Introduction to Waterborne Pathogens in Agricultural Watersheds
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Technical Note No. 9, September 2012
1. Introduction to Waterborne Zoonotic Pathogens

1.1 Introduction

The goal of this technical note is to provide a survey of the on-farm beneficial practices that help reduce waterborne pathogens at their source, thereby reducing the overall pathogen load and waterborne contamination within an agricultural watershed. Reducing the pathogen load and contamination in water should help reduce the risk of human and animal waterborne infections from irrigation, drinking, and recreational water sources. In addition, an overview is provided of the different types of waterborne pathogens that can cause illness in susceptible individuals. There is an emphasis on pathogens that are transmitted between animals and humans. Several key microorganisms that are proven causes of human illness are described in greater detail, including *Escherichia coli* O157:H7, *Cryptosporidium parvum*, and *Giardia duodenalis*. Indicator bacteria, such as fecal coliforms and enterococci, which are used as surrogates for microbial water quality, are also described. Lastly, information on the survivability of these pathogens and methods to reduce the risk of waterborne transport from fecal sources to bodies of water are also presented. This overview generates a deeper understanding of how to use on-farm beneficial practices to improve microbial water quality and minimize the risk of waterborne transmission of disease from agricultural watersheds. Agricultural runoff is a potential source of pathogen contamination to surrounding watersheds. This document is meant to complement and inform current conservation practices that have previously focused on nutrient contamination and illustrate ways they may be adapted to help control pathogen contamination.

Many waterborne pathogens can also function as foodborne pathogens; routes of transmission are the result of ingesting water or food contaminated with a pathogen. Human outbreaks of infection are defined as two or more individuals becoming infected in the same time frame with the same strain of pathogen. Many human disease outbreaks caused by pathogens are commonly associated with the foodborne routes of transmission. However, foodborne pathogens are not specifically described in this technical note. Moreover, there are numerous pathogens that affect animal but not human health and are beyond the scope of this technical note. Examples of these pathogens would be the various coccidial parasites like *Eimeria bovis*, or roundworm species that infect livestock such as *Ostertagia* and *Haemonchus*, and viruses that cause bovine viral diarrhea. A professional animal health advisor should be consulted to determine how to best control these diseases that are passed from animal to animal.

**What are waterborne zoonotic pathogens?**

A pathogen is an agent that causes disease in animals or plants. A zoonotic pathogen is a pathogen that is naturally transmitted between animals and humans. Pathogens comprise a wide variety of different organisms like bacteria, protozoa, viruses, helminthes, fungi, and prions. "Waterborne zoonotic pathogen" is a term used within this text to describe a pathogen that is transmitted among animals and humans via contaminated water. Transmission from livestock to humans is of particular concern and is emphasized in this technical note. Most waterborne zoonotic pathogens are excreted in human and animal feces (referred to as enteric pathogens) and enter water along various hydrologic pathways such as surface runoff during rainstorms. A smaller number of waterborne zoonotic pathogens are excreted by other routes, for example through urine, eye, or respiratory secretions. Important pathways for animal feces to connect with water bodies include direct defecation in a water body or the pathogen being carried in runoff or overland flow, interflow, or subsurface flow.

Waterborne zoonotic pathogens are a particular concern at the watershed scale because they can quickly amplify (i.e., increase their numbers) inside a host when they infect wildlife or livestock under certain conditions.
management practices, such as high animal densities on confined animal facilities (e.g., dairy, swine) or when large numbers of newborn animals are present. Understanding the basic biological characteristics of these organisms will permit individuals involved in watershed management to better plan and implement beneficial agricultural practices and avoid practices that could inadvertently promote pathogen replication, enhanced infectivity, and increased hydrological transport. For example, excessive moisture in a compost pile can reduce the internal temperature that is achieved during composting, leading to longer survival of bacterial and protozoan pathogens potentially contaminating soil, irrigation water, and crops (e.g., produce) when the compost is applied as a soil amendment.

**Interspecies barriers to pathogen transmission**

Many of the various pathogens shed by animals are not capable of infecting or causing illness in humans. The reason for this is that many of these animal-derived pathogens do not have the ability to attach or adhere to the walls of the human intestine or other surfaces such as eyes or skin, and even when they do, they are often not capable of growing or replicating to sufficient numbers to cause human harm. These pathogens that are limited to one or a few species of animals tend to be host-adapted, meaning they have evolved to preferentially infect a specific host or small number of hosts. A good example would be the many different types of viruses shed in the feces of livestock that for the most part are not able to attach or replicate inside the cells of humans. This phenomenon is referred to as an interspecies barrier to pathogen transmission. Other examples are the many pathogens that infect insects, mollusks, or plants that tend to not cause harm to humans.

**1.2 Sources of waterborne zoonotic pathogens**

Microbial pathogens such as bacteria and protozoal parasites can be excreted or shed in the feces or urine by many vertebrate species. Wildlife, companion animals, livestock, and humans are all possible sources of many different types of waterborne pathogens. It is important to note that most, if not all, species of pathogens shed in the feces or urine of infected humans are infectious for other susceptible humans, yet only a subset of pathogens shed by animals appear capable of infecting humans. This is due to interspecies barriers to transmission. For example, human waterborne outbreaks of enteric viruses in the United States are typically the result of human sources of contamination due to a limited number of viruses shed in the feces of animals that have been definitively shown to be infectious for humans through waterborne routes of exposure. Many serotypes of *Salmonella*, such as *Salmonella enterica* subspecies *enterica* serotype Dublin, are infrequent causes of human illness. Many of the enteric protozoa parasites shed by livestock (cattle, sheep, goats, horses, etc.) do not appear to be very infectious to humans given the rare occurrence of many subspecies (a.k.a. genotypes, assemblages) of livestock-derived protozoa as a cause of human infection. Numerous species of *Cryptosporidium* are shed in the feces of cattle, such as *Cryptosporidium parvum*, *Cryptosporidium andersoni*, and *Cryptosporidium bovis* to name a few, yet *Cryptosporidium parvum* appears to be the most common species of bovine-derived *Cryptosporidium* that is isolated from infected humans. Zoonotic pathogens such as rabies or malaria do not utilize water as the route of transmission for human infection; hence, these types of diseases are not covered in this technical note. Nevertheless, the threat of human waterborne illness from populations of livestock, companion animals, or wildlife contaminating either drinking, recreational, or irrigation water with infectious pathogens is very real and needs to be a serious management consideration.

Table 1 contrasts waterborne outbreaks of gastroenteritis associated with drinking water compared to recreational exposure and the pathogen causing the illness (causative agent), when known, for the outbreak. Examples of recreational exposure would be swimming in either untreated water sources, such as rivers and lakes, or treated water, such as municipal
Introduction to Waterborne Zoonotic Pathogens in Agricultural Watersheds

pools and recreational parks. Keep in mind that an outbreak of an infectious disease is two or more people, not necessarily a large number of people.

The National Water Quality Inventory Report to Congress

Agriculture is often suspected as a significant source of water quality impairments, with nutrients, bacterial indicators, or pathogens cited as the pollutant. This is in part due to The National Water Quality Inventory Report to Congress, which are key documents that inform Congress and the public about general water quality conditions in the United States. These reports are compiled by the U.S. Environmental Protection Agency (EPA) based on information submitted by the States, Tribes, and other jurisdictions regarding their water quality assessment reports. These reports are issued every even-numbered year and are often referred to as State 305(b) reports based on the section of the Clean Water Act (CWA) that describes this reporting mechanism. The National Water Quality Inventory, 1996 Report to Congress, listed bacteria as the third leading cause of impairment for rivers and streams, an improvement from being ranked as the leading cause in 1994. The 1998, 2000, and 2002 Reports to Congress list bacteria as one of the leading pollutants for rivers and streams. Bacterial impairment of water quality was of secondary importance for estuaries. Since the CWA was passed in 1972, States, territories and authorized Tribes are required to develop a list of impaired waters called the 303(d) list. The water bodies on the list do not meet water quality standards and installed pollution controls are not sufficient to attain or maintain applicable water quality standards. The law requires that these jurisdictions establish priority rankings for water bodies on the lists and develop action plans, called Total Maximum Daily Loads (TMDL), to improve water quality. According to the 303(d) list updated by EPA in 2006, pathogens are the leading cause of impairment of water bodies.

Table 1

<table>
<thead>
<tr>
<th>Type of organism</th>
<th>Agent</th>
<th>Number of outbreaks</th>
<th>Outbreaks associated with drinking water</th>
<th>Outbreaks associated with recreational water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>surface</td>
<td>ground</td>
</tr>
<tr>
<td>Protozoa</td>
<td>Giardia duodenalis</td>
<td>27</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Cryptosporidium spp.</td>
<td>21</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Bacteria with potential for infecting multiple species</td>
<td>Escherichia coli O157:H7</td>
<td>11</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Campylobacter jejuni</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Salmonella Typhimurium</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Salmonella Java</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Leptospira grippotyphosa</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bacteria infections associated with humans</td>
<td>Shigella sonnei</td>
<td>17</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Shigella flexneri</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Human viruses</td>
<td>Hepatitis A</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Norwalk-like viruses</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Small round-structured virus</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Unknown</td>
<td>Unidentified cause—many consistent with viral epidemiology</td>
<td>70</td>
<td>8</td>
<td>44</td>
</tr>
<tr>
<td>Other</td>
<td>Cyanobacteria-like bodies</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

1. Summary of MMWR surveillance summaries from the CDC.
2. *Giardia duodenalis* is also known as *Giardia intestinalis* and *Giardia lamblia*. The strain of *Giardia duodenalis* infecting humans during the 1989-1996 period of surveillance is presumed to be assemblage A or B, both of which can cause infection in humans.
3. During the period of surveillance, the species of *Cryptosporidium* that is adapted to humans, called *Cryptosporidium hominis*, had not been fully characterized, hence the species of *Cryptosporidium* isolated from earlier human infections was presumed to be *Cryptosporidium parvum* which is found in a variety of animal species and humans.
Human contamination or inadequacies at water treatment plants have been implicated in several large-scale waterborne outbreaks associated with drinking water. The ability of humans to function as a source of waterborne pathogens is supported by the observation of repeated waterborne outbreaks associated with swimming pools or other such communal bodies of water used for recreation. The definitive source of waterborne pathogens is often hard to identify for outbreaks associated with recreational exposure to rivers and lakes. The collaborative surveillance system maintained by the Center for Disease Control and Prevention (CDC), the EPA, and the Council of State and Territorial Epidemiologists (CSTE), for the occurrences and causes of waterborne disease outbreaks found that recreational water-related disease outbreaks appear to have been increasing in recent years. Many of these larger outbreaks occurred during summer months at public pools and water parks despite the water being treated with chlorine.

### 1.3 Drinking water

To ensure a safe drinking water supply, treatment normally involves filtration, disinfection, or both. Chlorination has been a common method to disinfect drinking water. While chlorine-based treatment systems can be effective for bacteria and viruses, it does not effectively inactivate parasites such as *Cryptosporidium*. The effectiveness of conventional (chlorination) and alternative (chlorine dioxide, ozonation, and ultraviolet irradiation) disinfection procedures for inactivation of *Cryptosporidium* has been the focus of much research due to the resistance of oocysts to many chlorine formulations. Table 2 lists waterborne-disease outbreaks associated with drinking water caused by different pathogens.

#### Table 2 Waterborne disease outbreaks (two or more people) associated with drinking water, 1999 to 2006

<table>
<thead>
<tr>
<th>Type of organism</th>
<th>Agent</th>
<th>Number of outbreaks</th>
<th>Water source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protozoa</td>
<td><em>Cryptosporidium</em> spp.</td>
<td>4</td>
<td>well</td>
</tr>
<tr>
<td></td>
<td><em>Giardia duodenalis</em></td>
<td>12</td>
<td>river, spring, stream, well, and unknown sources</td>
</tr>
<tr>
<td></td>
<td><em>Naegleria fowleri</em></td>
<td>1</td>
<td>well</td>
</tr>
<tr>
<td>Bacteria</td>
<td><em>Campylobacter</em> spp.</td>
<td>15</td>
<td>irrigation water, pond, river, spring, well</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em> O145</td>
<td>1</td>
<td>river</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em> O157:H7</td>
<td>7</td>
<td>river, irrigation canal, river, spring, well, and unknown sources</td>
</tr>
<tr>
<td></td>
<td><em>Helicobacter canadensis</em></td>
<td>1</td>
<td>well</td>
</tr>
<tr>
<td></td>
<td><em>Legionella</em> spp.</td>
<td>23</td>
<td>river, lake, reservoir, well, and unknown sources</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella</em> spp.</td>
<td>3</td>
<td>spring, well</td>
</tr>
<tr>
<td></td>
<td><em>Shigella</em> spp.</td>
<td>1</td>
<td>pond</td>
</tr>
<tr>
<td></td>
<td><em>Yersinia enterocolitica</em></td>
<td>1</td>
<td>well</td>
</tr>
<tr>
<td>Viruses</td>
<td>Hepatitis A</td>
<td>1</td>
<td>spring</td>
</tr>
<tr>
<td></td>
<td>Norovirus</td>
<td>14</td>
<td>pond, well</td>
</tr>
<tr>
<td></td>
<td>Small round-structure virus</td>
<td>1</td>
<td>spring</td>
</tr>
</tbody>
</table>

1. Summary of MMWR surveillance summaries from the CDC. Some outbreaks were caused by several pathogens at once; in this event we listed each pathogen separately as the cause of an outbreak. Web site: [http://www.cdc.gov/healthyswimming/surveillance_drinking_water.htm](http://www.cdc.gov/healthyswimming/surveillance_drinking_water.htm)
2. During the period of surveillance, the species of *Cryptosporidium* that is adapted to humans, called *Cryptosporidium hominis*, had not been fully characterized, hence the species of *Cryptosporidium* isolated from earlier human infections was presumed to be *Cryptosporidium parvum* which is found in a variety of animal species and humans.
3. *Giardia duodenalis* is also known as *Giardia intestinalis* and *Giardia lamblia*. The strain of *giardia duodenalis* infecting humans during the 1999-2006 period of surveillance is presumed to be assemblage A or B, both of which can cause infection in humans.
Waterborne outbreaks due to treatment plant failure

A collaborative voluntary surveillance system for reporting waterborne disease outbreaks in humans has been maintained by the CDC and the EPA since 1971. The results are used to assess the rate of water system deficiencies, determine the type of contaminants associated with waterborne disease outbreaks, and to improve water quality regulations. Throughout the early and mid-1990s these CDC and EPA surveys indicated a steady decline in the percentage of drinking water outbreaks associated with problems at the treatment plant for community water systems but more recently this percentage may have increased. Although these outbreaks were attributed to failures at the treatment plant, it would seem that pathogens were already present in the source water supply such that treatment failure allowed these waterborne pathogens to pass through to the consumer.

