Evaluating Microbial Pathogens in Reservoirs

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PURPOSE: This technical note describes the results of an investigation that examined recent progress in evaluating the presence of microbial pathogens in reservoirs. Overall guidance as well as pertinent technical advances are summarized in a form suitable for field use.

BACKGROUND: As part of its ongoing mission in water quality, the U.S. Army Corps of Engineers constructs and operates a wide variety of water resource projects along streams and rivers in the United States. These projects were constructed for one or more of the following purposes: navigation, hydropower, flood control, fish and wildlife, and recreation. While water supply and water quality are not stated purposes for reservoir projects, many projects serve as rural or municipal water supplies and are used to provide dilution water to improve poor downstream water quality resulting from seasonal low flows, acid mine drainage, municipal wastewater treatment plant discharges, and other problems. Water resources are potentially impacted by the presence of microbial pathogens. For this reason, possible sources and levels of microbial pathogens are an important operational consideration in water resource projects.

Alternatives for dealing with microbial pathogens are limited because inflows to reservoir projects are vulnerable to activities occurring in the watershed that result in fecal contamination to the inflows and, thus, to the project. District offices may have some control over point sources of pollution (i.e., outfalls of municipal wastewater treatment plants), but even within project boundaries, nonpoint pollution sources, such as broken sewer lines, sanitary sewer overflows, agricultural runoff, and waste holding tank discharges can introduce microbial contamination into the project. Given the inability to prevent fecal contamination from entering water resource projects, perhaps the most viable strategy for dealing with microbial pathogens is a thorough understanding of the microorganisms involved, their sources, their detection and behavior in the environment, and their ultimate fate in the watershed. This understanding, coupled with knowledge of the project, its history and operational characteristics, and the capabilities of mathematical models to predict the movement and dispersion of pathogens, will enable Corps personnel to anticipate problems and their impacts. Consequently, appropriate actions may be taken to reduce or eliminate problems resulting from the presence of microbial pathogens. In addition, state and local public health agencies may assist in regulating or eliminating external sources of microorganisms. In agricultural areas where runoff from feedlots or from fields treated with manure or sewage sludge is a problem, management practices that minimize or eliminate the opportunity for these materials to enter waterways during periods of high runoff should be encouraged.

Factors affecting the occurrence, detection, survival, and virulence of microbial pathogens, the biochemical mechanisms of pathogenicity involved in diseases, and the ability of sewage treatment plants to disinfect plant products prior to their discharge into receiving waters are under continuous investigation. Many environmental and public health services are trying to keep abreast of changes in technology to provide legislation to assist in regulating or eliminating discharges of these microorganisms. The present investigation provides Corps field offices with an overview of the

current state of knowledge of microbial pathogens, their detection, and assessment of their impact on Corps water resource projects.

MICROBIAL PATHOGENS OF RELEVANCE TO FRESHWATER SYSTEMS: Microbial pathogens include bacteria, protozoans, and viruses that are able to infect and produce disease. The principal concern is disease in humans and in animals used as food (livestock, poultry, fish).

Bacteria are unicellular microorganisms ranging in size from approximately 0.5 µ to approximately 10 µ in diameter and often possessing a cell wall (Brock 1970). Bacteria lack an organized nucleus and chlorophyll, but may possess flagella for motility. Bacteria possess single-stranded DNA and reproduce by binary fission to form two cells. Bacteria can be grouped by form into the general categories of rods (or bacilli), spheres (or cocci), curved rods (or vibrios), and spirals (or spirilla). A few bacteria can grow as filaments. *Enteric* bacteria occur in water contaminated by human wastes. Enteric bacteria multiply in the intestine and are discharged in the feces (Gaudy and Gaudy 1980). While the disinfection of community drinking water supplies has lowered the occurrence of waterborne diseases extensively, the potential for drinking water contamination is always present.

Protozoans are unicellular microorganisms that reproduce by fission and lack cell walls (Kudo 1971). Protozoa are often found in soil and aquatic environments, but at a minimum, require the presence of moisture to grow and function. Pathogenic protozoans comprise approximately 30 percent of the 35,000 known species (Mitchell 1974). Since 1981, enteric protozoans have been identified as the principal cause of waterborne disease outbreaks for which the causative agent has been established (Moe 1997). Pathogenic protozoans are able to survive in unfavorable environments as cysts or oocytes. Once ingested, these organisms can hatch and proliferate, often resulting in disease.

