and fecal coliform samples were collected and analyzed, and field measurements were made again.

Bacteria

Water samples for analysis of *Clostridium perfringens* spores were collected in sterile bottles from the center of flow. Samples were processed by methods described by Armon and Payment (1988). *Salmonella* were collected in sterile bottles from the center of flow, and cultured according to the 18th edition of Standard Methods (American Public Health Association, 1992). Water samples were immediately placed on ice and shipped, on ice, to the contract laboratory. Culturing began within 48 hours. *C. perfringens* and *Salmonella* were cultured by Biovir Laboratories of Benicia, California.

Protozoans

Water samples for *Cryptosporidium* and *Giardia* were collected according to USEPA Method 1623 with a Genera Filta-Max filter (U.S. Environmental Protection Agency, 2001*b*; Idexx Laboratories, 1998). A 10-L water sample was dipped from the center of flow, chilled, and shipped to the laboratory. The sample was filtered in the laboratory and the oocysts, cysts, and extraneous materials were retained on the filter. Oocysts and cysts were separated from other materials, stained, and examined with fluorescence and differential interference contrast microscopy. Oocysts and cysts were identified when the size, shape, color, and morphology matched specified criteria and examples in a photographic library; confirmed oocysts and cysts were counted. *Cryptosporidium* and *Giardia* analyses were done by Environmental Associates of Ithaca, New York.

Viruses

Enteric (intestinal) viruses were assayed by the Total Culturable Virus (TCV) method described in USEPA's Information Collection Rule (Environmental Associates, 2000*c*). At least 200 L of water were filtered on site to retain virus particles, which were shipped to the laboratory for all subsequent processing. Filters were eluted and viruses were concentrated. Sample concentrates were disinfected, inoculated onto cultured "Buffalo" African Green Monkey kidney cells, and monitored for cytopathic effects over a 14-day period. Viruses were quantified (Most Probable Number), based on confirmed positive and negative cultures. Enteric virus assays were done by Environmental Associates of Ithaca, New York.

Water samples (500 mL) for male-specific coliphage were dipped from the center of flow. Samples were added to an streptomycin- and ampicillin-resistant *E. coli* covering a Petrie dish (a bacterial "lawn") (Ijzerman and Hagedorn, 1992). Any coliphage particles present attached to *E. coli* cells and reproduced, eventually forming plaques, or bare spots in the bacterial lawn; these plaques were counted. Coliphage were cultured by BioVir Laboratories of Benicia, California.

RECONNAISSANCE FOR SELECTED PATHOGENS

Pathogens were detected at both sites (table 1). Empty (29 per 100 L) and amorphous (71 per 100 L) *Giardia* cysts were present in the Dunloup Creek sample. Empty *Giardia* cysts lack any internal structures, and amorphous *Giardia* cysts contain only deformed internal structures (Environmental Associates, 2000*a*). Only intact *Giardia* cysts are infectious. The presence of any cysts indicates that *Giardia*-infected animals are present in the stream's basin and that under other weather or flow conditions, the stream is likely to contain infectious *Giardia* cysts. Enteric viruses were present in samples from both sites, although they were much more abundant in Madam Creek than Dunloup Creek. Most Probable Numbers of enteric viruses were 280 per 100 L in the Madam Creek sample and 5.1 per 100 L in the Dunloup Creek sample. *Cryptosporidium* and *Salmonella* were not detected in samples from either site.

Clostridium perfringens (12 colonies per 100 mL) and male-specific coliphage (0.25 plaque-forming units per mL) were detected in the Madam Creek sample, but neither were detected in the Dunloup Creek sample. As spores, *C. perfringens* are resistant to chlorination and are sampled principally as indicators of chlorinated wastewater.

Indicator-bacteria concentrations at both sites exceeded standards or guidelines for a single sample of water used for contact recreation. All *E. coli* samples exceeded the USEPA guideline of 126 col/100 mL. The fecal coliform samples from Madam Creek exceeded the West Virginia standard for one fecal coliform sample, 400 col/100 mL. Other measured water-quality characteristics met all established guidelines.