1.4 Recreational water

Waterborne illness from swimming, bathing or related activities

Exposure to pathogens can occur during swimming or other water-related recreational activities, particularly when high concentrations of infected individuals are present in the water body. Exposure is related to ingestion, inhalation, or direct contact with contaminated water. Outbreaks have been traced to lakes and rivers (untreated water), swimming pools and water parks (treated water bodies). Outbreaks of disease caused by bacteria, protozoa, and viruses have all been documented as a cause of illness following recreational exposure to water. Fungi may also present a risk to users and employees of recreational water facilities. The source of these pathogens is typically other infected humans for treated water sources (pools, water parks) given the high density of swimmers and recreators at these locations. The source of outbreak-associated pathogens for untreated water supplies like lakes or rivers could be humans, or domestic animals, or wild animals. Whether humans, domestic animals, or wild animals function as the primary pathogen source is dependent on the land-use patterns of the contributing watershed (urban, rural, agricultural, and wild) and the various connections and links between fecal sources and recreational bodies of water. The EPA, in collaboration with the CDC, is continuing to research water quality criteria and to develop regulatory policies that will help States and Tribes strengthen their water quality standards for recreational water. The National Epidemiologic and Environmental Assessment of Recreational Water Study should provide data on new methods for monitoring water quality and their relationship to levels of gastroenteritis among swimmers and beach goers. Table 3 lists pathogens causing gastrointestinal illness associated with exposure to recreational water, with *Cryptosporidium* responsible for about 50 percent of these outbreaks and *Escherichia coli* (E. coli) O157:H7 and norovirus responsible for about 14 and 11 percent, respectively.

1.5 Pathogens of primary concern compared to secondary concern

Although there are possibly thousands of species of viruses, protozoa, bacteria, fungi, and helminthes (worms) that are shed in the feces or urine of animals, relatively few of these organisms have been determined to be the cause of waterborne disease outbreaks in humans in the United States. An outbreak is defined as two or more people involved in the waterborne disease event. It is likely that sporadic illness, where only a single case of illness has happened, occur every year at some background rate due to waterborne transmission from animals to humans for many of these pathogens. Documenting the cause of a single case of illness is problematic because linking an animal source within a watershed to a specific case of illness is difficult to accomplish. Hence, absence of evidence is not evidence of absence. The majority of human waterborne outbreaks are often, but not exclusively, spread through water that is fecally contaminated with intestinal organisms. Free-living pathogens that live in water or soil as their natural environment and do not require an animal or human host to sustain their population, such as the bacteria *Vibrio* spp. and *Legionella* spp., or the amoeba *Naegleria fowleri*, can under appropriate environmental and host conditions, result in skin, gastrointestinal, ocular, or other such infections for humans. Swimming or bathing with open wounds is a key risk factor for acquiring some of these more opportunistic infections.
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Table 4 lists some of the microbial zoonotic pathogens of concern that can be shed in the feces or urine of domestic and wild animals (including poultry) in the United States and can be potentially transmitted to humans through water. This list focuses primarily on pathogens from livestock because animal agriculture is often included as a key suspect source of waterborne pathogens for humans. Inappropriate disposal of companion animal (dog, cat) feces could also lead to waterborne illness in humans from, for example, certain types of Giardia or Campylobacter. Zoonotic pathogens of primary concern are defined in this review as those microbial species that were shown to be infectious to humans in laboratory studies (i.e., human volunteers ingest the pathogen) or were suspected of being infectious to humans (based on results of outbreak investigations). Alternatively, a pathogen shed by livestock whose molecular or DNA pattern is indistinguishable from the pathogen in an infected human, provided there exists an epidemiological link between the human cases and the animal source (e.g., humans swimming in a pond that livestock use as a watering source), is also considered of primary concern.

Pathogens of secondary concern are defined as those microbial species that are infrequently or incapable of being shed by livestock hosts or where documented cases of human waterborne infection are rare to nonexistent in the United States. For example, hepatitis E appears to be a cause of foodborne illness in Asia, but seems uncommon here in the United States.
## Introduction to Waterborne Zoonotic Pathogens in Agricultural Watersheds

**Table 4** An abbreviated list of microbial waterborne zoonotic pathogens excreted by livestock or poultry and potentially transmitted to humans through water

<table>
<thead>
<tr>
<th>Pathogens and levels of concern</th>
<th>Special concerns and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protozoa pathogens of primary concern</strong> 2</td>
<td></td>
</tr>
<tr>
<td><em>Cryptosporidium parvum</em></td>
<td>Low infectious dose; environmentally resistant oocysts; oocyst ~ 5 micron diameter; other species of livestock- or poultry-derived <em>Cryptosporidium</em> rarely isolated from human cases. <em>Cryptosporidium hominis</em> mostly shed by humans.</td>
</tr>
<tr>
<td><em>Giardia duodenalis</em> (a.k.a. <em>G. intestinalis, G. lamblia</em>)</td>
<td>Low infectious dose; environmentally resistant cyst; oval cysts ~ 12 × 15 microns; only a subset of strains or assemblages are infectious for humans.</td>
</tr>
<tr>
<td><strong>Protozoa pathogens of secondary concern</strong> 2</td>
<td></td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>Felines are the definitive host; there are no reports of documented waterborne outbreaks in humans in the United States, but outbreaks in Canada and Panama indicate cases may be occurring undetected.</td>
</tr>
<tr>
<td><em>Balantidium coli</em></td>
<td>Swine suspected but unclear role in human waterborne infection; human waterborne cases rarely documented in the United States.</td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>Unclear role for livestock as source of human waterborne infection; human waterborne cases rarely documented in the United States.</td>
</tr>
<tr>
<td><em>Cyclospora cayetanensis</em></td>
<td>Unclear role for livestock as source of human waterborne infection; human waterborne cases rarely documented in the United States; humans likely the source of oocysts.</td>
</tr>
<tr>
<td><em>Naegleria fowleri</em></td>
<td>Free living amoeba; unclear role for livestock; human waterborne cases rarely documented in the United States.</td>
</tr>
<tr>
<td><em>Blastocystis hominis</em></td>
<td>Unclear role for livestock as source of human waterborne infection; status as a pathogen debated; humans are presumed to be the source of infection, but zoonotic strains may exist.</td>
</tr>
<tr>
<td><strong>Bacterial pathogens of primary concern</strong></td>
<td></td>
</tr>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td><em>Campylobacter jejuni</em> can be common in livestock, birds, dogs; bacteria is susceptible to environmental inactivation</td>
</tr>
<tr>
<td><em>Salmonella</em> serotypes</td>
<td>Many serotypes found in livestock and poultry feces, but only a subset found in human cases.</td>
</tr>
<tr>
<td>Enterohemorrhagic <em>E. coli</em> (<em>E. coli O157:H7</em>, etc.)</td>
<td>Can be highly virulent for humans; low infectious dose; livestock shedding well documented for this pathogen.</td>
</tr>
<tr>
<td><strong>Bacterial pathogens of secondary concern</strong></td>
<td></td>
</tr>
<tr>
<td><em>Brucella</em> spp.</td>
<td>Waterborne transmission unclear; rare cases in the United States and human infection by direct contact with infected animals or food-borne transmission.</td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>Waterborne outbreaks documented, but unclear role for livestock and poultry as source of human waterborne infection; humans often considered the source.</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em> types A and C</td>
<td>Unclear role for livestock and poultry as source of human waterborne infection.</td>
</tr>
<tr>
<td><em>Legionella</em> spp.</td>
<td>Unclear role for livestock and poultry as source of human waterborne infection; free living bacterium; often associated with water distribution systems in a building complex.</td>
</tr>
</tbody>
</table>
## Introduction to Waterborne Zoonotic Pathogens in Agricultural Watersheds

**Leptospira spp.**

Human waterborne cases occur but are infrequent in the United States; human infection typically by direct contact with infective urine; increased concern for tropical watersheds; cattle, pigs, rats and dogs have been suspected sources in various waterborne outbreaks.

**Listeria monocytogenes**

Human cases linked to food-borne illness; waterborne route poorly documented. This bacteria can readily grow outside of animals.

**Mycobacterium (e.g., *M. avium* complex, *M. avium* subspecies *paratuberculosis* and *M. bovis*)**

Long-term survival in the environment and in water may facilitate waterborne transmission of various serotypes of *M. avium* complex and *M. paratuberculosis*. Controversy remains over the claim that *M. avium* complex *paratuberculosis* (Johne's disease) is the causative agent of Crohn's disease in humans; livestock disease eradication programs and milk pasteurization has reduced the incidence of *M. bovis* in humans.

**Plesiomonas shigelloides and Pseudomonas aeruginosa**

Unclear role for livestock and poultry as source of human waterborne infection; free-living bacterium; waterborne outbreaks can be associated with swimming or bathing in a spa.

**Vibrio spp.**

Many documented cases in humans, but unclear role for livestock and poultry as source of human waterborne infection; typical free living waterborne bacterium, including marine.

**Yersinia spp.**

Swine are considered a primary reservoir; human cases rarely documented in the United States; humans cases often linked to foodborne contamination.

### Viral pathogens from livestock or poultry that may have waterborne zoonotic potential

Examples include norovirus, adenovirus, hepatitis E, rotavirus, influenza A

Unclear role for livestock and poultry as significant source of human enteric waterborne viral infection in the United States; majority of illness in humans due to exposure to human-derived viruses, or direct contact with infected animals as in avian influenza. Future work may clarify viral zoonoses passed in water in the United States, such as certain strains of hepatitis E, rotavirus or norovirus shed by animals such as cattle and swine.

### Fungal pathogens from livestock or poultry

**Microsporidia (Enterocytozoon bieneusi, Encephalitozoon cuniculi, Encephalitozoon intestinalis)**

Several genotypes appear to be zoonotic, while other genotypes do not appear linked to human illness at this time; unclear role for livestock as source of human waterborne infection; spores range from 1.5 to 5.0 micron diameter.

**Dermatophytes or ringworm (Microsporum sp. and Trichophyton sp.)**

Unclear role for livestock as source of human waterborne infection; human illness linked to contact with infected humans or animals or contaminated surfaces like public shower stalls.

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1. Adapted from Atwill, 1997.
2. Pathogens of primary concern for this list are defined as those microbial species shown to be infectious to humans during experimental infections, suspected of being infectious to humans as the result of outbreak investigations, or where both the molecular pattern of the causative pathogen is indistinguishable between the pathogen shed by livestock and the infected human(s) and there exists an epidemiological link between the human case(s) and the animal source (e.g., humans swimming in a pond that livestock use as a watering source). Pathogens of secondary concern with respect to livestock or poultry as the source are defined as those microbial species that are infrequently or incapable of being shed by livestock or poultry or where documented cases of human waterborne infection are rare to nonexistent in the United States.
Characteristics of waterborne zoonotic pathogens

Many of our waterborne zoonotic pathogens of greatest public health concern have one or more of the following characteristics that enhance their ability to be transmitted via water:

- The organism is shed into the environment in high numbers by an infected host.
- The organism is highly infectious to humans and animals at low doses.
- The organism can survive and remain infectious in the environment for long periods of time.
- The organism is highly resistant to water treatment processes.
- The organisms can multiply outside of a host under favorable environmental conditions.

The impact of manure contamination in water sources

When accumulated amounts of manure or runoff from fecal waste are discharged into a waterway, large amounts of pathogens or bacterial indicators can quickly enter a water body. Examples of when this can occur include when a liquid manure storage lagoon is inadvertently discharged into a waterway following intense rainfall conditions, when manure is spread too close to a waterway during the rainfall or snowmelt season, placing livestock attractants such as salt too close to a waterway, or using a stream as the sole watering source for livestock. Once large amounts of manure or runoff from manure sources reach surface waters, it is possible that the concentration of waterborne pathogens becomes high enough to result in human or animal illness if this contaminated water is ingested or used to irrigate produce that is close to being harvested. In contrast, when feces or manure are deposited on the terrestrial or dry part of a landscape or captured in a manure management system, numerous natural processes, such as drying, heating, and composting can reduce these pathogen loads and thereby reduce the threat to human or animal health. For example, much of the environmental load of Cryptosporidium parvum that is deposited in bovine fecal pats during the warmer parts of the year may not survive long enough to be transported to a water body to cause human waterborne infection to downstream users. In contrast, during cooler seasons Cryptosporidium parvum can survive weeks to months, thereby increasing the likelihood of human infection. Waterborne pathogens captured in overland or shallow subsurface flow following rainfall are often reduced in concentration as the runoff moves through and over the soil. Nevertheless, given the real possibility that livestock or poultry are infected and shedding high concentrations of one or more zoonotic pathogens, it is prudent to minimize the possibility that animal feces come in contact with surface water sources whenever possible. Moreover, even if pathogen loads have been reduced by natural processes or manure management practices, nutrients and other contaminants may still be present that threaten water quality, hence, proper management of manure and fecal waste is always important.

1.6 References and further reading


2. Waterborne Zoonotic Protozoa

Protozoa are microscopic, single-celled organisms that belong to the kingdom Protista. Although they are single-celled, their structure is complex. Over 50,000 species have been described, of which 10,000 are parasitic. There are several species that are important disease-causing parasites in humans. Parasitic protozoa occur in many different species of animals, including humans. Many protozoa are host-specific, but some, such as Cryptosporidium parvum, can infect a variety of unrelated host species (mice, cattle, humans, etc.). Protozoa may complete their life cycle within one animal or depend on a series of hosts. Some parasitic protozoa are passed from animal to animal directly, while others form cysts that are resistant to environmental stressors, such as temperature, moisture, and pH, and can be passed from host to host via water or food. Protozoa are divided into four principal classes according to their mechanism of locomotion (table 5).

A protozoan life cycle represents a series of stages through which the organism passes in relation to its environment. In simpler cases, an organism reproduces by simple division when nutritional supplies and other conditions are favorable. As conditions change, such as decline of food supply or an increasingly harsh environment, the organism stops dividing and encysts by secreting an outer covering (cyst wall). This cyst wall can be durable. The encysted stage remains until changing conditions (varies by species) induce hatching of the cyst (excyst).

Waterborne zoonotic protozoa of primary concern on watersheds under the influence of agriculture, especially animal agriculture, would include Cryptosporidium parvum and Giardia duodenalis. Cryptosporidium hominis, shed mostly by humans and occasionally by other animals, is also a protozoa of key concern for watersheds or recreational water bodies under the influence of human fecal contamination (e.g., downstream of a sewage treatment plant, municipal pools, water parks). These protozoa cause symptoms such as mild to severe diarrhea, nausea, abdominal cramps, and vomiting in immunocompetent individuals and life-threatening infections for immunocompromised individuals such as those with Acquired Immunodeficiency Syndrome (AIDS). These protozoa are found throughout the world, can coexist in the same host, and are commonly transmitted by the fecal-oral and waterborne routes of transmission in animals and humans. High levels of infection can occur under crowded host conditions, such as dense concentrations of dairy calves. Similarly, high levels of infection occur with dense populations of children, such as at day care centers or municipal pools in summer. A distinguishing feature of these protozoa, compared to many waterborne bacterial pathogen species, is that neither Cryptosporidium nor Giardia can reproduce outside the host. Once the fecal load of protozoa is deposited in the environment by animals or humans, their numbers begin to decline. Another distinguishing feature compared to many waterborne bacterial pathogen species is that illness in humans can be caused by relatively

<table>
<thead>
<tr>
<th>Protozoan Class</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciliophora</td>
<td>Cells with short, hair-like projections called cilia. Major pathogen is Balantidium coli, which causes dysentery in humans.</td>
</tr>
<tr>
<td>Mastigophora</td>
<td>Flagellated cells for motility; major pathogens are Trypanosoma, Leishmania, Trichomonas, Giardia.</td>
</tr>
<tr>
<td>Sarcodina</td>
<td>Amoeba-like motility. Major pathogen is Entamoeba hystolytica, the cause of amoebic dysentery.</td>
</tr>
<tr>
<td>Sporozoa</td>
<td>All are nonmotile, animal parasites with a complex life cycle that may require a different host for sexual and asexual reproduction. Do not engulf particulate matter. Major pathogens are Plasmodium (malaria), Toxoplasma (toxoplasmosis), and Cryptosporidium.</td>
</tr>
</tbody>
</table>
few numbers of these protozoa in water, with as few as 10 *Cryptosporidium* oocysts capable of initiating human infection. In other words, the infectious dose required to infect 50 percent of the people who ingest the pathogen, referred to as ID50, is very low.