Viruses are the smallest pathogens, ranging in size from extremely small (example: 25 mµ for the poliomyelitis virus) to large (250 mµ for the smallpox virus) (Mitchell 1988). Viruses occur as infectious particles, or virions. In its dormant (outside of host) state, each virus possesses a single strand of genetic material composed of either DNA or RNA (in the case of some viruses, double-stranded DNA) surrounded by a protein coat, or capsid. Since viruses are unable to grow and synthesize organic compounds on their own, reproduction only occurs in host cells. Upon invasion of the host cell, viruses redirect the cellular activities of the host to produce new viral particles, which are subsequently released upon the death of the host cell. The most significant waterborne viruses are the enteric viruses that infect the gastrointestinal tract of their hosts.

Table 1 is a list of predominant microbial pathogens of concern in water resource projects and includes representatives of all three groups of microorganisms. Many of the same pathogens infect other animals as well as humans. The normal pathogenic microorganisms excreted by humans cause diseases of the gastrointestinal tract, although other organs, including the liver, may also become infected.

As understanding of human health and the environment increases and detection methods become more sensitive, we are gradually becoming aware of microorganisms that were previously unknown. Examples of some of these emerging pathogens described by Moe (1997) include several enteritis-causing strains of the bacterium *Aeromonas*, enterohemorrhagic *Escherichia coli* O157:H7, the

Table 1
Microbial Pathogens of Concern to Quality of Water Resource Projects (Modified from Metcalfe and Eddy (1991))

Category	Disease	Effects
Bacteria		
Aeromonas hydrophila *Aeromonas salmonica*	Furniculosis (Lesions on fish)	Lesions, death
Campylobacter spp.	Gastroenteritis	Vomiting, diarrhea, death
Clostridium botulinum	Botulism	Tetany, death
Escherichia coli	Gastroenteritis	Vomiting, diarrhea, death
Legionella pneumonophila	Legionellosis	Acute respiratory illness
Leptospira	Leptospirosis	Jaundice, fever
Pseudomonas aeruoginosa	Lesions in fish	Skin ulcerations, death
Salmonella typhi	Typhoid fever	Fever, diarrhea, ulceration of small intestine
Salmonella	Samonellosis	Diarrhea, dehydration
Shigella	Shigellosis	Dysentery
Vibrio cholerae	Cholera	Extreme diarrhea, dehydration
Yersinia enterolitica	Yersinosis	Diarrhea
Viruses		
Adenoviruses	Respiratory disease	
Enteroviruses	Gastroenteritis	Heart anomalies, meningitis
Hepatis A	Infectious hepatitis	Jaundice, fever, eventual death
Norwalk/Snow/Mountain/small round viruses	Gastroenteritis	Vomiting, diarrhea
Reovirus	Gastroenteritis	Vomiting, diarrhea
Rotavirus	Gastroenteritis	Vomiting, diarrhea
Protozoans		
Cryptosporidium	Cryptosporidiosis	Diarrhea
Entamoeba histolytica	Giardiasis	Mild to severe diarrhea, nausea, indigestion
Giardia lamblia	Giardiasis	Mild to severe diarrhea, nausea, indigestion
Naegleria fowleri	Amoebic meningoencephalitis	Fatal brain inflammation

epidemic viral gastroenteritis-producing Norwalk-like viruses, and the cyanobacteria. This list does not include the waterborne pathogens recently recognized in developing countries.

SOURCES OF MICROBIAL PATHOGENS: Several sources may introduce microbial pathogens into water resource projects. Identifying each potential source and establishing the loadings contributed to the project or project watershed are vital. Microbial pathogens and their indicator species may be introduced into water from both point and nonpoint sources (Metcalf and Eddy 1991; Hurst 1997c; Moe 1997; Toranzos and McFeters 1997). Potential point sources of microbial pathogens include wastewater treatment plants, combined sanitary overflows, and industries concerned with the slaughter and processing of meat, poultry, fish, and shellfish. Tanning, textile, pulp, and paper facilities are sometimes also included as point sources, although these industries do not generally release high numbers of microbial pathogens. Nonpoint sources of microbial pathogens include urban runoff contaminated with pathogens from litter and refuse, sanitary sewer overflows produced during storm events, leaking sewage and septic systems, livestock wastes released into pastures and feedlots, poultry wastes from large production farms, soil surface applications of manure and sewage sludge, and wastes pumped from holding tanks.