REVIEW OF PERTINENT LITERATURE

Published accounts of all pathogen methods used in this study include important caveats. The "pathogens" collectable with these methods include some organisms that are not pathogenic in humans, are rarely transmitted through water, or cause diseases considered to be eradicated in the United States. Methods to detect some of the pathogens thought to most frequently cause waterborne disease are not available from commercial laboratories. No published information about pathogen concentration in waters used for contact recreation appears to be available for comparison to the results of this study, and information about infectious doses is available for only some pathogens. These caveats and complications make the results of this study ambiguous to interpret in terms of human health risk; therefore, expanding the scope of the present study by using the present methods at more sites may be of dubious value.

The World Health Organization has published recommendations for microbiological monitoring to protect human health at beaches. These recommendations could be easily adapted for a riverine setting and might be applied to a study of the New River Gorge National River.

Detectable Pathogens and Known Sources of Error in Methods

Concentrations of fecal-indicator organisms vary greatly, in complex ways, with changes in streamflow and season: relations between pathogen concentrations and other environmental factors undoubtedly are also complex, so that isolated samples provide temporally limited information for a given stream. The methods for *Giardia* and especially *Cryptosporidium* poorly recover laboratory spikes (deliberate inoculations of organisms into quality-control samples). Precision and recovery experiments from *Cryptosporidium*-oocyst spikes of 10-L volumes of reagent-grade water had averages of 39 percent and 47 percent with membrane disk and capsule filter variations, respectively, for USEPA Method 1622; oocyst recoveries from stream-water samples averaged only 22 percent and 12 percent with the membrane disks and capsule filters, respectively (Simmons and others, 2000). In source-water samples (the source was unspecified, and may have been either ground water or surface water), the Genera Corporation reported use of its proprietary Filta-Max filter increased mean recovery of spiked *Giardia* by 12 percent, from 42 percent to 60 percent, but increased mean recovery of *Cryptosporidium* by only 2 percent, from 38 percent to 40 percent (Environmental Associates, 2000*b*). Poor recovery of laboratory-spiking samples suggests a potential for "false negatives," or that the method might not detect organisms actually present in the sample.

Table 1. Water-quality data for two New River Gorge tributaries, West Virginia

[E, estimated; L, liters; ft^3/sec, cubic feet per second; mg/L. milligrams per liter; µS/cm, microsiemens per centimeter; °C, degrees Celsius; col/100 mL, colony-forming units per 100 milliliters; --, not analyzed; MPN/100 mL, Most Probable Number per 100 milliliters; PFU/mL, Plaque Forming Units per milliliter; MPN, Most Probable Number; <, less than; %, per cent]

Station	Station number	Date	Discharge (ft^3/s)
Madam Creek at Brooklin, W.Va.	374023080534101	September 7, 2000	2.9
Madam Creek at Brooklin, W.Va.	374023080534101	September 14, 2000	E3.0
Dunloup Creek near Thurmond, W.Va.	375635081051601	September 6, 2000	20.0

Station	Dissolved oxygen concentration (mg/L)	pH (units)	Specific conductance (µS/cm)
Madam Creek at Brooklin, W.Va.	9.7	7.9	148
Madam Creek at Brooklin, W.Va.	8.6	7.7	178
Dunloup Creek near Thurmond, W.Va.	9.8	8.0	529

Station	Temperature (°C)	*E.coli* (col/100 mL)	Fecal coliform (col/100 mL)
Madam Creek at Brooklin, W.Va.	15.4	130	1900
Madam Creek at Brooklin, W.Va.	19.3	280	3200
Dunloup Creek near Thurmond, W.Va.	15.5	330	230

Station	*Clostridium perfringens* (col/100 mL)	*Salmonella* sp. (MPN/100 mL)	Male-specific coliphage (PFU/mL)
Madam Creek at Brooklin, W.Va.	--	--	--
Madam Creek at Brooklin, W.Va.	12	<2.2	0.25
Dunloup Creek near Thurmond, W.Va.	<1	<2.2	<0.5

	Giardia cysts, per 100 L		
Station	Empty	with amorphous structure	with internal structures
Madam Creek at Brooklin, W.Va.	<6.3	<6.3	<6.3
Madam Creek at Brooklin, W.Va.	--	--	--
Dunloup Creek near Thurmond, W.Va.	29	71	<5.9