During the past decade, these protozoal parasites were the cause of numerous waterborne disease outbreaks of gastroenteritis associated with drinking water from contaminated surface or groundwater sources and from recreational exposure to treated or untreated water venues. Depending on the year, *Cryptosporidium* or *Giardia* is often the leading cause of parasite-associated outbreaks of gastroenteritis associated with drinking water. *Cryptosporidium*, compared to *Giardia*, appears to be the more common cause of gastroenteritis associated with exposure to treated recreational water venues (municipal pool, water park). This is likely the result of *Cryptosporidium* oocysts being more resistant to chlorine-based disinfectants commonly used in pools and parks compared to the cysts of *Giardia duodenalis*. Prevention is particularly difficult because it requires improved filtration methods, selected inactivation methods, as well as education of patrons about hazards associated with fecal accidents, especially in pools frequented by diaper-aged children.

**How infectious are waterborne pathogens for humans?**

The infectious dose, which is the total number of individual microorganisms needed to infect a susceptible human, is different for each pathogen species. A common way to compare the infectivity of different pathogens for humans is to compare their ID50, which is the infectious dose required to infect 50 percent of the people who ingest or otherwise come into contact with the pathogen. Depending on which statistical model and which human infection data we use to make the calculation, the ID50 is about 165 cysts for *Cryptosporidium parvum* oocysts (eggs), about 900 colonies for *Campylobacter jejuni*, and about 750 organisms for *E. coli* O157:H7. Figure 1 shows the estimated infectious dose curve for each of these pathogens for susceptible humans. Ingesting low numbers of each pathogen results in a low risk or probability of infection. The risk of infection rapidly increases as one approaches and exceeds the ID50 value until you reach the point where so many pathogens are being ingested that the risk of infection is about 100 percent on average. For example, ingesting 10 oocysts of *Cryptosporidium parvum* results in a ~4-percent probability of infection; ingesting 100 oocysts results in a ~34-percent probability of infection. Because it is hard to enroll large numbers of humans to volunteer to ingest these various pathogens for research purposes,
there is typically very little data to estimate the ID50. Therefore, there is a lot of uncertainty and error in calculating these curves and values. Moreover, there is rarely any data on how the ID50 would change for more susceptible humans such as young children, elderly, and anyone with a weakened immune system. In addition, there are literally hundreds to thousands of strains with small individual differences for each pathogen species, with each one slightly more or less infectious for humans, so these values for the ID50 are only rough approximations.

2.1 Cryptosporidium parvum

_Cryptosporidium parvum_ is a tiny parasite that is a leading cause of human waterborne illness which is shed in the feces of livestock (especially young stock), some wildlife, and humans. It can live a long time in cool moist conditions and be carried by water long distances in rivers and lakes. Very few parasites are needed to infect humans. It cannot grow or replicate out in the environment. There are numerous other species of _Cryptosporidium_ shed by animals that do not appear to be a significant cause of human illness and in some cases these animal-derived parasites may not be able to infect humans.

_Cryptosporidium parvum_ is a protozoan or single-celled parasite that infects humans and a variety of other mammalian species, including different species of livestock (cattle, sheep, goats, and horses) and various other domestic and wild animals. What was once classified as _Cryptosporidium parvum_ infection in a variety of different hosts in the past has been shown more recently to be different species of _Cryptosporidium_ based on DNA comparisons and animal infectivity studies. Some of these new species appear to be relatively host adapted, such as _Cryptosporidium hominis_ limited primarily to humans, and _Cryptosporidium andersoni_ common to cattle. If such host-adapted species are found to be the cause of a waterborne outbreak, such as _Cryptosporidium hominis_ at a municipal pool, the source of the fecal contamination is likely to be the preferred host for that species of _Cryptosporidium_. This process of naming new species of _Cryptosporidium_ has created much confusion and may occur for some time, given the large numbers of subspecies or genotypes that have been discovered for _Cryptosporidium_ that still have no species designation. This has large ramifications for how we rank and prioritize different mammalian hosts as possible sources of _Cryptosporidium parvum_ on a watershed. Each time the parasitology community decides that a subset of cryptosporidial infections are not _Cryptosporidium parvum_, but perhaps some other species of low zoonotic potential (i.e., uncommon cause of human infection), it lowers the human health-risk profile for that host as a major contributor of zoonotic _Cryptosporidium_ for agricultural watersheds. Moreover, it suggests that historical surveys of the prevalence of infection (percent of individuals infected) conducted prior to the present version of the taxonomy of _Cryptosporidium_ are now biased too high. What was assumed to be _Cryptosporidium parvum_ was probably in many cases a different species of _Cryptosporidium_, causing investigators to overestimate the occurrence of _Cryptosporidium parvum_ in livestock, humans, and wildlife populations.

What is a life cycle?

A life cycle refers to the entire period in which a new generation of an organism is produced, through asexual or sexual reproduction.

**Life cycle of _Cryptosporidium parvum_**

The life cycle of _Cryptosporidium parvum_ can be completed in just a few days, but varies with host species. The small, colorless, ovoid-to-spherical oocyst (the environmental infectious state), containing three sporozoites which are the actual entity that initiates infection (fig. 2), is shed in the feces and is immediately infective to a susceptible animal. When the oocyst is ingested by the host, the sporozoites are released once

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Figure 2  _Cryptosporidium_ oocysts (top) and sporozoites (bottom) released from an oocyst during excystation

Courtesy University of California, Agriculture and Natural Resources (UCANR)
the oocyst shell opens, and these sporozoites then parasitize the cells lining the intestine to start a new cycle of infection. In many ways this process is like a science fiction movie where the alien egg lies dormant until a host draws too close, only to be quickly infected as the egg hatches and the alien newborn invades the body of its new host. Ingestion of only a few oocysts can ultimately lead to a heavy infection due to the parasite replicating many times within the infected host. Oocysts can be shed in feces for several days to over a week by an infected individual, with up to 10 million oocysts per gram (about 280 million per ounce) of feces during the peak of shedding. A recent study reported that an infected 6-day-old calf would produce as much as \(3.9 \times 10^{10}\) oocysts until 12 days old. This level of oocyst production is similar to what has been observed in humans, with infected persons capable of shedding up to \(10^8\) to \(10^9\) per bowel movement and to excrete oocysts for up to 50 days. Given the low infectious dose of Cryptosporidium for humans, this high amount of oocyst production by infected animals underscores the need to minimize any connection between the feces of infected animals and drinking water sources.

**Cryptosporidium as a cause of human waterborne outbreaks of illness**

Waterborne outbreaks of cryptosporidiosis have been reported in the United States since 1984. The annual surveillance data compiled by the CDC and EPA indicates that during 1997 through 2004, Cryptosporidium was an important cause of waterborne disease for drinking water and exposure to recreational water in the United States. This protozoan parasite was the most frequently identified agent for outbreaks in recreational waters in the United States from 1971 to 2000, and remained a leading cause of illness associated with exposure to recreational water during 2003, 2004, and 2006. The leading suspected sources of contamination or contributing factors, regardless of pathogen species for outbreaks associated with recreational exposure, was overcrowded bathing conditions, human fecal or sewage contamination, and deficient management of water treatment facilities, with animal feces suspected to have been the cause for 2 percent of outbreaks with treated water and 18 percent of outbreaks with untreated water. Despite many advances in water treatment technology and an increase in public awareness on how to prevent recreational exposure to this protozoan parasite, a relatively large number of cryptosporidium caused outbreaks occurred across the United States in 2006, with 18 waterborne outbreaks officially reported as of July 24, 2007, to the CDC Waterborne Disease and Outbreak Surveillance System.

Earlier studies found that Cryptosporidium oocysts were present in 39 to 87 percent of surface water (rivers, lakes) tested throughout the United States from 1988 to 1993. The source or sources of the oocysts was typically unknown. These pioneering studies were often done without the application of DNA fingerprinting technology and prior to the current list of Cryptosporidium species, many of which do not appear to be significant causes of human illness. It is likely that if these large surveys of waterborne Cryptosporidium were conducted today, they would reveal the presence of a variety of host-adapted species of Cryptosporidium or species of low zoonotic potential. Nevertheless, Cryptosporidium parvum and Cryptosporidium hominis are apparently common enough in untreated water supplies given the continuing occurrence of this parasite as a cause of human illness associated with improperly treated drinking water or recreating in untreated surface water.

**Infectious dose of Cryptosporidium**

A series of experimental studies in healthy humans determined that the infectious dose at which 50 percent of subjects acquired infection (ID\(_{50}\)) ranged from about 10 to just over 1,000 oocysts, depending on the isolate of Cryptosporidium parvum. As few as 50 oocysts of Cryptosporidium parvum are infectious for young dairy calves. The ID\(_{50}\) for people with preexisting antibodies to the Iowa strain of Cryptosporidium parvum was about 20 times higher, meaning it took much larger amounts of oocysts to initiate an infection in previously exposed individuals. An ID\(_{50}\) of about 10 oocysts was also estimated for Cryptosporidium hominis (formerly known as Cryptosporidium parvum genotype I) for healthy adults, revealing just how infectious this species of Cryptosporidium can be during crowded swimming or bathing conditions and the frequent isolation of this species of Cryptosporidium during outbreaks of gastroenteritis associated with treated recreational water.

**Cryptosporidium shedding in animals**

The age of cattle that have the highest prevalence of Cryptosporidium parvum infection (percent infected) and the highest daily environmental loading rate (oocysts per animal unit per day) in the United States are young calves. Fecal shedding of Cryptosporidium parvum can occur in older cattle, but usually at lower prevalence and low concentration. Many of the earlier studies were conducted prior to the decision to name Cryptosporidium bovis as a species distinct from Cryptosporidium parvum; hence, prevalence estimates of Cryptosporidium parvum in cattle populations from these earlier studies are likely overstated. Fecal shedding of Cryptosporidium oocysts...
around the time of giving birth has been documented in ewes. Other species such as horses, goats, pigs, geese, and chickens, are also capable of excreting Cryptosporidium oocysts, but some of these infected animals appear to shed host-adapted strains of Cryptosporidium that are rarely isolated from human cases of cryptosporidiosis (Cryptosporidium felis from cats, Cryptosporidium canis from dogs, Cryptosporidium suis in pigs, Cryptosporidium meleagris from birds, etc.). Although most species of mammalian wildlife are infected with strains of Cryptosporidium that currently seem to be of low public health concern, wildlife contribute to the overall pool of oocysts identified in environmental samples, which can lead to regulatory demands for new treatment plants for municipal water treatment systems.

Unique types of Cryptosporidium in wildlife

Cryptosporidium in wildlife remains poorly studied given the large number of wildlife species in the United States. In many cases Cryptosporidium shed by wildlife appears to be different from the types shed by livestock and humans. Moreover, it is uncommon for many of these unique types to be identified as the cause of human infection. For example, California ground squirrels which are ubiquitous throughout California at lower elevations shed high concentrations of several unique types of Cryptosporidium but to date they have not been shown to be a cause of human infection. Over time there may be clarification which of these new strains or new species of wildlife Cryptosporidium are a threat to public health.

Cryptosporidium in human sewage

Previous research in the United States showed that 40 percent of reclaimed effluent samples collected in six locations were positive for Cryptosporidium and the average concentration was seven oocysts per liter. Using quantitative polymerase chain reaction method, the concentration of Cryptosporidium parvum oocysts were estimated to be 129 oocysts per liter of municipal water treatment sludge samples. Depending on the depth in the filtration system in a treatment facility, Cryptosporidium oocysts concentration were estimated at 25 to 800 oocysts per 100 liters of reclaimed effluents. The occurrence of Cryptosporidium in five sewage treatment plants was found in all samples from all plants throughout the year, with mean values ranging from 103 to 139 oocysts per liter and a minimum and maximum of 40 and 340 oocysts, respectively. It is unclear what percentage of these oocysts from sewage effluent and biosolids are infectious for humans, but agricultural use of human sewage biosolids as a soil amendment may need to take into account the possibility that infectious amounts of Cryptosporidium are present in this material and take the necessary precautions to prevent water contamination and animal infection.

2.2 Giardia duodenalis

Giardia duodenalis is a tiny parasite that is a leading cause of human waterborne illness. Only certain strains, called assemblages, can infect humans. These human-infectious strains can be shed in the feces of some livestock, some wildlife species, companion animals like dogs, and humans. It can survive a moderate amount of time in cool moist conditions and can be transported by rivers and across lakes. Very few parasites are needed to infect humans. It cannot grow or replicate out in the environment.

G. duodenalis is generally similar to C. parvum in that it infects the intestinal tract of a wide variety of mammals, such as humans, dogs, cats, cattle, sheep, horses, and a variety of wildlife species such as rats, muskrats, and beaver. This species of Giardia is further divided into subgroups referred to as Assemblages A through G. Assemblage A infects humans and other primates, livestock, companion animals, rodents, and other mammals; Assemblage B infects humans, other primates, and dogs; Assemblages C and D infect dogs; Assemblage E infects cattle and other hoofed livestock; Assemblage F infects cats; and Assemblage G infects rats. Of particular concern for agricultural watersheds is Assemblage A because this type of Giardia is infective to humans and also shed by livestock, dogs and cats, humans, and some wildlife species. Some surface water surveys have shown that Giardia cysts are more widespread than Cryptosporidium. This protozoan parasite is a common cause of waterborne outbreaks of enteric disease, linked to both exposure to recreational water and from drinking contaminated or improperly treated water. Along with Cryptosporidium, Giardia has been one of the most frequent parasites to cause waterborne outbreaks of enteric disease in recent years. The source of infection for sporadic (nonoutbreak) cases of waterborne human Giardia infection is often unknown, most likely due to the variety of mammals that can shed human-infective cysts of Assemblage A.
Introduction to Waterborne Zoonotic Pathogens in Agricultural Watersheds

**Biology of Giardia**

*Giardia* (fig. 3) is a flagellated protozoan with two forms: a motile form or trophozoite, and a cyst. Trophozoite attaches to the surface of the epithelial cells of the upper small intestine near the stomach where it feeds and reproduces. The cyst is ovoid to ellipsoidal and is the environmentally resistant stage that is able to infect a new host. When cysts are ingested by a susceptible host, the life cycle takes about a week to be completed (fig. 4). Trophozoites leave the cysts and attach to the surface of the epithelial cells of the small intestine by an adhesive disc. Here they feed and reproduce asexually. The trophozoites form into cysts in the lower part of the small intestine. Cysts leave the host in the feces and may or may not be immediately infective to an animal. Cysts are shed in feces intermittently, with infected individuals such as children able to excrete up to 108 to 109 cysts per day. Ingesting as few as 10 cysts has been reported to result in infection.