INDICATOR SPECIES FOR MICROBIAL PATHOGENS: Indicator species are used to establish the potential presence of fecal contamination and subsequent degradation of potable and recreational waters. Unfortunately, ability to establish the microbiological safety of water is limited by the fact that hundreds of different kinds of microorganisms cause gastroenteritis, including all three groups of microorganisms examined here. Consequently, a search for all pathogens possibly present in water supplies is impractical on a routine basis. Further, not all of the waterborne microbial pathogens causing gastroenteritis can be cultured or similarly identified. For this reason, several groups of microorganisms are used as keys, or indicator species, to establish the microbiological safety of waters.

Toranzos and McFeters (1997) indicate that successful indicators of disease-causing microorganisms must represent groups of microorganisms excreted by healthy humans and animals and that fecal contamination must be demonstrated. By contrast, specific pathogens are excreted only by infected individuals, and the numbers of these microorganisms thus depend on the excretion level for the pathogen times the number of infected individuals present. Successful indicator species must correlate well with the type and amount of microbial pathogens present in the body of water and thus give an indication/prediction of the risk of waterborne disease.

The following groups of microorganisms serve as common bacterial indicators:

a. *Total coliforms*: These microorganisms are aerobic, or facultatively anaerobic, gram-negative, nonspore- forming, rod-shaped bacteria able to ferment lactose with gas and acid production in 24 to 48 hr at 35 °C (Mack 1997). This group of microorganisms has traditionally been used as an indicator of fecal contamination/water pollution from sewage. However, these bacteria can also originate from the intestines of animals.

b. *Fecal coliforms:* This group is a subset of the total coliform group that is more reliable as an indicator of fecal contamination from warm-blooded animals. These bacteria meet all of the criteria for total coliforms, but also are able to produce gas and acid at 44.5 ± 0.2 °C (Dufour 1977). For this reason, the occurrence of these bacteria is highly

correlated with fecal contamination from warm-blooded animals. Nonetheless, fecal coliform bacteria meeting this definition have been obtained from environmental samples lacking any source of fecal pollution (Dufour 1977, Rivera,Hazen, Toranzos 1988). The type species for fecal coliforms is *Escherichia coli* which, although also sometimes found in pristine areas, has been used in Europe and is now used in U.S. drinking water standards as a specific indicator of fecal contamination.

c. *Fecal streptococci and enterococci*: These gram-positive bacteria are commonly found in the intestinal tracts of humans and other animals (Clausen, Green, and Litsky 1977). These bacteria also are able to survive over a similar range of conditions as waterborne pathogenic bacteria (Richardson, Stewart, and Wolfe 1991), especially certain enterococci, such as *Streptococcus faecalis*. This microorganism is considered more representative of human fecal pollution than other streptococci (Toranzos and McFeters 1997).

d. *Enteric viruses and protozoans*: Unfortunately, the indicator species for these microorganisms are often the actual pathogens. Indirect evidence provided by the bacterial indicators of fecal pollution must be relied on to suggest that enteric viruses are present. Procedures to test for the presence of viruses are limited to detection of the poliovirus, and the enteroviruses (Hurst 1997a). For the protozoa, finding species of *Cryptosporidium* and/or its oocysts or *Giaradia* sp. and/or its cysts is considered evidence that the waterbody may be contaminated with the infectious form of these agents [*Cryptosporidium parvum* and *Giaradia lamblia*, respectively (Schaefer 1997)]. Detection methods for each of these groups of microorganisms are considered in the next section.

e. *Other indicators*: Toranzos and McFeters (1997) list other types of indicators for bacterial pathogens, each having its own strengths and weaknesses as microbial indicators. These include (1) sulfite-reducing Clostridia (*Clostridium perfringens* and *Clostridium welchii*), which are found in the feces of all warm-blooded animals; (2) *Pseudomonas* spp; (3) heterotrophic plate counts, which suggest the richness of the system in bacteria rather than being a direct indication of microbial pathogens; (4) fecal sterols, a chemical method utilizing coprostanol as a biomarker of fecal pollution from humans and animals in sediment; (5) the fecal coliform to fecal streptococci ratio (FC/FS ratio), where a ratio of 4 or greater indicates human fecal contamination, while a ratio of 0.7 or less indicates animal waste; (6) bacteriophages specific for *E. coli*, *Salmonella*, and other species, and (7) hydrogen-sulfide-producing bacteria. The reader is referred to Toranzos and McFeters (1997) for a detailed discussion of these indicators and their limitations.