	Cryptosporidium oocysts, per 100 L		
Station	Empty	with amorphous structure	with internal structures
Madam Creek at Brooklin, W.Va.	<6.3	<6.3	<6.3
Madam Creek at Brooklin, W.Va.	--	--	--
Dunloup Creek near Thurmond, W.Va.	<5.9	<5.9	<5.9

	Total culturable virus, per 100 L		
Station	Lower 95% confidence limit	MPN	Upper 95% confidence limit
Madam Creek at Brooklin, W.Va.	140	280	540
Madam Creek at Brooklin, W.Va.	--	--	--
Dunloup Creek near Thurmond, W.Va.	2.30	5.1	8.8

The USEPA Method 1622 and 1623 assays for *Giardia* and *Cryptosporidium* are sensitive at the genus level (Environmental Associates, 2000*a*). Both genera contain multiple species, not all of which are infectious to humans, but that are indistinguishable by these methods. *Giardia* assays detect both *G. lamblia* and *G. muris*, although only *G. lamblia* is known to infect humans. In addition to humans, *G. lamblia* also infects mammals including cattle, goats, sheeps, dogs, pigs, and beavers (Maier and others, 2000). Although strains hosted in other animals have not been directly shown to infect humans, evidence suggests that strains of *G. lamblia* from other animals are infectious to humans. Beavers have been infected with *G. lamblia* obtained from humans, but not vice versa; molecular studies have found no difference between *G. lamblia* cysts obtained from beavers and humans (Maier and others, 2000). *Cryptosporidium* species and hosts include *C. parvum* (mammals, including humans); *C. baileyi* and *C. meleagridis* (birds); *C. muris* (rodents); *C. serpentis* (reptiles); and *C. nasorum* (fish) (Environmental Associates, 2000*a*). In most areas, cattle are thought to be the major reservoir of *C. parvum,* the only *Cryptosporidium* species infectious to humans (Maier and others, 2000).

Because USEPA Methods 1622 and 1623 for *Giardia* and *Cryptosporidium* rely on visual examination rather than culturing, they provide no information on the infectivity of the intact cysts and oocysts collected (Environmental Associates, 2000*a*). These methods are considered to be most useful for assessing effectiveness of treatment practices, because of their nonspecificity with respect to infectivity, viability, host species of origin, or species of protozoan.

Humans are believed to be the only source of human enteroviruses (one of eight genera of enteric viruses), which are considered to be the only viruses detectable by TCV assays. Positive results on TCV assays demonstrate the survival of viruses and the presence of human feces in streams. The TCV assay detects enteroviruses for three of the five groups of enteroviruses, the coxsackieviruses, echoviruses, and polioviruses (U.S. Environmental Protection Agency, 1995; Maier and others, 2000). Enteroviruses are readily detected in environmental water samples (Maier and others, 2000). Waterborne enterovirus transmission is difficult to document, largely because most infections caused by enteroviruses are subclinical (although the virus is propagated and shed by the host, the host does not develop symptoms). Some enteroviruses are capable of causing a wide variety of clinical symptoms, from asymptomatic to disabling or fatal, but under most circumstances most enteroviruses do not cause overt disease. Polioviruses are one of the major groups of enteroviruses assayed by the TCV test. Paralytic poliomyelitis is among the diseases caused by polioviruses, and waterborne transmission of paralytic poliomyelitis has been strongly suspected. The United States is certified as free of paralytic poliomyelitis (World Health Organization, 2001). Paralytic poliomyelitis has been nearly eradicated since the development of the polio vaccine in the 1950's; humans who have been vaccinated may shed large numbers of polioviruses (Maier, 2000).

Caliciviruses, including the Norwalk virus, are a group of enteroviruses not detected by the TCV assay (Maier and others, 2000). The Norwalk virus is very small, with a diameter on the order of 26-35 nm, and cannot be propagated in a conventional cell culture. Norwalk virus is the most common cause of waterborne viral disease in the United States. Although causes for about half of waterborne gastroenteritis outbreaks in the United States are not found, epidemiologists suspect that many or most of them are caused by caliciviruses in general and the Norwalk virus in particular (Fout and others, 2000). The Norwalk virus can be detected by electron microscopy, but it and several other small viruses, the Small Round Structured Viruses (SRSV's), are poorly understood because of their size, low numbers in feces, and lack of suitable reagents for their detection (Maier and others, 2000).