**Is Giardia from animals infectious for humans?**

For well over a decade, it has been debated how important zoonotic transmission (pathogens transmitted between animals and humans) is in the annual incidence of human waterborne giardiasis. There is growing evidence that dogs and humans may be capable of sharing specific assemblages of *G. duodenalis*, but the relationship between livestock infection and human waterborne illness is still not clear. Although both beef and dairy cattle have been shown to be commonly infected with *G. duodenalis* for many decades, it would appear that the majority of infections in cattle are of Assemblage E (non-zoonotic) and a smaller percentage of Assemblage A (human infective). Recent studies at the USDA Agricultural Research Service (ARS) have shown that 13 to 15 percent of calves, 3 percent of yearlings, and 2 percent of adult dairy cows were shedding cysts of Assemblage A. The prevalence of fecal shedding is much higher for Assemblage E compared to Assemblage A, which may have led early investigators and public health officials to overestimate the importance of livestock as a source of zoonotic *Giardia*. The study of recent reviews is encouraged to better understand the ongoing challenge of establishing a definitive role for livestock as a cause of human giardiasis. The zoonotic genotypes of *Giardia duodenalis* have been detected in several wildlife species that encompass nearly all mammalian orders including Artiodactyla, Rodentia, Primates, Carnivora, and Hyracoidea, while novel *Giardia* genotypes are still being discovered in wildlife.
2.3 Waterborne protozoa of secondary zoonotic concern

Waterborne protozoa of secondary importance with respect to this document are those whose transmission to humans does not appear to have a major livestock component or where a waterborne route is rarely documented. According to the CDC, wild and domestic cats serve as the host for *Toxoplasma gondii*; infections which are attributable to undercooked meat exposure, or to feline fecal exposure and contaminated water and soil exposure. Hence, neither livestock nor poultry are involved in the contamination of water with this protozoan. Furthermore, it would appear that only a few outbreaks of toxoplasmosis in humans have been linked to a waterborne route of exposure in North America, such as in Chicago and British Columbia. *Balantidium coli*, a ciliated protozoan found in the intestines of humans, pigs, and a few other mammals, is rarely reported in the United States. The potential for this pathogen to be transmitted from pigs to humans remains a subject of debate. One source of the intestinal amoeba, *Entamoeba histolytica*, is thought to be humans. Livestock have no clear role in human infection to date, but future work may clarify this association. Similarly, it is unknown if data exists that clearly implicates livestock or poultry as the source of a waterborne outbreak of *Cyclospora cayetanensis* in humans. Furthermore, a detailed fecal survey of domestic animals in an area with active human infection did not find infected pigs, cattle, horses, goats, dogs, cats, chickens, turkeys, or other such animals, suggesting that humans were the only source of infection. *Naegleria fowleri* is a free-living amoeba and the cause of primary amoebic meningoencephalitis, whereby the amoeba enters the brain through the nasal cavity leading to high case-fatality. Infection is often associated with bathing in thermal pools. There exists controversy regarding the human pathogenicity of the amoeba, *Blastocystis hominis*, with indirect evidence that *B. hominis* shed by animals is infectious for humans via direct contact (animal handlers) but little evidence that a waterborne route of zoonotic transmission is involved in the epidemiology of human infection.

2.4 References and further reading


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3. Waterborne Zoonotic Bacteria

While this publication focuses on several species of bacteria that can be shed by livestock or poultry and are potentially harmful to humans, many bacteria are beneficial for decomposing dead material and releasing nutrients back into the environment for sustenance of plants and animals.

What are bacteria?
Bacteria are a group of microorganisms that lack membrane-bound organelles, and are considered simpler than plant and animal cells. They have a cell wall and some have an outer protective layer. Bacteria are unicellular and have various shapes; including, spherical (coccus), rod-shaped (bacillus), comma-shaped (vibrio), spiral (spirillum), or corkscrew-shaped (spirochete). Generally, they range from 0.5 to 5.0 micrometers. Motile species (those that can move on their own) bear at least one fine hair (flagella) arising from their surface. Many possess an outer, slimy capsule, and some have the ability to produce an encysted or resting form (endospore). Bacteria reproduce by simple division of their cell.

3.1 Escherichia coli O157:H7 (E. coli O157:H7)

*E. coli* O157:H7 is one of many strains or serotypes of this bacterium (fig. 5). There are other Shiga-toxin producing *E. coli* (STEC) that can cause human illness (e.g., O26, O103, O111), but *E. coli* O157:H7 is the most frequent serotype of this group of bacteria detected as a cause of waterborne infection in humans in the United States. This serotype can be passed from animal to human or human to human via several routes of transmission, such as ingesting contaminated food or water, direct contact with infected animals, direct contact with animal’s bedding or their pens, and person-to-person direct contact transmission. When *E. coli* is used as an indicator of water quality, *E. coli* refers to the mutualistic or generally harmless serotypes that help maintain normal intestinal functions and are considered to be one of the better, but far from perfect bacterial indicators for public health protection. In contrast, *E. coli* O157:H7 can be a virulent bacterium that can cause a variety of clinical symptoms, among which are bloody diarrhea and hemorrhagic colitis, dehydration, and hemolytic uremic syndrome. It is a highly infectious organism for humans, in that ten to several hundred bacteria are capable of causing illness.

Young children and the elderly are often the groups most susceptible to STEC infections. The combination of letters and numbers in the name of the bacterium refers to the specific O and H antigen markers found on cell surface structures that distinguish it from other strains or serotypes of *E. coli*. Although most strains are harmless and live in the intestines of healthy humans and animals, many of the *E. coli* O157 and non-O157 enterohemorrhagic strains carry a variety of virulence factors, such as the genes that encode Shiga toxin 1 or 2.

Risk factors for human outbreaks of *E. coli* O157:H7

According to the CDC, approximately 73,000 cases of *E. coli* O157:H7 infection occur each year. With respect to human outbreaks of disease caused by this pathogen during 1982 to 2002, consumption of contaminated food accounted for 52 percent of the 350 outbreaks and 61 percent of the 8,598 outbreak-associated human cases. Contaminated hamburger and contaminated produce were responsible for 20 and 21 percent, respectively, of these outbreak-associated human cases. Among produce-related foodborne illness, about half the cases were due to cross-contamination in a kitchen, with the other half due to produce contaminated prior to purchase by such possible processes as bacterial contamination of irrigation water, animal or human defecation in the field, and contamination during processing or handling.
Waterborne transmission accounted for about 18 percent of the 8,598 outbreak-associated human cases, with the majority of these infections due to contaminated drinking water (15 percent of cases) compared to recreational exposure to lakes, ponds, and pools (3 percent of cases). Person-to-person transmission can occur if infected persons, especially food handlers, do not wash their hands. Direct contact with infected animals is generally a minor cause of outbreaks, though five E. coli O157 outbreaks related to animal exposure were reported by the EPA from 2006 to 2008.

To identify risk factors for cases involving one person (sporadic human cases and not an outbreak of two or more people), a case-control study was performed from 1996 to 1997 and found that ingestion of undercooked hamburger, eating at a restaurant, exposure to cattle, visiting a farm, and other nonwaterborne risk factors were associated with infection.

While the incidence of infection may have declined in recent years compared to a 1996 through 1998 survey by the EPA, outbreaks of E. coli O157:H7 have continued to occur since 2006, with several major outbreaks associated with contaminated food, such as fresh produce and raw or undercooked meat.

During the late summer and early fall of 2006, a multi-state foodborne outbreak of E. coli O157:H7 occurred in association with the consumption of bagged spinach grown from at least one field in San Benito County, CA. Over 200 cases of human illness and three deaths occurred. During the investigation E. coli O157:H7 with a similar fingerprint to the human cases and contaminated spinach was isolated from river water, cattle feces, and wild pig feces, but the final conclusion of this investigation was that, "No definitive determination could be made regarding how E. coli O157:H7 pathogens contaminated spinach in this outbreak."

In response to this foodborne threat, the leafy green industry of California adopted a marketing agreement that, among other things, put forth a series of good agricultural practices (GAPs) designed to reduce the risk of E. coli O157:H7 contamination of leafy greens. For example, fields of leafy greens may not be located closer than 400 feet from a confined animal feeding operation; surface water supplies should not be located closer than 100 feet to untreated manure; irrigation water should not have more than 126 colony forming units (cfu) per 100 milliliter (mL) of commensal E. coli. Many of these GAPs attempt to reduce waterborne transport of pathogens into the produce production environment. Yet, at the same time, growers struggle with the decision to remove water quality practices because they may function to attract wildlife to habitat adjacent to the produce fields, or worse, encourage wildlife to enter a produce field where they might defecate and create a food safety hazard. This potential conflict in resource goals (food safety versus wildlife habitat and water quality) highlights an urgent need for research to clarify which practices improve, have no effect, or degrade produce food safety.

### Fecal shedding of E. coli O157:H7 by animals

Numerous surveys have shown that beef and dairy cattle, sheep, horses, and other such domestic animals can shed low to high concentrations of E. coli O157:H7 in their feces, with the percentage of infection for livestock ranging from less than 1 to over 90 percent. The percentage of individuals shedding this bacterium in their feces is dependent in part on such factors as age of animal (often higher in young stock), season (often higher in summer), management factors, and diagnostic test used to detect this bacterium. When livestock become infected with this bacterium, the concentration of E. coli O157:H7 in feces can vary widely, ranging from 102 to over 106 bacteria or cfu per gram (g) feces (cfu/g). Fecal shedding is often sporadic from day to day, fluctuating from several hundred cfu/g feces to 200,000 cfu/g feces in as few as 2 days. The duration of fecal shedding is also highly variable, with some cattle and sheep shedding from a few days to weeks while other cattle or calves shed intermittently for up to 27 weeks following experimental infection. Livestock manure containing E. coli O157:H7 has been linked to or suspected as the cause of a variety of food- and waterborne outbreaks of human illness over the past 20 years, indicating the need to carefully handle and dispose of stored manures and to encourage proper grazing management so as to eliminate this route of transmission to humans. Additionally, E. coli O157:H7 has been shown to persist in water trough sediment for at least 4 months. Inoculating calves with 106 cfu/liter (L) of water resulted in several weeks to over a month of fecal shedding of E. coli O157:H7 at concentrations of 100 to 106 cfu/g feces.

In addition to cattle and sheep, E. coli O157:H7 has been isolated from a wide variety of animals, including dogs, horses, white-tailed deer, elk, coyotes, raccoon, various species of birds such as starlings, gulls, crows, and geese, feral pigs, flies, and others. Many species of wildlife have not been systematically examined for E. coli O157:H7 (all seasons, different age groups, different climates, etc.), hence, there is no clear understanding of how wildlife populations on agricultural or rural watersheds participate in the environmental cycling of this pathogen.
3.2 Campylobacter

Campylobacter is a common cause of bacterial gastrointestinal illness in humans and can be transmitted when contaminated water is consumed. It is shed by humans and a wide variety of animals, including livestock, companion animals such as dogs, and wildlife, especially birds. It typically does not survive as long as the protozoal parasites nor as long as E. coli O157:H7 or Salmonella, but it has a fairly low infectious dose (see fig. 1; page 12). The species that causes the most human illness is called Campylobacter jejuni.

Campylobacter is one of the most common causes of human bacterial gastroenteritis in the United States, with several species of Campylobacter capable of causing infection in humans (e.g., C. jejuni, C. coli, C. laritii). C. jejuni accounts for almost all of human-diagnosed cases, with C. coli being the second most common species associated with human illness. As few as 500 to 800 organisms appear sufficient to cause clinical illness in humans. Much of human campylobacteriosis occurs as sporadic cases as opposed to outbreaks involving large numbers of people, with 80 percent of total cases estimated to be from a foodborne route of transmission. There are relatively few reported outbreaks of Cryptosporidium associated with waterborne transmission compared to the protozoal parasites, C. parvum and G. duodenalis, and the bacterial parasite E. coli O157:H7 (tables 1 and 2; pages 3 and 4). Key risk factors for sporadic campylobacteriosis are consumption or exposure to contaminated foods (e.g., poultry products, raw milk), exposure to pets and farm animals, especially when they have diarrhea, and drinking untreated surface water. Intense rainfall may have been a predisposing factor for a significant outbreak of campylobacteriosis in Canada. Direct human-to-human transmission is uncommon, but direct animal-to-human transmission via contact with calves has been implicated in some human cases.

Fecal shedding of Campylobacter by livestock and wildlife

C. jejuni is frequently shed in the feces of livestock, poultry, wild mammals, and birds. A recent multistate survey in dairy cattle found about 51 percent of animals tested positive for either C. jejuni or C. coli. Similar results were found in 1996 where 38 percent of dairy cattle in the United States were shedding C. jejuni and 2 percent C. coli. Similarly, 31 percent of dairy cattle in Washington State tested positive for C. jejuni and 6 percent for C. coli. There is some evidence that calves are rapidly colonized soon after birth, with peak shedding occurring a few months later. A survey done in California found that 5 percent of adult beef cattle shed Campylobacter in their feces, but the prevalence of this bacterium was only 0.5 percent in fecal pats that had aged several days from the same herd. Additional food animals found to shed Campylobacter include sheep, chickens, turkeys, ducks, and swine. A wide variety of wildlife are known to shed one or more species of Campylobacter, including, but not limited to, crows, common gulls, pigeons, puffins, ducks, Canada geese, sandhill cranes, rats, starlings, and sparrows. Companion animals such as dogs can shed several different species of Campylobacter and are a risk factor for human infection.

C. jejuni is frequently cultured from a variety of surface water supplies, stream sediments, sewage effluents, and manure slurries. Concentrations in freshwater have ranged from less than one to several hundred most probable number (MPN)/100 mL, with 10-fold higher levels observed in sewage effluents. Effluent from poultry processing facilities have been shown to contain C. jejuni that in some cases are similar to human isolates. One study found that sewage sludge contained Campylobacter in a concentration of 10^3 to 10^5 cfu per 100 mL on average.

3.3 Salmonella enterica

Salmonella is a common cause of bacterial gastrointestinal illness in humans. Most infections are foodborne, but it can also be transmitted when we drink contaminated water. It is shed by humans and a wide variety of animals, including livestock, companion animals such as dogs, and wildlife but especially birds. It has the ability to survive fairly long, even under dry conditions. Unlike the protozoal parasites Cryptosporidium parvum and Giardia duodenalis, it can replicate and increase its numbers in the environment. It is easily killed using standard water treatment methods or by boiling water at home.

There are more than 2,500 reported strains or serotypes of Salmonella enterica found in a wide variety of host species, including humans, livestock, companion animals, reptiles, avian species, and mammalian wildlife. The nomenclature for naming of different strains or serotypes of S. enterica has undergone considerable revision this past decade and the reader is referred to Brenner et al., 2000, J. Clin. Microbiol. 38:2465–2467, for an explanation of the current system. Briefly, there are six subspecies of S. enterica (named or assigned a Roman numeral): enterica (or I), salamae (or II), arizonae (or IIIa), diarizonae (or IIIb), houtenae (or IV), and indica (or VI). In addition, each isolate of S. enterica is labeled according to its O and H antigens, and other possible features.
The convention for naming is the following: *S. enterica* subspecies [space] O antigens [colon] phase 1 H antigen [colon] phase 2 H antigen. So, for example, *S. enterica* subsp. *enterica* 4,12:i:1,2 has the O 4 and 12 antigens and the i and 1,2 complex for phase 1 and phase 2 H antigens, respectively.

**Salmonella serotypes that cause human infection**

Over 90 percent of human, avian, and other mammalian infections are attributed to serotypes from *S. enterica* subspecies *enterica*, with the majority of human infections in the United States caused by a small number of serotypes within this subspecies, such as *S. enterica* subsp. *enterica* serotypes Typhimurium and Enteritidis (see fig. 6). It is estimated that 1.4 million human infections and 400 to 600 deaths occur each year from these serotypes of *S. enterica* subsp. *enterica* serotypes, with 95 percent of these infections due to a foodborne route of transmission. These infections can be fatal in immunocompromised persons, young children, and the elderly. Most clinical illness appears as individual cases apparently unrelated to outbreaks, and the majority of outbreaks are associated with the foodborne route of transmission. Foods often implicated in outbreaks include poultry and poultry products, meat and meat products, dairy products, seafood, and fresh produce.