DETECTION METHODS

Bacteria. The detection of indicator microorganisms in fresh water has been examined extensively by Toranzos and McFeters (1997). General techniques for isolating and enumerating the groups of indicator bacteria are available in *Standard Methods* (American Public Health Association (APHA) 1992). Briefly, these include collection of the water sample, followed by transport at or below 10 °C, and processing within 24 hr. The level of total coliforms is established with the most probable

number (MPN) technique based on gas and/or acid production in lactose or lauryl tryptose broth incubated at 35 ± 0.5 °C. Total coliforms are confirmed by establishing growth with gas and/or acid production in brilliant green lactose bile broth and lauryl tryptose broth, also at 35 ± 0.5 °C. The level of fecal (thermotolerant) coliforms is determined by MPN with gas and/or acid production upon incubation at 44.5 ± 0.2 °C in Bacto-E C medium (EC medium) (Difco Laboratories, Inc., Detroit, MI). This is confirmed by inoculation into a second EC medium followed by observations of gas and/or acid production upon incubation at 44.5 ± 0.2 °C. Levels of Enterococci are established by MPN based on growth in azide dextrose broth at 35.0 ± 0.5 °C. This is then confirmed by growth at 35.0 ± 0.5 °C in selective *Enterococcus* medium, followed by production of brownish colonies with halos. In each case, the number of microorganisms obtained in the MPN test is based on the number of positive tubes out of the total number of tubes inoculated (normally 5 or 10) at each dilution. The numerical value is obtained from a lookup table (APHA 1992).

Each of the procedures listed for the MPN methods has a corresponding procedure carried out with the use of micropore (0.45-µm) filters and corresponding media. These are collectively termed the MF method (APHA 1992). The technique is faster and more accurate than the MPN method, but also requires greater training and skill. Briefly, the technique traps the bacteria on a micropore-sized filter (0.45 µm), and the filter is placed on a medium appropriate for the microorganisms being detected. Unfortunately, the method is limited to waters lacking high levels of materials that can plug the filter and having only low levels of nontarget (nonfecal) microorganisms, as these can overgrow colonies of the target organisms. For the total coliform test, the medium can be m-Endo LES agar, m-Endo Total Coliform broth, or m-Coliform broth (all products of Millipore Corporation, New Bedford, MA), and the resulting colonies have a unique coloration (APHA 1992). Confirmation is performed using broth with the same procedure as the MPN test. M-fecal coliforms are detected by placing the filter bacteria on M-FC agar (Millipore Corporation) and incubating at 44.5 ± 0.2 °C. Positive colonies are detected by appearance and counted at 24 hr after inoculation. Enterococci are recovered by placing the filter on mE agar (Millipore Corporation) containing nalidixic acid plus triphenyl tetrazolium chloride, incubating at 41 °C and inspecting at 24 and 48 hr. Following incubation, the membrane filter is transferred to a second medium (EIA medium) (Millipore Corporation) and incubated for 20 min at 41 °C. Positive results are indicated by production of pink to red colonies developing a black or red-brown precipitate on the underside of the filter.

Recently, new criteria based on the application of rapid presence-absence (P-A) tests have been added to the traditional procedure for determining total coliform bacteria. These tests do not provide quantitative information on the bacteria cultured and, therefore, cannot indicate the magnitude of the total coliform problem. However, P-A methods are valuable in indicating the prevalence of problems with microbial pathogens in a watershed system and can be used to assess compliance with U.S. regulations (Toranzos and McFeters 1997). Briefly, the P-A analysis consists of adding 100 mL of sample to 50 mL of a triple-strength P-A broth, incubating and looking for color change and gas production. Confirmation is performed as for the MPN test using brilliant green lactose bile broth and lauryl tryptose broth. Recent advancements in detecting coliform bacteria have resulted in requirements for coliform bacteria based on the presence of an enzyme. The assay defines the coliform group of bacteria and *E. coli*. The assay is presently built on the activity of the enzyme ß-galactosidase for the total coliform bacteria and a second enzyme, ß-glucuronidase, for *E. coli*. Procedures are available as an alternative method in the current (18^{th}) edition of *Standard Methods*

(APHA 1992). In addition, some investigators have proposed the number of heterotrophic bacteria as an indicator of general microbiological water quality. The cellular enzyme 4-methylumbelliferyl-heptanoate hydrolase (MUHase) activity is used to rapidly determine the equivalent heterotrophic plate count (Tryland and Fiksdal 1998). Future methods may apply ratios based on the results of such enzyme assays to characterize the sources of fecal pollution to waters, as is presently done with the fecal coliform/fecal streptococci ratio.