Other pathogenic viruses that can be transmitted by water that are not detected by the TCV assay include astroviruses, adenoviruses, Hepatitis A virus, Hepatitis E virus, and rotaviruses. Most of these viruses can be detected by molecular methods, although these methods are not quantitative. It is unknown whether the same factors that affect the viability of any of these viruses also affect the viability of the viruses detectable with the TCV assay.

Salmonella bacteria were not detected in this study. *Salmonella* is a group of rod-shaped, gram-negative bacteria comprising more than 2,000 known serotypes that are members of the Enterobacteriaceae (Maier and others, 2000). All these serotypes are pathogenic to humans. In the United States today, salmonellosis is primarily due to foodborne transmission, because the bacteria infect beef and poultry and grow in these foods; surface waters receiving domestic-

sewage discharges, meat-processing wastes, or stockyard wastes are most likely to be contaminated with *Salmonella*.

Guidelines, Pathogen Infectivity, and Frequency of Waterborne Disease

The Information Collection Rule (ICR) of the USEPA was designed largely to collect baseline data on extent of microbiological contamination of drinking water (U.S. Environmental Protection Agency, 1995). The ICR called for large water suppliers to sample each month for a year for the broadest suite possible (in 1996) of waterborne pathogens, and for 18 months of additional sampling at plants with *Cryptosporidium* or *Giardia* concentrations in source water exceeding 10 cysts or oocysts per L, or TCV concentrations exceeding 1 per L.

Studies in which healthy human volunteers were given *C. parvum* have shown large variations in the number of oocysts from different isolates needed to cause either infection or disease (U.S. Environmental Protection Agency, 1999*b*). As few as 10 oocysts of one isolate infected 50 percent of human volunteers, while other isolates required a dose of 1,100 oocysts to achieve the same number of infections. Similar human-volunteer studies showed the 50-percent-infectivity rate for *Giardia* to be as low as 110 cysts, although some isolates of the parasite are much more virulent than others (Rendtorff, 1978).

Infectivity of about six viruses is well known, but it is unknown how well this infectivity represents that of the 150 or so viruses that are not well understood (U.S. Environmental Protection Agency, 1999*b*). The genetic makeup of viruses determines how infective they are. Some people may be genetically more susceptible to particular viruses, and genetically sensitive individuals may not produce antibodies to a virus.

The great majority of cases of waterborne disease are related to drinking water contamination rather than recreational exposure, and the majority of outbreaks of disease from recreational exposure result from children defecating in swimming pools or shallow water at beaches. From 1988-1998, 6 of 141 waterborne disease outbreaks (four percent) reported to the Centers for Disease Control and Prevention (CDC), representing only 25 of 15,000 reported cases (0.16 percent), were traced to recreational exposure to pathogens in rivers and streams; none of these were from West Virginia (Barwick and others, 2000; Centers for Disease Control and Prevention, 1991, 1993; Kramer and others, 1996; Levy and others, 1998). Outbreak statistics underreport the incidence of waterborne disease because individual cases of a disease do not meet the definition of an outbreak, and small outbreaks of relatively mild diseases are likely to go untreated by physicians and thus unreported to the CDC. Also, tourists who developed a waterborne disease after returning home would seem unlikely to be treated by the same physician, so that an outbreak among them would be particularly unlikely to be noted and reported to the CDC.

The number of diarrhea cases caused by waterborne diseases in the absence of outbreaks in the United States is not known, but is estimated to be much less than the 30-40 percent of an annual total of 370 million cases diarrhea cases thought to be caused by foodborne diseases (U.S. Environmental Protection Agency, 1999*b*). Foodborne disease has been better studied than waterborne disease, largely because foodborne disease poses a significantly greater public health problem in the United States than waterborne disease. About 14 percent of diarrhea cases in Canada are related to waterborne causes (Payment, 1997). Payment, however, discusses waterborne causes of diarrhea exclusively in terms of drinking water.