*S. enterica* subsp. *enterica* serotype Enteritidis, now one of the more common serotypes isolated from human cases, is associated with ingestion of contaminated shell eggs. Interestingly, relative to the protozoal parasites, *Cryptosporidium parvum* and *Giardia duodenalis*, and *E. coli* O157:H7, few outbreaks of *S. enterica* have been associated with exposure to recreational water or to drinking water (tables 1 and 2; pages 3 and 4), despite its widespread occurrence in domestic and wild animals. Many of the serotypes of *S. enterica* shed by domestic animals or wildlife are infrequent causes of human illness, such as *S. enterica* subspecies *enterica* serotype Dublin. This is a result of failing to detect these rare serotypes of *S. enterica* in infected humans, humans not coming into contact with the infected host species, or an interspecies barrier existing for many of these serotypes of *S. enterica* in that they are not infectious for the majority of humans.

**Shedding of Salmonella by livestock**

Animals used for food production are common carriers of numerous serotypes of *S. enterica*, with a range of prevalence occurring in different livestock species depending on animal species, *Salmonella* serotype, livestock production practices, and other such factors. A survey of layer facilities in the United States found that 7.1 percent were positive for *Salmonella* Enteritidis, with this bacterium frequently present in the litter. It was estimated that 27 to 31 percent of dairy herds across the country have one or more cattle shedding *Salmonella*, with the herd-level animal prevalence ranging from 0 to 37 percent for lactating dairy cattle. In a study investigating the prevalence of *Salmonella* in diverse environmental farm samples, the prevalence of *Salmonella* was 57 percent in swine farms, 18 percent in dairy farms, and 16 percent in poultry farms. In Midwest swine farms, mean individual pig prevalence for *Salmonella* was 5 percent. In New York dairies (440 dairy farms enrolled) *Salmonella* was isolated from 1.5 percent of milk filters. Serotypes of *S. enterica* in poultry farms in the United States included Typhimurium, Montevideo, Kentucky, and Enteritidis.

**Salmonella shedding in wildlife add recent work from USDA**

*S. enterica* is shed in the feces of a wide variety of wildlife, ranging from avian species (e.g., Western scrub jay, sparrows, crows), mammals ranging from carnivores (e.g., coyotes), omnivores (e.g., feral pig, striped skunk, opossum), and herbivores (e.g., deer, elk), wide variety of rodent species (e.g., deer mice, California ground squirrels, meadow vole), and reptiles (e.g., snakes and turtles). Among wild birds trapped on California dairies, about 3 percent of cowbirds and house sparrows, 2 percent of Brewer's blackbirds and house finches, and 1 percent of starlings, red-winged blackbirds, and pigeons shed various serotypes of *Salmonella*. Mice can shed up to $10^5$ *Salmonella* per dropping. In one study, the prevalence of *Salmonella* ranged from 1 to 7.7 percent in white-
tailed deer, with serotypes known to be pathogenic to humans. More recently, 4 to 5 percent of wild rodents trapped along the edges of produce fields have been found to shed *Salmonella*, along with other pathogens such as *Campylobacter jejuni*, *E. coli* O157 and *E. coli* O26.

**Salmonella common in human sewage**

Human sewage effluents are known to carry *Salmonella*, with municipal waste discharges potentially linked to infecting wildlife and a nearby commercial layer flock with *Salmonella enteritidis*. This suggests that serotypes shared between food animals and humans such as *Salmonella enteritidis* may circulate between human and animal populations if human foods of animal origin and human sewage are not properly handled so as to prevent *Salmonella* contamination and environmental dissemination. In six different existing wastewater treatment systems, *Salmonella* densities in municipal sludge were found to be 217 to 1,000 MPN/g in primary biosolids, 400 to 750 MPN/g in waste-activated sludge, and 4 to 208 MPN/g in anaerobically digested biosolids.

3.4 Waterborne bacteria of secondary zoonotic concern

This group includes waterborne bacteria of secondary importance. This ranking of lower concern for this group of bacteria is because waterborne transmission to humans does not appear to have a major livestock component or the waterborne route has been rarely documented.

**Brucella and Listeria**

The waterborne route of transmission is not known to play a significant role in human infection. For example, 99 percent of the ~2,500 cases per year of *Listeria monocytogenes* are estimated to be from foodborne infections, leaving only 25 annual cases to be associated with other routes of transmission such as contaminated water.

**Yersinia**

The estimated number of human infections with *Yersinia enterocolitica* (fig 7.) in the United States is about 100,000 per year, with 90 percent of these cases associated with a foodborne route of transmission. This is far less than the reported number of campylobacteriosis and salmonellosis cases. Most human cases of sporadic yersiniosis in the United States are caused by the 4/O:3 bioserotype of *Y. enterocolitica*.

Although much of the epidemiology of human infection is poorly understood, consumption of contaminated food, especially pork meat products, is considered a primary route of transmission. Numerous studies have documented fecal shedding of this bacterium in pigs, but dogs, cats, sheep, cattle, and rodents can also be sources of *Yersinia. Y. enterocolitica* has been identified from streams in urban and agricultural watersheds. This bacterium was capable of surviving in excess of 1 year in sterile spring water held at 4 °C (39 °F), but reduced levels are observed when water is held at higher temperatures.

**Leptospira**

Human infections with different species of *Leptospira* are uncommon in the continental United States, but clinical cases continue to occur within the Pacific Islands, such as the Hawaiian Islands. From 1999 through 2006, slightly fewer than 300 cases of leptospirosis were reported within the State of Hawaii. Although human infections are confined mostly to direct contact with infected animals, as might occur among veterinarians and slaughterhouse workers, ingestion or contact with contaminated water has been associated with numerous outbreaks in the United States. For example, flooding in Iowa was associated with human leptospirosis. Cattle, pigs, rats, and dogs have all been suspected sources of various waterborne outbreaks in the past. The Hawaii State Department of Health provides the following synopsis, "Leptospirosis is a bacterial disease that is primarily carried by rats and mice, although dogs, pigs, cattle, and horses can also become infected. The disease is generally transmitted to humans by exposure to fresh water that is contaminated with urine from infected animals. Infection can take place when contaminated water enters the body through the mouth, nose, eyes or open wounds."

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**Figure 7** Wayson stain of *Yersinia pestis* with the characteristic "safety pin" appearance of the bacteria

*Courtesy of CDC*
Hawaii State Department of Health provides the following recommendations to reduce the risk of contracting leptospirosis in their State:

- Do not swim, wade or play in fresh water or mud when you have cuts or abrasions.
- When swimming, do not place your head underwater.
- Do not drink stream water without boiling or chemically treating it first.
- Keep water catchment collection areas free from overhanging branches and prevent access to these areas by animals.
- Drain potentially contaminated areas of standing water.
- Control rats, mice, and mongooses around the home and at work sites.
- Vaccinate pets and farm animals.

**Legionella, Shigella, Plesiomonas, Psuedomonas, Vibrio**

The role of livestock and wildlife as a host of these waterborne pathogens is not well documented and possibly of low significance in the ecology of these pathogens. Species of *Legionella*, *Shigella*, *Plesiomonas*, *Psuedomonas*, and *Vibrio* are typically soil or aquatic free-living bacteria and do not require a mammalian host to sustain their populations. *Vibrio* spp. are a common cause of foodborne illness associated with consumption of raw oysters, indicating their common presence in marine shellfish-harvesting locations.

**Mycobacterium (M. avium complex, M. avium subspecies paratuberculosis, M. bovis)**

Human infection with *M. bovis* in the United States has been substantially reduced by the implementation of nationwide disease eradication programs in cattle and milk pasteurization that can kill the organism prior to human consumption. Waterborne transmission may be possible, however, when humans ingest water contaminated with these organisms from infected domestic or wild animals. Serotypes of *M. avium* complex (MAC) are widespread in the environment and both MAC and *M. avium* subspecies *paratuberculosis* (Johne’s disease in cattle) can survive extended periods of time in soil, in the environment, and in aquatic systems, which leads to the possibility of waterborne transmission (fig. 8). Humans infected with HIV are at risk of waterborne transmission from *M. avium* complex. Considerable debate exists regarding the assertion that *M. avium* subspecies *paratuberculosis* from cattle is the causative agent of Crohn's disease in humans: future research will hopefully clarify this association.
**Bacterial Insights**

Bacteria are so widespread that only the most general statements can be made about their life history and ecology. They are everywhere on Earth, even in the most hostile of environments. Some live in soil, plants, or water; others live within or on humans, animals, and plants. Bacteria that live within or on the surface of their host can either cause harm to their host, be beneficial to their host, or cause no harm nor help their host, referred to as commensal bacteria. Many of the bacteria that harm their hosts do so by producing toxins that affect one or more cellular functions of their host.

Bacteria have a wide range of environmental and nutritional requirements. They can be classified into three groups based on their need for oxygen.

- **Aerobic bacteria** thrive in the presence of oxygen and require it for continued growth and existence.
- **Anaerobic bacteria** thrive in oxygen-free environments.
- **Facultative anaerobes** can survive in either environment, although they prefer to grow in the presence of oxygen.

Cyanobacteria, commonly called blue-green algae, are a separate group of bacteria that deserves mention here. Cyanobacteria have the ability to fix atmospheric nitrogen into usable organic molecules. They have chlorophyll and are photosynthetic. Although they are not pathogens themselves, they can produce toxins, such as anatoxin and microcystins, that have been implicated as the cause of human waterborne disease outbreaks. Many regulate their buoyancy, often floating to the surface of a water body where livestock have easy access to concentrated populations of organisms. Cattle drinking water contaminated with cyanobacterial toxins can die if sufficient toxin is consumed. Drinking water facilities are often faced with trihalomethane problems associated with the necessity to chlorinate water that has excess algal growth. Excess algae becomes a major problem in open bodies of water such as lakes or ponds when cell growth stops and the organisms begin to die. As this happens, decomposing bacteria consume oxygen (respiration) as they break down the dead cyanobacteria cells, causing oxygen depletion of the water body. This may lead to fish mortality and other negative consequences caused by low oxygen.

### 3.5 References and further reading


Introduction to Waterborne Zoonotic Pathogens in Agricultural Watersheds


4. Other Waterborne Pathogens of Secondary Zoonotic Concern

4.1 Enteric viruses

Viruses are tiny agents of disease that infect plants, animals, and even bacteria. They use their hosts’ cells for reproduction and are unable to reproduce outside their host. When viruses are outside host cells, they exist as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) surrounded by a protein coat or capsid ranging from 20 to 300 nanometers (1 nanometer = one billionth of a meter). No other structures are typically found in viruses, such as a nucleus or chloroplast. Because a virus does not have any of these organelles, it has no metabolism.

Viruses are not active unless they are within living cells. When in contact with a host cell, the virus can inject its genetic material into the cell, leaving its protein shell behind. Once within the host cell, the virus uses the cell’s own cellular processes, such as protein synthesis, to produce more viruses. In essence, the virus forces the cell to replicate the virus’ own genetic material and protective shell. Once replicated, the new viruses leave the host cell and are ready to invade others. Some viruses may remain dormant inside their host cell (lysogenic phase) for long periods, causing no obvious change in the host cell. However, when a dormant virus is stimulated (lytic phase), new viruses are formed and burst out of the host cell, killing it and going on to infect other cells.

Hundreds of known viruses cause a wide range of diseases in humans, other animals, insects, bacteria, and plants. Within a species, 100 or more types of viruses may infect that species alone. Most viruses are specific to a single host species. A few are more general and are capable of infecting one or more animal species, including humans.

Waterborne transmission of human-excreted enteric viruses is well documented and a serious cause of gastrointestinal illness in the United States. Enteric viruses are viruses shed from the gastrointestinal tract and typically excreted in the feces. Examples of such human-derived waterborne viruses include norovirus (previously named Norwalk-like virus), enterovirus, hepatitis A & E, rotavirus, and astrovirus. Other types of viruses target other locations of the host’s body, such as the respiratory tract (influenza virus), skin (papilloma virus), and brain (rabies). Cases of human waterborne infection from livestock or poultry shedding animal-derived strains of these enteric viruses appear to occur infrequently in the United States, likely due to a high degree of host specificity leading to interspecies barriers for these viruses. In other words, animal-derived enteric viruses present in the United States do not appear to be common causes of human infections when shed into the environment. The evidence supporting their zoonotic potential is based on noroviruses isolated from swine sharing some genetic similarity with strains of human noroviruses. In addition, norovirus has been shown to replicate in pigs. In contrast, noroviruses from cattle appear dissimilar to human strains, leading some investigators to claim that bovine noroviruses are unlikely to be a human health risk. Hepatitis E virus from domestic and feral pigs appears to be infectious for humans, with strong evidence for foodborne transmission. It is less clear if hepatitis E virus from domestic and feral pigs is being transmitted to humans through water, though given the large amounts of virus that are shed in the feces of infected pigs, waterborne transmission seems possible. Strains of influenza A virus from avian and swine have been isolated from human cases, but the role of waterborne transmission is unclear in the epidemiology of human infections. Runoff from agricultural fields that have received surface applied municipal sludge may be a source of waterborne viruses for humans on agricultural watersheds. In addition, septic tank effluent may be a source of pathogenic viruses in the subsurface environment. Some authors have argued that livestock-derived rotaviruses are generally not considered a source of human waterborne infection. Table 6 lists some enteric viruses that can cause waterborne disease in humans.
Table 6  Partial list of waterborne human enteric viruses ¹

<table>
<thead>
<tr>
<th>Group</th>
<th>Type</th>
<th>Key Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>ds DNA</td>
<td>Commonly isolated from sewage effluents, frequent cause of infection in young children</td>
</tr>
<tr>
<td>Astrovirus</td>
<td>ss RNA</td>
<td>Isolated from sewage effluent, polluted rivers, shellfish, wading pools; common infection in young children</td>
</tr>
<tr>
<td>Saporovirus</td>
<td>ss RNA</td>
<td>Sewage effluent and polluted rivers; milder infection in children</td>
</tr>
<tr>
<td>Hepatitis A and E</td>
<td>ss RNA</td>
<td>Sewage effluent and polluted rivers; waterborne outbreaks and shellfish consumption due to Hepatitis A (HAV); Hepatitis E (HEV) from domestic and feral pigs appear infectious for humans; HEV infections rare in the U.S.</td>
</tr>
<tr>
<td>Norovirus</td>
<td>ss RNA</td>
<td>Very common foodborne infection; can cause serious vomiting; isolated from sewage effluent, water contaminated with sewage; human cases documented from drinking water and recreational exposure to untreated water</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>ds RNA</td>
<td>Infected humans shed high concentrations in feces; common cause of diarrhea in children; isolated from sewage, rivers and lakes, estuarine and marine water</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>ss RNA</td>
<td>Sewage effluent and polluted rivers; shellfish; milder symptoms</td>
</tr>
</tbody>
</table>

1. Adapted from (1) Carter, 2005, and (2) Fong and Kipp, 2005.
2. ds = double-stranded RNA or DNA, ss = single-stranded RNA or DNA.

4.2 Enteric fungi

Fungi are a group of eukaryotic organisms that possess a chitinous cell wall. The majority of species grow as multicellular filaments called hyphae forming a mycelium, and some fungal species also grow as single cells. Sexual and asexual reproduction of the fungi is commonly via spores. Fungi live in soil or in organic matter, or live as symbionts of plants or animals. They are important for ecosystems by decomposing organic matter and are indispensable in nutrient cycling and exchange. Most fungi are invisible to the naked eye but some fungi become visible when fruiting, such as mushrooms and molds.