Viruses specific for certain kinds of bacteria, the coliphages, that use *E. coli* as the host bacterium have been suggested as indicators of fecal contamination in water (Toranzos and McFeters 1997). In this case, the number of coliphage is assumed to be proportional to the number of host *E. coli* present. However, the numbers of the viruses obtained from the environment depend on the specific host bacterium employed and the virus may not be detected if it has too great a host specificity.

Methods for detection of waterborne coliforms and *Escherichia coli* are continuously being modified and upgraded to include more sensitive media or techniques. These include, among others, better media for use with the membrane filter method (Brenner et al. 1993, Ciebin et al. 1995), and a spectrofluorometric assay for total and fecal coliforms (Park et al. 1995). One recent advance in membrane filter technology allows simultaneous detection of both total coliforms and *Escherichia coli* in water using the same medium (Brenner et al. 1993). Each of these methods also has limitations, such as underestimation of bacteria unable to grow well on the media, a long analysis period, and artifacts resulting from densities of nontarget species (Van Poucke and Nelis 1997, Tryland and Fiksdal 1998).

Modern molecular techniques are being applied in identifying and enumerating pathogenic bacteria. This is a particularly important development, since not all microbial pathogens or their indicator species can be recovered from the environment. For example, recent research has shown that these microorganisms often enter a nonculturable, but viable (NCBV) state in which they can cause infection, but cannot be detected by techniques dependent on culture of the microorganism (Rollins and Colwell 1986; Morgan, Rhodes, and Pickup 1993). NCBV organisms are presently detectable only with the molecular techniques that focus on DNA content. A variety of such molecular techniques have emerged in the last 5 years. For example, a combination of rRNA-targeted polymerase chain reaction (PCR) and in situ hybridization using fluorescently labeled oligonucleotides have been used to detect pathogenic species of *Yersinia* (Trebesius et al. 1998). These techniques are presently in the developmental stage and are being applied to spiked throat and stool samples where concentrations of the target bacteria are normally very high. However, the technology has the potential for further development and at some point may be suitable for application to very dilute environmental samples. In like manner, some investigators are actively developing and applying fluorescent antibodies to detect and determine survival and persistence of indigenous bacteria and microbial pathogens in water (Bottger et al. 1987, Faude and Hofle 1997, Buswell et al. 1998). Since they are very sensitive and highly specific, monoclonal antibodies specific for individual groups and species of microbial pathogens may make detection of the target organisms possible, even directly in water samples. Monoclonal antibodies specific to individual microbial pathogens will be most useful when the specific kinds of pathogens sought are known.

Viruses. Present procedures for detecting viruses in water samples are based on concentration by passive or directed adsorption, ultrafiltration, direct physicochemical flocculation and phase

separation, or affinity chromatography. Following this, the virus particles are eluted from the filter or other sorbant, reconcentrated, and infectivity is established by inoculation into cultures of either human or animal cells in a cytopathogenicity assay (Hurst 1997a). Application of the PCR reaction and reverse-transcriptase-polymerase chain reaction (RT-PCR) to detect Norwalk and enteric viruses in environmental samples is becoming a common technique (Moe et al. 1994; Schwab et al. 1998; Abbaszadegan, Stewart, and LeChevallier 1999). Eventually, methods based on these procedures may be applied to detection of multiple pathogens from the same samples.