As part of a microbiology pilot program for the National Water-Quality Assessment Program (NAWQA), the USGS collected representative, flow-weighted indicator-bacteria samples from three to five stream sites in six study units, the Kanawha-New River Basin, Lake Erie-Lake St. Clair Basin, the Santee Basin and Coastal Drainage, the Puget Sound Basin, and the Eastern Iowa Basin (Francy and others, 2000). Study-unit selection criteria included a broad geographic coverage of the United States and a range of hydroclimatic and land-use settings. Land-use settings of the sites included agricultural row crops, agricultural row crops with animal feedlots, urban, suburban, mixed, recreation, and forested. Median indicator-bacteria concentrations from the New River at Thurmond, W.Va., were the lowest of any site where bacteria were collected in the five NAWQA study units (Donna Francy, U.S. Geological Survey, oral commun., March 2000).

Published Recommendations for Sanitary Water-Quality Monitoring

Tests for specific pathogens are expensive and difficult if they exist at all, but indicator organisms have been used successfully for a long time to signal the protential presence of pathogens (World Health Organization, 1999). Several indicator organisms are available for cost-effective determination of both the presence of feces and of wastewater in streams, and no single indicator or approach is likely to represent all the facets and issues associated with contamination of recreational water with feces. *E. coli* and enterococcus bacteria have been positively correlated with cases of diarrhea among swimmers, and the USEPA and World Health Organization recommend these organisms for microbiological monitoring in recreational waters (U.S. Environmental Protection Agency, 1999*a*; World Health Organization, 1999). *Clostridium perfringens* is an indicator for fecal organisms remaining in treated wastewater after its discharge to a stream (Maier and others, 2000). Humans and dogs are the only important hosts of *C. perfringens,* so its presence in streams is a specific indicator of residential land uses. Coliphage are bacterial viruses that are pathogenic to *E. coli.* They are specific indicators of fecal contamination, and more importantly, are thought to remain viable under conditions similar to those in which pathogenic viruses remain viable (U.S. Environmental Protection Agency, 2001*a*).

Several investigators have reported successfully linking strains of bacteria collected from environmental waters to host animals (Dombek and others, 2000; Wiggins, 1996; Samadpour and Chechowitz, 1995). These researchers have used several different source-tracking methods that have not yet been cross-tested in published studies, and many other questions remain concerning the host specificity of bacterial strains and the spatial and temporal applicability of bacterial source libraries. When these questions have been answered, bacterial source tracking may prove to be a useful tool for managing watersheds to improve sanitary water quality. A study began in 2001 in Berkeley County, West Virginia, to validate and compare five bacterial source tracking methods (Hugh Bevans, U.S. Geological Survey, oral commun., September 2001).

The World Health Organization has proposed a set of potential improvements to monitoring and managing recreational waters with respect to microbial contamination (World Health Organization, 1999). The document, produced by a group co-sponsored by the World Health Organization and USEPA, is written from the perspective of a beach-resource manager; but it contains some recommendations specific for rivers, and most other recommendations are readily applicable to rivers. The most fundamental recommendation is that categorization of beach quality be changed from meeting or failing to meet a single numeric standard, to belonging to a quality class (poor through excellent) based on the number of samples with concentrations of multiple indicators falling into appropriate numeric ranges, and that procedures be developed for closing beaches when they have poor sanitary quality. Reconnaissance of sources contributing feces to the water resource is also recommended; an accounting of sources was considered to be adequate to determine the dominant source of indicator organisms. Regular monitoring of multiple indicator organisms was recommended, as was the development of monitoring protocols and schedules that take into account the location and timing of water-resource use. In cases where monitoring finds water bodies to have sanitary water quality unacceptable for their uses, the recommended next step is to develop site-specific regression models that correlate concentrations of indicator organisms with conditions of precipitation, streamflow, land use, and other factors that are quick, cheap, and easy to measure. One ongoing project, for instance, reports fecal bacteria concentrations modeled in real time, using turbidity as the principal explanatory variable (Christensen and others, 2000). At beaches where such regression models based on quickly and cheaply measured explanatory variables closely fit observed indicator organism concentrations, beach closings may be based on these models. In cases where water bodies and their contributing catchments can be understood well enough to develop a regression model, microbial budget studies were recommended as a subsequent step that could inform managers in planning actions to improve sanitary water quality.

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