Microsporidia are single-celled, obligate intracellular microorganisms that were previously classified as protozoa. However, in recent years studies have suggested that microsporidia are more related to and have evolved from fungi, resulting in their recent reclassification as fungi. Approximately 1,200 species of microsporidia have been identified and most of these organisms infect invertebrates and fish. Fourteen species of microsporidia are known to infect humans and most cases are related to Enterocytozoon bieneusi and Encephalitozoon spp. Enterocytozoon bieneusi has been found in cattle, muskrats, beavers, foxes, otters, raccoons, dogs, pigs, and goats. Encephalitozoon intestinalis (formerly known as Septata intestinalis) has been confirmed in pigs in Europe, and Encephalitozoon hellem infection was mostly found in birds.

Enterocytozoon bieneusi has been detected in tertiary sewage effluent, surface water and groundwater, in swimming pools, and in the Seine River. Encephalitozoon intestinalis has been detected in tertiary sewage effluent, surface water and groundwater, in drinking water, and in irrigation water used for crops. A waterborne outbreak of microsporidiosis was documented in France and lake contamination was suspected. The role that livestock or wildlife have in infecting humans via the waterborne route of exposure is unclear at this time, but further research may clarify the waterborne route of transmission for human infection.

4.3 Worms (helminthes)

Helminthes are worms that may be free-living or parasitic in plants and animals. The parasitic worms of greatest concern in water are Platyhelminthes, or flatworms (flukes and tapeworms), and Nematoda (roundworms). Most flukes and tapeworms require several hosts to complete their life cycles. For some worms, humans are needed to complete the worm's life cycle (humans are the definitive host), for example, Taenia saginata, or the beef tapeworm, must develop in humans to produce eggs. When humans are the definitive host, the worm matures and sheds...
eggs in the human intestine. In other cases, humans are an accidental host, through which the developing worm may form into a tissue cyst when lodged in the liver or other locations. Examples of these species are *Echinococcus multilocularis* or hydatid disease and *Fasciola hepatica* or fasciolosis. Because the developing worm lodges in an organ, its cyst causes clinical symptoms due to damage to the organ. Infection with one or a few roundworms, or *Ascaris* sp., may not be noticed. Infection with numerous roundworms may result in pneumonia during the migratory phase when larvae that have hatched from the ingested eggs penetrate into the lungs. Vague digestive tract discomfort sometimes accompanies intestinal infection, but small children that have more than a few worms may have intestinal blockage because of the large size of the worms.

*Ascaris lumbricoides* is one of the largest parasitic roundworms and is the most common parasite found in humans where they function as the definitive host. It is estimated that 20 to 25 percent of the world’s population is infected with this nematode. The adult female of this species can measure up to 18 inches long, with males generally shorter. The adult worms live in the small intestine and eggs are passed in the feces. A single female can produce up to 200,000 eggs each day. *Ascarus suum*, a round worm common in pigs, has larvae that will migrate to the lungs and die. This can cause a particularly serious form of pneumonia. Adult worms of this species may develop in young children's intestines. Nematode eggs, such as those of *Ascaris* sp., can contaminate crops when irrigation water has been inadvertently contaminated with human sewage or minimally treated biosolids. Sewage sludge from human feces may also be a source of crop contamination if inadequate pretreatment is used. Humans can be infected if they eat raw produce that is contaminated with live ascaris eggs. Hookworm is a nematode that is endemic in moist tropical and subtropical regions. When inadequately treated sewage is used on crop-lands, in combination with the naturally high soil moisture, one can expect hookworm infection. The infection can be contracted by persons walking barefoot over contaminated soil.

### 4.4 References and further reading


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5. Harmful Algae and Pfiesteria

Algae are a natural part of marine and freshwater environments. They help form the base of the food web and provide oxygen to the water. Some harmful algal blooms, like toxic Pfiesteria outbreaks, can be detrimental at low concentrations. In other cases, like certain red and brown tides, harmful effects occur when the algae reach concentrations high enough to discolor the water. However, not all algal blooms that discolor the water are harmful. Many red tides have no negative effects on marine life, people, or the environment.

Some kinds of algal blooms, like some kinds of red tides, are harmful because the algae produce one or more toxins that poison fish or shellfish. They also can pose human health risks when people come in contact with affected water. These toxic algal blooms may also kill seabirds and other animals indirectly when the toxins are passed up the food chain. Certain kinds of these toxic algal blooms can cause human health problems via consumption of contaminated seafood. Ciguatera fish poisoning, amnesic shellfish poisoning, and paralytic shellfish poisoning are various examples of toxic algae. Most algal blooms however, are not toxic, but are still considered harmful if they reduce the amount of light or oxygen in the water, consequently killing sea grasses, fish, or other marine life.

Recent attention has been focused on Pfiesteria piscicida, which has been associated with fish lesions and fish kills in coastal water from Delaware to Alabama. These organisms are believed to be native and are probably common inhabitants of estuarine water within their range. These microbes have not been found in freshwater lakes, streams, or other inland waters.

Pfiesteria identification

Pfiesteria belongs to the dinoflagellates group of algae. These algae are microscopic, normally free-swimming, single-celled organisms (fig. 9). Although many dinoflagellates are plant-like and obtain energy by photosynthesis, others, including Pfiesteria, are more animal-like and acquire some or all of their energy by eating other algae and often incorporating their prey's chloroplast into their own cells. The vast majority of dinoflagellates are not toxic. Pfiesteria, however, is a known toxin-producing dinoflagellate. Discovered in 1988 by researchers at North Carolina State University, Pfiesteria piscicida is now known to have a highly complex life cycle, with a number of life stages capable of producing toxins. Pfiesteria was named in honor of the late Dr. Lois Pfiester, who made substantial contributions to our current knowledge of the complex lifestyles of the dinoflagellates.

Pfiesteria and human health problems

Preliminary evidence suggests that exposure to water where toxic forms of Pfiesteria are active may cause memory loss, confusion, and a variety of other symptoms including respiratory, skin, and gastrointestinal problems. It has been shown that similar human health effects can be caused by exposure to Pfiesteria toxins in a laboratory setting. To date, other Pfiesteria-like organisms have not been shown to cause human illness. Pfiesteria is not contagious or infectious and cannot be caught like a cold or flu. No evidence shows that Pfiesteria-related illnesses are associated with the consumption of finfish, shellfish, or crustaceans, such as crabs, lobsters, and shrimp. Any human health problems associated with the microbe stem from its release of toxins into river and estuarine water and human contact with that water, rather than the organism infecting a person.

The CDC, in cooperation with State health departments in Delaware, Florida, Georgia, Maryland, North Carolina, South Carolina, and Virginia, have established a surveillance system to collect reports of hu-
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man illness thought to be related to exposure to Pfiesteria and Pfiesteria-like organisms in estuarine water. This and other ongoing research efforts are expected to further delineate the nature, extent, and duration of any Pfiesteria-related human health effects.

**Nutrients and Pfiesteria**

Nutrients, such as nitrogen and phosphorus, are thought to encourage the growth of Pfiesteria populations by stimulating the growth of algae that Pfiesteria feeds on when in its nontoxic forms. Some evidence suggests that nutrients may also directly stimulate the growth of Pfiesteria, but more research is needed to show this conclusively. At this time the precise role that nutrients and other factors may play in promoting toxic outbreaks of Pfiesteria is not clear and is an area of active research. Excess nutrients are common pollutants in coastal water. Chief sources of nutrient pollution in coastal areas are sewage treatment plants, septic tanks, polluted runoff from suburban landscapes and agricultural operations, and air pollutants that settle on the land and water.

**Causes of toxic Pfiesteria outbreaks**

The exact conditions that cause toxic outbreaks of Pfiesteria to develop are not fully understood. Scientists generally agree that a high density of fish must be present to trigger the shift of Pfiesteria cells into toxic forms. However, other factors may contribute to toxic Pfiesteria outbreaks by promoting the growth of Pfiesteria populations in coastal water. These factors include warm, brackish, poorly flushed water and high levels of nutrients.

5.1 References and further reading


6. Bacterial Indicators of Fecal Contamination

Bacterial indicators used to monitor water quality

The direct identification and counting of microbial pathogens in water typically is not practical because of the cost of pursuing the many pathogenic bacteria and protozoa that may be present in a water sample. Identification of the different bacterial species often relies on unique growth requirements and metabolic functions of specific bacterial species plated onto selective culture that favors the growth of one type of bacteria over the other. In addition, a variety of new technologies have been developed, such as DNA-based methods like quantitative polymerase chain reaction (qPCR) that allow detection and possibly quantification of specific species of bacteria in water. For most water quality monitoring applications, simple culture methods are used to enumerate key organisms common to fecal material. These key organisms are indicator bacteria that are used to signal the potential presence of waterborne pathogens. Although indicator bacteria are typically not pathogenic in and of themselves, high numbers may indicate fecal contamination from leaky septic tanks, animal manure, or faulty wastewater treatment facilities. However, some of these indicator bacterial species also live in soil and on plants, and their presence in water does not correlate with fecal contamination, thereby creating a false alarm for the presence of microbial contaminants. In addition, numerous outbreaks have occurred when bacterial indicators were within acceptable levels, indicating that false negatives also occur with these procedures.

Bacterial indicators

Total coliforms include a large number of different types of bacteria found both in feces and in the environment. They are not good indicators of waterborne pathogens. Fecal coliforms are a subset of the larger group of total coliforms that are found in feces and in the environment. They are not a very good indicator of waterborne pathogens most of the time. *Escherichia coli* (*E. coli*) is one of several species within the fecal coliform group. It is found in high concentrations in feces of almost all animals and humans, but it is also found in the environment. Sometimes *E. coli* is a good indicator of waterborne pathogens, but many times it does not correlate very well with the occurrence of waterborne pathogens. Typically, even when *E. coli* is present in water, there are often few waterborne pathogens of primary concern, such as *Cryptosporidium parvum* or *Salmonella* at detectable levels.

Fecal bacteria have traditionally been used as an indicator of the possible presence of pathogens in surface water and to signal an elevated risk of enteric disease for swimmers and bathers. Both *E. coli* and Enterococci are considered to have a higher degree of association with outbreaks of gastrointestinal illness than fecal coliforms or total coliforms and are recommended by the EPA as more appropriate bacteria-indicator organisms for monitoring water quality.

Total coliforms

Total coliform (table 7) is a broad category of indicator bacteria and was originally believed to indicate the presence of fecal pollution. One criticism of this group is that too many species of bacteria occur in the environment in the absence of fecal contamination; hence, it is too generic.

Fecal coliform

Fecal coliform can be thought of as a subgroup of total coliforms, with many of the species comprising this group present in moderate to high concentration in fecal material from wildlife, livestock, companion animals, sewage, and sometimes soil. This subgroup is commonly used as an indicator of fecal and bacterial contamination in watersheds. One criticism, similar to the criticism for total coliform, is that several of the bacterial species included in this group can grow and survive in the environment without fecal input, thereby falsely indicating the presence of fecal contamination. Bacteria known for this ability to grow in the environment are species of *Klebsiella*. The presence of *Cryptosporidium* and *Giardia* are often not correlated.

*E. coli*

*E. coli* is a member of the fecal coliform group. This bacterial species is in high concentration in fecal material, especially in mammals. It is not uncommon to find tens of millions of *E. coli* in a gram of fresh feces, similar to the volume of a thimble (fig. 10). As a consequence, it does not take a large amount of fecal contamination to elevate this bacterial species in confined bodies of water (ponds), creeks or small streams. Under appropriate environmental conditions (warm, moist, available nutrients), *E. coli* can rapidly grow or replicate outside of its host and as a result can correlate poorly with enteric pathogens. This can occur in more tropical watersheds or during summer in temperate climates when warmer conditions prevail.
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Table 7  Comparison of commonly used fecal bacteria indicators for water quality monitoring

<table>
<thead>
<tr>
<th>Microbial indicator</th>
<th>Designation Properties</th>
<th>Federal standard</th>
<th>Primary (^2/)Secondary (^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coliforms (TC)</td>
<td>• Originally believed to indicate the presence of fecal pollution&lt;br&gt;• Widely distributed in nature: soils, water, flora, fauna&lt;br&gt;• Contains members of <em>Escherichia</em>, <em>Citrobacter</em>, <em>Klebsiella</em>, and <em>Enterobacter</em> identified by incubation at 35 °C (95 °F)</td>
<td>1,000 cfu/100mL</td>
<td>2,000 cfu/100mL</td>
</tr>
<tr>
<td>Fecal coliforms (FC)</td>
<td>• Subgroup of Total coliforms that can originate from intestinal tracts of animals; can be found in soils and plants&lt;br&gt;• Cultured by increasing the incubation temperature to 44.5 °C (112 °F)&lt;br&gt;• Remains the predominant indicator used to assess bacterial pollution in watersheds</td>
<td>1,000 cfu/100mL</td>
<td>2,000 cfu/100mL</td>
</tr>
</tbody>
</table>

**General Standard**

| *Escherichia coli*        | • Member of the FC group<br>• Presence can correlate with gastrointestinal illness associated with swimming in either fresh and marine water<br>• *E. coli* O157:H7 is rare, oxin-producing strain of this common bacterium                                                                                   | 126 cfu/100mL    |                |

| Enterococci              | • Various species contained within this group, *E. faecalis*, *E. faecium*, and *E. avium*<br>• Commonly found in intestinal tracts of mammals, including humans<br>• Presence can correlate with human gastrointestinal illness associated with swimming in either fresh and marine water | Fresh water 33 cfu/100mL | Marine water 35 cfu/100mL \(^4\) |

1. Individual States may have higher standards, but not lower<br>2. Primary recreational contact includes activities such as swimming and fishing.<br>3. Secondary recreational contact includes limited water contact, such as boating.<br>4. Ambient water quality criteria for bacteria.

For example, most studies show that adult cattle in California are only infrequently infected with *C. parvum*, yet every animal is likely shedding millions of *E. coli* in its feces, indicating that the presence of waterborne *E. coli* from grazing of adult cattle is unlikely to correlate with the presence of bovine-derived *C. parvum*. Enterococci are an additional group of indicator bacteria not associated with fecal coliforms or *E. coli*. Similar to *E. coli*, this group of bacteria can be in high concentrations in mammalian fecal material (often millions per gram of feces) and given their resistance to salt, are often used as an indicator of marine water quality.

**Water quality standards**

The Federal water quality standards for indicator bacteria are shown in table 8. EPA is encouraging States and authorized Tribes to use *E. coli* or Enterococci as the basis of their water quality criteria for monitoring freshwater. For marine recreational waters, EPA recommends the use of Enterococci as the basis for water quality criteria for bacteria. Further, for coastal recreational waters (i.e., marine waters, coastal estuaries, and the Great Lakes), States were required to adopt bacteriological criteria using EPA’s Clean Water Act, Section 304(a) recommendations by April 10, 2004. The final rule, Water Quality Standards for Coastal and Great Lakes Recreation Waters, was promulgated on November 16, 2004. Through this final rule, EPA
Figure 10  The concentration of indicator E. coli and Enterococcus from 91 fresh fecal pats from beef cattle grazing rangeland in the southern Sierra Nevada foothills, Madera County, California.