Monoclonal antibodies are effective for identification of viruses as well as bacteria (Rigonan, Mann, and Chonmaitree 1998), and techniques presently being developed for identification of enterovirus isolates from humans in medical microbiology may eventually be applicable for analysis of virus filter concentrates from water samples. In the long run, the need for concentration by filtration may also be eliminated. For example, antibodies specific for detection of hepatitis A virus have been attached to special magnetic beads in a process termed immunomagnetic capture (IC). When the beads are added to water and sewage samples, the virus particles are concentrated so they can then be detected in RT-PCR assays (Jothikumar, Cliver, and Mariam 1998). Culturable enteroviruses (F-specific RNA bacteriophages) can be detected by using the concentration techniques described above and inoculating the concentrates into a special bacterial cell line. Therefore, enteric virus concentrations in water and sewage samples may eventually become predictable (Havelaar, van Olphen, and Drost 1993; Puig, Queralt, Jofre, and Araujo 1999).

Protozoan Parasites. The following procedure is summarized from the description presented by Schaefer (1997). Parasitic forms of protozoa are normally quite sparse and suspended in the water column. Therefore, large volumes of water must be concentrated by a yarn-wound filter to recover the cysts and oocysts. Eventually, more effective immunomagnetic separation techniques described above may replace the older style yarn filters. The particles, including the protozoan cysts, are eluted from the filter, reconcentrated by centrifugation, and separated by flotation at the interface of a Percoll-sucrose solution. The interface is placed onto a membrane filter, stained with a fluorescent antibody, and examined under a UV microscope. Cysts and oocytes are determined by their size and shape, as well as fluorescence properties. Confirmation requires the eye of a trained specialist who can look for internal structural characteristics in *Giardia* cysts and sporozoites in *Cryptosporidium* oocysts.

These detection processes continue to be modified and improved. For example, Pernin et al. (1998) have examined the efficiency of replacing filtration with centrifugation (Pernin et al. 1998). Other groups have demonstrated the use of randomly amplified polymorphic deoxyribonucleic acid PCR and reverse-transcription-polymerase chain reaction technologies to enhance detection of low numbers of viable *Cryptosporidium parvum* strains and oocysts in creek and river water samples (Shianna, Rytter, and Spanier 1998; and Kaucner and Stinear 1998, respectively). New commercially available enzyme-linked immunosorbent assays (ELISA) are also becoming available for the parasitic protozoans and have the promise to be extremely sensitive and rapid (Aldeen et al. 1998). While presently applicable only to direct use on fecal samples, these ELISA systems may become suitable for use with environmental samples, particularly if the samples are concentrated prior to analysis.

FACTORS IMPACTING SURVIVAL OF MICROBIAL PATHOGENS: Many factors affect the survival and detectability of microbial pathogens. The most important are described below.

Age. The recency of fecal contamination and whether steady, intermittent, or a one-time event have a strong bearing on the isolation of microbial pathogens downstream from the source. Once fecal contamination enters the aquatic environment, the opportunities for growth are likely to diminish due to lower nutrient concentrations and other factors unfavorable for growth (Burton, Gunnison, and Lanza 1987). The microorganisms will gradually die off, either from starvation or from the negative impacts of other factors listed below. In addition, research has shown that some microorganisms achieve the NCBV state within a few days after exposure to lake water (Morgan, Rhodes, and Pickup 1993). The survival of microorganisms is expressed as a general exponential decay equation (Hurst 1997b) having the form:

$$\frac{dN}{dt} = -KN$$

Where: t = time, K = rate constant, and N = number or concentration of microorganisms. The negative sign associated with the rate constant indicates a net loss in number of microorganisms with time.

pH. Acidity is a critical factor impacting the viability of many bacteria, including those of interest here. Most natural environments have pH values in the range of 5 to 9. A few microorganisms can grow below pH 4 or above pH 10 (Brock 1970).

Sunlight. The ultraviolet (UV) component of sunlight is highly lethal to microorganisms because of the tendency for this type of light to damage nucleic acids (Auer and Niehaus 1993; Sinton, Davies-Colley, and Bell 1994). Some microorganisms are capable of limited repair of damaged nucleic acids. Nonetheless, microorganisms capable of avoiding direct exposure to sunlight, either by remaining below the soil/sediment surface or by remaining at lower depths in the water column, will have a better chance of survival (Burton 1985).

Temperature. Temperature generally has an inverse relationship to survival. The die-off rate for bacteria doubles for each 10 °C rise in temperature. Thus, bacteria survive longer at lower temperatures (Rollins and Colwell 1986, Buswell et al. 1998), as do the enteric viruses (Moe 1997). Parasitic protozoa such as *Cryptosporidium* and *Giardia* also do better if their oocysts and cysts remain at cooler temperatures, preferably below 15 °C (Moe 1997, Schaefer 1997).