Table 8  Federal water quality standards for indicator bacteria

<table>
<thead>
<tr>
<th></th>
<th>Steady state geometric mean indicator density (cfu/100mL)</th>
<th>Single sample maximum allowable density (cfu/100mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Designated beach area</td>
</tr>
<tr>
<td>Freshwater</td>
<td></td>
<td>Moderate full body contact recreation</td>
</tr>
<tr>
<td>Enterococci</td>
<td>33</td>
<td>Lightly used full body contact recreation</td>
</tr>
<tr>
<td>E. coli</td>
<td>126</td>
<td>Infrequently used full body contact recreation</td>
</tr>
<tr>
<td>Marine water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococci</td>
<td>35</td>
<td>104</td>
</tr>
<tr>
<td>E. coli</td>
<td>126</td>
<td>235</td>
</tr>
</tbody>
</table>

Technical Note No. 9, September 2012
established Federal standards for those States and territories with coastal recreation waters that had not yet adopted bacteria criteria into their water quality standards as protective of health as EPA 1986 criteria.

One important point to note on bacterial indicators is that waterborne pathogens, such as Cryptosporidium parvum, Shigella sp., and E. coli O157:H7 may be present in water that meets all bacterial water quality standards yet not be in high enough concentration to cause human illness. This clearly indicates that better detection methods are needed for pathogens in water, such as EPA Method 1623 for Giardia and Cryptosporidium.

Why do bacterial indicators often poorly correlate with waterborne zoonotic pathogens in mammals?

Bacterial indicators, such as indicator E. coli and fecal coliforms, are typically in high concentrations in fresh feces from livestock and mammalian wildlife. Indicator E. coli is the background E. coli that is naturally present in mammalian and other species’ intestines, with usually ~100 percent of mammals, including humans, having these bacteria in their intestinal tract. Figure 11 shows the range of concentration of indicator E. coli and Enterococcus in a herd of adult beef cattle grazing rangeland in the California foothills. Concentrations of E. coli per gram feces ranged from a few million to over 100 million bacteria. In contrast to this common occurrence of indicator E. coli in the intestines of mammals, only a subset of mammals will be infected at any point in time with such pathogens as Cryptosporidium parvum or Salmonella. Furthermore, when a mammal is infected with a microbial pathogen, it will shed from low to high quantities of the pathogen depending on the immune status of the mammal and its ability to fight off the infection. For example, only 5 to 10 percent of adult beef cattle on rangeland, and cattle in feedlots in the western United States have been shown to shed Cryptosporidium, and when an animal is infected it typically sheds only 1 to 50 eggs or oocysts per gram feces. Hence, when beef cattle feces are deposited in a waterway, this can elevate waterborne concentrations of indicator E. coli but to a much lesser degree elevate waterborne concentrations of Cryptosporidium in proximity to the cattle if the cattle are infected. Said another way, for every Cryptosporidium deposited in adult bovine feces, there are usually hundreds of thousands of indicator E. coli also deposited, creating the possibility that the water sample will exceed water quality standards for indicator E. coli but without the presence of measurable Cryptosporidium in the water sample. Similar trends occur for the relationship between indicator E. coli and various pathogens such as E. coli O157:H7, Salmonella, or the protozoa parasite, Giardia duodenalis.

6.1 References and further reading


7. Survival of Pathogens in the Environment

Once an enteric pathogen leaves the host's gastrointestinal track or urinary system, it must adjust to ambient conditions that are substantially different from the host (e.g., temperature, moisture, pH, nutrients, and oxygen). Biological, chemical, and physical stressors interact in complex ways and affect the survival and viability of pathogens along the transport pathway. Figure 11 illustrates the stresses and processes the organisms are subjected to outside the host. Certain pathogens exit the host in an environmentally resistant form (e.g., cyst) that can remain viable in the environment for significant periods of time. This allows the organism an opportunity to be ingested by another host and repeat its life cycle. Some pathogens are shed in great numbers, with the random chance that some organisms will find a new host to repeat the life cycle. In either case, the survival of a pathogen outside its host is dependent on media type and many environmental factors.

Figure 11  Potential inactivation process during various transport pathways for waterborne pathogens in watersheds

7.1 Media type and factors affecting survival of pathogens

Types of environmental media relevant to agriculture include manure, slurry, plants, soil, and water. Major environmental factors affecting pathogen survival include but are not limited to temperature, pH, salinity, nutrients, organic matter, presence of oxygen, presence of native microorganisms, soil type and composition, and moisture content, etc. Effects of these factors on pathogen survival vary with media types and pathogens.

Bacterial respiration and waste digestion

Anaerobic literally means "without air" while aerobic means "requiring air" (where "air" generally means oxygen). The term "anaerobic organism" refers to any organism that does not require oxygen for growth and metabolism, while "aerobic organism" refers to any organisms that require oxygen for growth and metabolism. Anaerobic digestion is a series of processes in which microorganisms break down biodegradable...
material in the absence of oxygen. It is widely used to treat wastewater sludges and organic wastes because it provides volume and mass reduction of the input material. Biogas typically refers to a gas produced by the biological breakdown of organic matter in the absence of oxygen. Thermophiles are organisms that thrive at relatively high temperatures, between 45 °C and 80 °C (113 °F and 176 °F). Mesophiles are organisms that grow best in moderate temperature, neither too hot nor too cold, typically between 15 °C and 40 °C (77 °F and 104 °F). Psychrophiles are organisms that are capable of growth and reproduction in cold temperatures. Thermophilic anaerobic digestion is a process that uses heat-loving bacteria in an oxygen-free environment to break down organic waste.

**Solid animal manure**

The floors of a livestock production facility or animal feeding operation are a major collection point for fecal wastes, and as a consequence, an area where bacteria and parasites accumulate. The total solid content of manure depends on the animal species, housing conditions, and the type of bedding used. The addition of other waste and water all influence the total solid content of manure and thus influence the survivability of pathogens in manure. Generally, manure is considered to be in a solid form when solids comprise at least 20 percent of its mass. The survival of pathogens in solid manure, particularly in solid manure stored anaerobically, is of great interest and concern because many microorganisms survive longer in manure stored anaerobically than in aerobic conditions. This is partially because the heat generated by bacterial breakdown of organic material in openly stored manure and manure compost is high enough to reduce bacterial survival. Consequently, most pathogens decline in solid manure storage. Waterborne pathogens of primary concern are inactivated (effectively killed) at varying rates by the spontaneous generation of heat in stacked manure, with the rate of inactivation dependent on such factors as moisture content, the amount of bulk material, and the amount of aeration. Sufficient turning of stacked manure and even active or piped aeration is essential to properly compost the waste and significantly reduce the survival of pathogens.

**Liquid manure and slurry**

Liquid manure and slurries are mixtures of feces, urine, wash water, rainwater, and varying amounts of feed and animal bedding. The defining difference between the two is their ratio of solids to liquids, slurries have a moisture content of 75 to 95 percent while liquid manure has >95 percent moisture. Some waste management systems for liquid manure and slurries include anaerobic digestion as a component. Thermophilic anaerobic digestion at 49 °C to 54 °C (120 °F to 129 °F) can greatly reduce the number of pathogens; however, mesophilic anaerobic digestion only attains a temperature of 30 °C to 38 °C (86 ºF to 100.4 ºF), which does not destroy many pathogens. Table 9 illustrates the factors affecting survival of pathogens in liquid manure and slurry.

### Plants

Pathogens that make their way to plant surfaces are a growing concern for the produce industry. Visible light, ultraviolet radiation, temperature, and drying are some of the factors that affect pathogen survival on plants; another is plant species. Stems and broad leaves can provide refuge via shading from ultraviolet radiation and can extend the viability of bacteria. Survival of pathogens on plants may also depend upon the height of plants and possibly the indigenous bacterial flora living on the surface. Differences in survival rates of pathogens on plant surfaces can be the result of multiple factors, including humidity, competing microbrial flora, exposure to solar radiation, shading, and access to nutrients that can allow bacterial replication. Rainfall can extend pathogen life by providing needed moisture for microbial survival.

### Soils

Application of manure and sewage sludge onto crop land soil has the potential to disseminate pathogens into soil. The survival of various microorganisms in soil can vary from less than 30 days to more than a year, depending in part on the type of pathogen. Soil composition, temperature, moisture content, dryness, pH, indigenous microflora, and other environmental conditions have been found to affect the survival of pathogens. Organic and clay particles in soil can effectively trap viruses, bacteria, and protozoa, and longer-term attachment to soil can lead to mortality of pathogens. Periods of drying between irrigation or during the dry summer season can increase the rate of pathogen inactivation in the soil.

### Water

The survival of most pathogens in water is highly variable depending upon the quality of the water and many other factors that contribute to the rate of die-off or inactivation of pathogens. Water turbidity, temperature, pH, oxygen levels, presence of pesticides and nutrients, organic matter content, and solar (e.g., ultraviolet) radiation can affect the survival of pathogens in water.

The normal pH range for most water bodies is close to 7 (neutral) which would not affect pathogen survival.
More extreme ranges of pH, such as less than 4.0 or greater than 8.0, can result in higher rates of inactivation for many bacterial species. Nutrient enrichment of water may play an important role in survival or growth of pathogens. Nitrogen is important for the survival of bacteria in water, allowing cells to survive the competition from indigenous bacterial flora and to go through periods of dormancy that prolong their viability. Physical treatments including freezing, heating, filtration, sedimentation, and ultraviolet (UV) irradiation affect survival or removal of protozoan parasites. Visible light was also found to affect pathogen survival, where both *E. coli* and *Enterococcus faecalis* can be reduced when exposed to visible light in both freshwater and marine systems.

Many protozoa feed on bacteria, including bacterial pathogens, and many invertebrates feed on both bacteria and protozoa. Bacterial flora native to water and sediment may be more adept at extracting nutrients than enteric bacteria, which can subsequently affect the survival of enteric bacteria in the aquatic environment.

### Table 9  Factors affecting survival of pathogens during the storage of animal waste as slurry

<table>
<thead>
<tr>
<th>Factor</th>
<th>Consideration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogen loading</td>
<td>Percent of herd infected (higher potential for shedding)</td>
</tr>
<tr>
<td>Frequency of waste additions (continued supply of pathogens)</td>
<td>Amount of dilution (lower concentration, less reproductive success for bacteria)</td>
</tr>
<tr>
<td>Length of storage</td>
<td>Longer storage generally leads to increased inactivation</td>
</tr>
<tr>
<td>Treatment</td>
<td>Inactivation rate dependent on temperature and duration (anaerobic, aerobic, aeration and composting processes) disinfectants, metals and/or pesticide content</td>
</tr>
<tr>
<td>Incidental treatment in storage</td>
<td>Drying (die-off from desiccation), freeze/thaw cycles</td>
</tr>
<tr>
<td>Slurry characteristics</td>
<td>Available oxygen, pH, temperature, nutrients, total solids content (important to some organisms), inhibitors (antibiotics, potential antibiotic resistance)</td>
</tr>
<tr>
<td>Pathogen survival mechanisms</td>
<td>Vegetative form vs. spore, endospore, or cyst (higher resistance to external environment)</td>
</tr>
</tbody>
</table>

1. Adapted from Kelly, 1978
can resemble an exponential decay curve. To better explain this trend, assume that for every unit of time (hour, day, etc.) about 90 percent of the number of indicator E. coli die regardless of how many are started with. If the initial count is 1,000,000 E. coli per-gram feces, as shown in figure 9, then after one unit of time there will be 100,000 E. coli per-gram feces, after two units of time only about 10,000 E. coli per-gram feces; and so on until after six units of time there will be no remaining viable E. coli. A single log reduction of the number of infective pathogens is when the number of infective pathogens is reduced by 90 percent, leaving only 10 percent (i.e., 100 microbes reduced to 10 microbes, or 10 percent of the original amount). The decimal reduction time is very similar: It is the duration of time needed for a 90-percent reduction in the number of pathogens, leaving only 10 percent of the original number still infective. For example, beginning with X numbers of infective pathogens, after one unit of decimal reduction time there will be 0.1X remaining infective pathogens (0.1 = 10 percent), or a one log reduction.

7.2.1 Protozoa

Cryptosporidium

A variety of processes, such as solar radiation, temperature variations, and drying can inactivate Cryptosporidium oocysts in agricultural operations and in manure. Temperature is one of the most critical factors governing the survival of oocysts in the environment, with rapid inactivation occurring once the temperature exceeds 40 ºC (104 ºF). Inactivation at higher temperatures can result from oocysts encysting and releasing their sporozoites, which rapidly use up their energy reserves if they cannot find a host to infect. Much like mammals during hibernation, cooler temperatures slow metabolic rates, extend energy reserves, and do not stimulate excystation of the oocyst. The protection of cool temperatures ends once they fall below the point of freezing; inactivation at freezing temperatures can result in physical damage to the oocyst.

Differences in the terms infectivity, inactivation, viability and survival time

The infectivity of a pathogen is defined as the ability of a disease causing agent to enter the host, attach and often invade one or more cells of an organ or tissue, and then multiply in the newly infected host. In other words, infectivity is the measure of a pathogen's ability to infect a host. This is typically measured by a test that involves infecting a new host or infecting a culture of cells in the laboratory and then monitoring for infection. To inactivate a pathogen is to destroy the pathogen's ability to infect a new host, such as killing the pathogen through heat. Practices that inactivate pathogens may also be said to decrease the infectivity of a pathogen, however this is rarely confirmed via some bioassay. Inactivation (or die-off) is typically expressed as a rate (k) (e.g. 0.05 log/day). Although many authors use the term viability as equivalent to infectivity, the term viability is more properly thought of as the ability of a pathogen to conclude a subset of the steps required for infectivity, but not necessarily all of the steps needed to infect a host. Using baseball as an analogy, we would be viable if we could pick up the bat and swing it at a ball, but infectivity is the ability to handle a bat, hit the ball, run the bases, and get back to home base. The survival time of a pathogen is a more loosely defined term. Survival time may be expressed in units of time needed to inactivate all pathogens in a media, or as a percentage of pathogens that are still viable or infective after a given time. All four of these terms are used to express components of the rate of survival of pathogens in the environment and can be found throughout the remainder of this chapter.

The importance of temperature in survival is apparent across multiple media types, from manure to soil to water, though the other factors affecting survival are much more complex in manure and soil than in water. Pasteurization of milk and cooking food is a time-tested method of inactivating pathogens so that they cannot infect us in the food we eat. Therefore, good animal waste management practices, such as stacking manure from dairy young stock (including neonatal calves) and composting manure solids to ensure that thermophilic processes occur, will lead to increased rates of inactivation of C. parvum oocysts. Table 10 illustrates the survival of Cryptosporidium oocysts in water at different temperatures.

When feces are deposited on dry land by an infected host (livestock, wildlife, humans), there are a variety of pathways by which oocysts can reach a body of water, and numerous processes that effectively reduce the number of infective oocysts that ultimately reach a water body. Studies have shown that most oocysts (>90 percent) are retained in fecal pats during natural rainfall or in simulated rainfall conditions, thereby not washing into nearby waterways. This suggests that there is an immediate water quality benefit from making sure animal feces are deposited on land instead of in a waterway because there is a >90-percent chance that oocysts are prevented from reaching a water body under most climate conditions. This underscores the recommendation that cattle should be discouraged
from defecating directly into waterways. The oocysts that are released from fecal pats (<10 percent of total) and able to reach a water body can remain infective for weeks to months, provided ambient conditions remain cool and moist. Oocysts can be inactivated, however, if exposed to intense solar radiation. Table 11 illustrates the survival of Cryptosporidium oocysts in different types of agricultural media.