Moisture. Moisture is vital to the survival of bacteria, and to protozoan cysts and oocysts (Moe 1997, Schaeffer 1997). Viruses are also negatively impacted by desiccation (Hurst, Gerba, and Cech 1980). Since the aquatic environment is of concern here, moisture is not a limiting factor unless the source material is subject to desiccation before entering the watershed.

Salinity. Salinity tends to increase the loss rate of total coliform bacteria and may nearly double the loss rate from freshwater at the same temperature (Burton 1985, Moe 1997).

Sediment. Both bacteria and viruses have a high affinity for clay particle and organic matter in soils and sediments (McDonald, Kay, and Jenkins 1982; Buckley et al. 1998). Microorganisms possess an electrostatic charge by which they are attracted and adsorbed to the surface of charged environmental particles. Sorption to particles improves the survival of microorganisms by (1) hiding the microbe from predators, (2) diminishing the impact of toxins by sorbing toxins before they can reach the microbe, and (3) facilitating microbial access to nutrients. The fate of sorbed microorganisms becomes tied to the fate of the particles, meaning that when the particles settle out of the water column, the microorganisms do likewise, and when scouring processes resuspend the particles, the associated microorganisms are resuspended and able to move. The movement process may also scour the microorganisms off of the particles through grinding action occurring when particles rub against each other. Several factors regulating survival in soil and sediment have implications similar to those in water - i.e., acidic pH values tend to decrease survival times, while both temperature and texture impact survival (Burton, Gunnison, and Lanza 1987). In certain instances, some bacteria are able to obtain sufficient nutrients to support regrowth (Burton 1985).

Predation. Both bacteria and protozoans have a wide variety of natural enemies. Bacteria are parasitized by other bacteria (example, *Bdellovibrio*) and viruses (bacteriophages) and are grazed by protozoans (Brettar and Hofle 1992). Protozoa may be attacked by other protozoa, parasitic fungi, viruses, and bacteria. Depending on the type of water encountered (pristine versus heavily sewage-polluted), predation may be light, nonexistent, or severe. Resistance to grazing is one important factor in survival strategies for certain bacteria (Weinbauer and Höfle 1998).

Survival Mechanisms (spore formation, encystation). Certain parasitic protozoans and bacteria (*Bacillus* spp., *Clostridium perfringens*), but not the traditional indicator species, are able to form survival devices (spores, cysts, oocysts), which improve survival over harsh times (Moe 1997). These microorganisms are likely to persist when other microorganisms die. However, the protozoan survival structures lose viability after a time, particularly at temperatures above 35 °C.

Interaction of Factors and Reservoir Conditions. Factors impacting survival never occur alone. All of the factors listed above are present in reservoirs and may enhance or degrade the ability of microorganisms to survive, depending on location in the body of water. The presence of nutrients also plays a major role in survival (Brettar and Hofle 1992), as does the presence of antagonistic microbial products, such as enzymes and antibiotics (Hurst 1997b). The microorganisms are constantly aging as they move down the watershed and through the reservoir. Opportunities for exposure to unfavorably high temperatures, sunlight, predation by other microorganisms, nutrient deficiences, and toxic materials are also likely to increase. In addition, exposure to microbe-absorbent particles and the subsequent settling means that microbes are continually (although not necessarily permanently) being removed from the system. Resuspension may add some microorganisms back into the system at a later time. The sum total of all these factors is to diminish the overall concentration of microorganisms in the system.

Assessment of the Sources of Microbial Pathogen Problems: The threat of fecal pollution and the high priority given to protecting human health has resulted in monitoring programs and water treatment facilities. The U.S. Environmental Protection Agency (USEPA) (1986) has established criteria to determine impairment of bodies of water. Commonly used measurements for pathogens are supplied by EPA as guidance to states for the establishment of standards to identify

water pollution problems (Table 2). The recreation levels were developed as national bacteriological ambient water quality criteria (USEPA 1986), as required under section 304(a) of the Clean Water Act. Since levels of enterococci are most strongly correlated with gastroenteritis, these criteria were designed to indicate concentrations of enterococci and *E. coli* that could result in illness rates of 8 illnesses per 1,000 swimmers at freshwater beaches. The status of reservoir data relative to the criteria in Table 2 will indicate whether or not the reservoir is impaired.

In order to understand the relationship of the various sources of microbial pathogens in the watershed to their levels in the project, the contribution of each of the possible sources of loadings must be known. Sources of microbial pathogens to water resource projects were presented above. Whether or not these sources threaten a given project is determined by several factors. To fully understand the impact of microbial pathogen loadings to the project, the locations and relative magnitudes of each of the loadings, the transport and dispersion mechanisms active in the watershed, and the duration and frequency of pathogen addition to the watershed must be known. This means that the field office must obtain a complete inventory of all sources of microbial pathogens and the relative magnitude of each. The inventory should emphasize principal sources of human pathogens to the system. Sources of contamination not identified from existing records may be obtained by examination of recent maps, available data, and recent reports on the project and the watershed, field surveys, and interviews with individuals and agencies knowledgeable about the area. The amount of data and other information available about the project and its watershed may be quite limited. As a result, the field office may need to conduct monitoring and statistical analyses of the watershed and reservoir. Suitable water quality models such as CEQUAL-R1 and WD-2 (available on WES home page at *http://www.wes.army.mil/el/elmodels.index.html*) can be used to draw the assembled information together. The models can give a good representation of where the pathogens are coming from, their respective loadings, and what the resulting concentrations will be at given points in the reservoir. The models require appropriate advection and dispersion process data. The models include die-off computation. As development of these models continues, other factors that hinder pathogen proliferation will be added. Risk assessments and establishment of high risk areas should currently be possible using a simple model with a multiple-attribute rating technique, such as that proposed by Venter, Kühn, and Harris (1998).

CONCLUSIONS: Microbial pathogens are a potentially serious problem for reservoirs and their tributaries. Microbial indicator species continue to be used as a means to monitor water for fecal pollution that may contain microbial pathogens. Much of the technology comprising the standard methods used for identifying and enumerating pathogens is changing. The older plate count method has been deemphasized in favor of membrane filter techniques, presence-absence tests, and enzyme assays. As time continues, current innvotions are also likely to be supplemented or replaced by emerging molecular techniques employing PCR or RT-PCR assays and monoclonal antibodies. In addition to identifying and enumerating pathogenic and indicator microorganisms, data on the sources of contamination and their relative contributions must be determined in order to establish the volume and input locations. This information, together with suitable data on the factors impacting microbial survival in reservoirs, can be placed into modern water quality models to predict the impact of microbial pathogens on the reservoir(s) of interest.

Table 2
Federal 304(a) Criteria for Determining Water Resource Project Impairment by Pathogens (modified from USEPA (1986))

Designated Use	Pathogens Evaluated	Criteria
Drinking water	Total coliform	Ninety percent of all raw water samples taken contain no greater than 100 CFU per 100 mL.
	Fecal coliform	Ninety percent of all raw water samples taken contain no greater than 20 CFU per 100 mL.
	Eschericha coli	Ninety percent of all raw water samples taken contain no greater than 20 *E. coli* per 100 mL.
	Cryptosporidium cysts	Ninety percent of all raw water samples taken contain a density no greater than 1000 *Cyrptosporidium* cysts per 100 L.
Recreation Primary (swimming, surfing, diving) Secondary (wading, boating)	*E. coli*	Geometric mean of 126 CFU per 100 mL based on no less than 5 samples equally spaced over a 30-day period. No sample is to exceed a one-sided confidence limit (CL) based on the following: designated bathing beach - 75% CL; moderate bathing use - 82% CL; light use for bathing - 90% CL; infrequent use for bathing - 95% CL
	Entrococci	Geometric mean of 33 CFU per 100 mL, based on no less than 5 samples equally spaced over a 30-day period; no sample should exceed a one-sided confidence limit (CL) based on the following: designated bathing beach - 75% CL; moderate use for bathing - 82% CL; light use for bathing - 90% CL
	Fecal coliform	Geometric mean of 200 CFU per 100 mL based on no less than 5 samples equally spaced over a 30-day period. No more than 10 percent of the samples should exceed 400 CFU per 100 mL during any 60-day period.

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> Gunnison, D. (1999). "Evaluating microbial pathogens in reservoirs," *Water Quality Technical Notes Collection* (WQTN PD-03), U.S. Army Engineer Research and Development Center, Vicksburg, MS. *www.wes.army.mil/el/elpubs/wqtncont.html*

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