**Giardia**

Giardia cysts, much like Cryptosporidium oocysts, can survive for varying amounts of time in the environment, depending on prevailing ambient conditions such as temperature. Giardia cysts are sensitive to cold temperatures in both soil and feces. Cysts do not appear to survive in frozen soil over the winter regardless of the soil characteristics. During one study of cattle feces, Giardia cysts were rendered noninfective within 1 week after freezing at -4 ºC (25 ºF) but remained infective after 1 week at 4 ºC and 25 ºC (39 ºF and 77 ºF). Similar results have been shown for survival in soil. In aquatic environments, Giardia cysts can survive at low, but nonfreezing water temperatures (0.5 ºC or 33 ºF) for extended periods of time (months), but are usually noninfective after 2 weeks at 25 ºC (77 ºF).

Many of the recent waterborne outbreaks caused by Giardia were associated with treated water in swimming and wading pools. This fact is consistent with the observation that cysts are moderately resistant to chlorine, particularly at low temperatures (5 ºC or 41 ºF). Hence, at low water temperatures, where killing of Giardia requires higher chlorine concentrations and longer contact times, are particularly conducive to the long-term survival of Giardia cysts and an increased probability if infecting humans.

**Other protozoa**

Toxoplasma gondii oocysts are able to survive in soil under natural conditions (seasonal temperature and rainfall variations) and maintain infectivity for more than a year. However, survival of sporulated T. gondii oocysts in water is directly related to temperature; table 12 demonstrates the survival time of oocysts across a wide range of temperatures. Another protozoa, Blastocystis hominis can survive 3 weeks at 18 ºC to 20 ºC (64 ºF to 68 ºF) but less than 1 week at 4 ºC to 6 ºC (39 ºF to 43 ºF) in laboratory settings.

---

**Table 10** Survival of Cryptosporidium oocysts in water 1

<table>
<thead>
<tr>
<th>Water type</th>
<th>Temperature ºC (ºF)</th>
<th>Exposure time</th>
<th>Loss of viability or infective oocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>River water</td>
<td>-20º (-4)</td>
<td>1 week</td>
<td>100% loss of infective oocysts</td>
</tr>
<tr>
<td></td>
<td>4 (39)</td>
<td>14 weeks</td>
<td>~0.7 log loss of infective oocysts</td>
</tr>
<tr>
<td></td>
<td>10 (50)</td>
<td>14 weeks</td>
<td>~0.4 log loss of infective oocysts</td>
</tr>
<tr>
<td></td>
<td>21 to 23 (70-73)</td>
<td>12 weeks</td>
<td>2.6 log loss of infective oocysts</td>
</tr>
<tr>
<td>Natural mineral waters</td>
<td>4 (39)</td>
<td>12 weeks</td>
<td>minimal change in viability</td>
</tr>
<tr>
<td></td>
<td>20 (68)</td>
<td>12 weeks</td>
<td>22-60% loss of viability</td>
</tr>
<tr>
<td>Ground and surface waters</td>
<td>5 (41)</td>
<td>16 weeks</td>
<td>1 log inactivated, (0.0088 log/day)</td>
</tr>
<tr>
<td></td>
<td>30 (86)</td>
<td>5 days</td>
<td>1 log inactivated, (0.20 log/day)</td>
</tr>
<tr>
<td>Chlorinated tap water</td>
<td>4 (39)</td>
<td>8 weeks</td>
<td>30% loss infectivity</td>
</tr>
<tr>
<td></td>
<td>10 (50)</td>
<td>8 weeks</td>
<td>67% loss infectivity</td>
</tr>
</tbody>
</table>

## Table 11 Examples of survival of Cryptosporidium oocysts in agricultural media or fecal material

<table>
<thead>
<tr>
<th>Media</th>
<th>Treatment</th>
<th>Temperature °C (°F)</th>
<th>Exposure time</th>
<th>Inactivity or loss of viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swine manure slurry</td>
<td>Anaerobic digestion</td>
<td>20 (68)</td>
<td>20 days</td>
<td>100% loss of viability</td>
</tr>
<tr>
<td>Sludge</td>
<td>Thermophilic aerobic</td>
<td>55 (131)</td>
<td>24 hours</td>
<td>100% inactive</td>
</tr>
<tr>
<td></td>
<td>Pasteurization</td>
<td>55 (131)</td>
<td>24 hours</td>
<td>100% inactive</td>
</tr>
<tr>
<td></td>
<td>Mesophilic reaction</td>
<td>35 (95)</td>
<td>18 days</td>
<td>90% loss of viability</td>
</tr>
<tr>
<td>Bovine fecal pats</td>
<td>Simulated solar radiation</td>
<td>40 to 70 (104 to 158)</td>
<td>1 day</td>
<td>&gt;99.9% inactivation</td>
</tr>
<tr>
<td>Sludge-treated soil</td>
<td>Raw sludge</td>
<td>10 (50)</td>
<td>30 days</td>
<td>25% loss of viability</td>
</tr>
<tr>
<td></td>
<td>Treated sludge</td>
<td>10 (50)</td>
<td>30 days</td>
<td>20% loss of viability</td>
</tr>
<tr>
<td>Soil</td>
<td>Freeze/thaw cycles, low soil moisture</td>
<td>-10 to 20 (14 to 68)</td>
<td>12 days</td>
<td>99% inactivation</td>
</tr>
<tr>
<td></td>
<td>Freeze/thaw cycles, high soil moisture</td>
<td>-10 to 20 (14 to 68)</td>
<td>27 days</td>
<td>99% inactivation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-4 (25)</td>
<td>&gt;12 weeks</td>
<td>100% loss of viability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 (39)</td>
<td>8 weeks</td>
<td>100% loss of viability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 (77)</td>
<td>4 weeks</td>
<td>100% loss of viability</td>
</tr>
<tr>
<td>Bedding in calf housing facility</td>
<td>Enclosed calf housing, bedding changed out</td>
<td>Winter months</td>
<td>8 weeks</td>
<td>80-100% loss of viability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~8 (44.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spring/Summer</td>
<td>8 weeks</td>
<td>90-100% loss of viability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~18 (64)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solar calf housing, bedding added to</td>
<td>Winter months</td>
<td>8 weeks</td>
<td>80-100% loss of viability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~3 (37)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spring/Summer</td>
<td>8 weeks</td>
<td>90-100% loss of viability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~17 (62)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**7.2.2 Bacteria**

Many enteric bacterial pathogens of zoonotic concern are sensitive to temperatures exceeding 50 ºC to 60 ºC (122 ºF to 140 ºF). Bacterial pathogens that produce resistant endospores or have thick cell walls may survive higher temperatures and may only be killed by prolonged heating at temperatures in excess of 100 ºC (212 ºF). The ability of bacteria to survive within various types of media and across multiple environmental conditions can vary greatly between species.

**E. coli O157:H7**

Numerous studies have shown that *E. coli* O157:H7 can readily survive weeks to months, and in a few cases, in excess of a year in the various media common to agricultural environments (water, feces, soil, feed, manure lagoon, etc.). Although *E. coli* bacteria may thrive during warm weather, they do become susceptible to inactivation when exposed to excessive heat. Elevated temperatures, either due to ambient conditions (summer) or due to composting, are generally associated with elevated rates of inactivation in feces, manure slurries, and soil. This correlation between higher rates of inactivation and higher temperature underscores the general recommendation to both age manure prior to spreading and to spread stored manure slurries in the warmer months of the year.

*E. coli* O157:H7 declines over time in surface water including river water, lake water, standing puddle water, and animal drinking troughs. However, in one study the organism was still detectable in 45 percent of nonsterile water after 2 months at 10 ºC (50 ºF). *E. coli* O157:H7 survives longer in filtered, autoclaved municipal water than in lake water, perhaps due to competition or predation. One study found that survival times at 8 ºC (46 ºF) were longer than at 25 ºC (77 ºF), regardless of water source.

In cattle manure and manure slurries, studies have shown exponential decay occurring for *E. coli* O157:H7 stored at 4 ºC, 20 ºC, or 37 ºC (39 ºF, 68 ºF, and 98.6 ºF). Decimal reduction times can range from 6 days to 3 weeks in manure and from 2 days to 5 weeks in manure slurry, with the most rapid inactivation occurring at 37 ºC (98.6 ºF) compared to the cooler temperatures. In soils, *E. coli* O157:H7 are able to survive for weeks to months, though the number of organisms will likely decline substantially over time. *E. coli* O157:H7 can survive up to 99 days in soil under fluctuating environmental temperatures (-6.5 ºC to 19.6 ºC, 20.3 ºF to 67.3 ºF). Additionally, *E. coli* O157:H7 grown in waste-amended soil in the root mass of crops can survive for over 5 weeks. The pH and fiber content of manure are significant factors influencing survival and are positively correlated with the rate of inactivation of bacteria.

**Campylobacter**

*Campylobacter jejuni* survival in manure varies between manure sources. Survival was about 1 week in stacked manure from dairy cattle, pigs, or poultry; about 30 days in stacked sheep manure; and about 60 days in stacked beef cattle manure. In stored slurries, *Campylobacter* may survive for up to 3 months, declining more rapidly at 17 ºC (63 ºF) than at 4 ºC (39 ºF). In one study, anaerobic digestion had little effect in reducing the viable numbers of *C. jejuni*, with a mean decimal reduction time of about 440 days.

In agricultural surface waters, *Campylobacter* survive better at cooler temperatures. The organism is often detected throughout the year, with recovery rates found more often during the colder months. *Campylobacter* can survive in a nonculturable state in cold water and still be infectious to livestock. Unchlorinated drinking water has been identified as a source of infection to cattle herds. Table 13 shows the survival of *Campylobacter jejuni* in water at different temperatures. In addition to the effects of temperature, higher

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**Table 12** Survival of *Toxoplasma gondii* oocysts in water at different temperatures

<table>
<thead>
<tr>
<th>Temperature °C (°F)</th>
<th>Survival Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>-5 and -10 (23 and 14)</td>
<td>106 days</td>
</tr>
<tr>
<td>0 (32)</td>
<td>13 months</td>
</tr>
<tr>
<td>4 (39)</td>
<td>54 months</td>
</tr>
<tr>
<td>10, 15, 20, 25 (50, 59, 68, 77)</td>
<td>200 days</td>
</tr>
<tr>
<td>30 (86)</td>
<td>107 days</td>
</tr>
<tr>
<td>35 (95)</td>
<td>32 days</td>
</tr>
<tr>
<td>40 (104)</td>
<td>9 days</td>
</tr>
<tr>
<td>45 (113)</td>
<td>1 day</td>
</tr>
<tr>
<td>50 (122)</td>
<td>1 hour</td>
</tr>
<tr>
<td>55 (131)</td>
<td>2 minutes</td>
</tr>
<tr>
<td>60 (140)</td>
<td>1 minute</td>
</tr>
</tbody>
</table>

2. Survival of oocysts was determined by mouse bioassay.
dissolved organic carbon can promote the survival of C. jejuni. Also, the origin of the strain can be a factor for the survival of C. jejuni in drinking water. For instance, poultry isolates appear to survive longer compared to strains isolated from humans, water, and cattle.

### Other bacteria
Survival of both Yersinia enterocolitica and Listeria monocytogenes in beef cattle slurry is temperature dependent with die-off occurring more rapidly at 17 ºC (63 ºF) than at 4 ºC (39 ºF). Leptospira interrogans serovar pomona survived for at least 42 days in New Zealand soil under simulated winter field conditions. Listeria survived in slurries for up to 3 months, and can survive longer than 1 month following land application of manure.

A common question among landowners, private industry, and regulatory agencies is the question of how long a pathogen survives under specific environmental conditions, for example, E. coli O157:H7 in a tail water pond. Survival of pathogens, or alternatively, their rate of die-off or inactivation, is often described by a numerical rate regardless of how many pathogens are started with. So for a given rate of inactivation, the more pathogens at the start, the longer it will take for them to die-off. Hence, asking how long pathogens survive is a trick question. To properly answer the question one needs to know, at a bare minimum, two pieces of information: how many pathogens you are starting with and the rate of inactivation. That is why the rate of inactivation rather than an absolute time needed to inactivate 100 percent of a pathogen is more informative, given that it requires more time to inactivate large numbers of pathogens than a small number of the same pathogen.

### Estimating the die-off rate for enteric bacteria
Moore et al. (1998) provided a simple way of estimating die-off or inactivation rates for bacteria, based in part on Chick's Law, which is a simple first-order reaction in chemical kinetics. The equation is defined as \( N_t = N_0 e^{-kt} \), where \( N_t \) is the number of bacteria remaining at time \( t \), \( N_0 \) is the number of bacteria at time \( t = 0 \) (i.e., the initial load), \( t \) is time in hours or days, and \( k \) is the first order inactivation rate (i.e., die-off rate). Because the equation uses a base of 10, this model predicts the log reduction in bacterial counts per unit time, whereby the time needed for a single log reduction (reduction of 90 percent) equals the decimal reduction time. There are many variations of this basic equation that provide a more accurate prediction of bacterial die-off rates, but this simple equation is often accurate enough for estimating field conditions. Table 15 provides a variety of die-off rates for various bacteria in manure or water. For example, using the die-off rate of 0.066 for fecal coliforms in a pile of dairy manure in winter (see the first entry in table 15) after 10 days residence time we would expect only 22 percent of the original amount of bacteria would still

### Table 13
Survival of Campylobacter jejuni in water at different temperatures

<table>
<thead>
<tr>
<th>Water type</th>
<th>Temperature °C (°F)</th>
<th>Survival time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>4 (39)</td>
<td>202 hours</td>
</tr>
<tr>
<td></td>
<td>10 (50)</td>
<td>176 hours</td>
</tr>
<tr>
<td></td>
<td>22 (72)</td>
<td>43 hours</td>
</tr>
<tr>
<td></td>
<td>37 (98.6)</td>
<td>22 hours</td>
</tr>
<tr>
<td>Stream water</td>
<td>6, 16 (43, 61)</td>
<td>Days to weeks</td>
</tr>
<tr>
<td>River water</td>
<td>5 (41)</td>
<td>60 days</td>
</tr>
</tbody>
</table>


---

**Salmonella**

Given the widespread occurrence of different serotypes of Salmonella enterica in livestock and poultry populations, reasonable care should be taken to prevent environmental dissemination of these bacteria through the improper disposal or use of livestock manures or poultry litter. Survival of Salmonella in soil and manure slurry varies with factors that include but are not limited to seasonal temperatures, moisture, compositions of soil or manure, and the initial concentration of bacteria. Increasing the temperature of manure is usually associated with substantially reduced survival durations, as is typical for many zoonotic enteric pathogens. Table 14 shows the survival of Salmonella in manure or slurry at different seasons or temperatures. Survival of Salmonella in water varies with water type and temperature. Generally in ground water, inactivation rates of Salmonella ranged from 0.07 to 0.1 log per day. In untreated river water, the number of viable Salmonella was reduced by more than 3 logs after 45 days at room temperature.
Table 14  Survival of *Salmonella* in manure slurries

<table>
<thead>
<tr>
<th><em>Salmonella</em> serotypes</th>
<th>Media type</th>
<th>Season/ temperature °C (°F)</th>
<th>Exposure time</th>
<th>Loss of viability</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em> Typhimurium</td>
<td>Pig slurry</td>
<td>Summer</td>
<td>19 to 34 days</td>
<td>100% loss of viability</td>
</tr>
<tr>
<td></td>
<td>Pig slurry</td>
<td>Winter</td>
<td>24 to 58 days</td>
<td>100% loss of viability</td>
</tr>
<tr>
<td></td>
<td>Pig slurry</td>
<td>Summer</td>
<td>26 days</td>
<td>0% loss of viability</td>
</tr>
<tr>
<td></td>
<td>Pig slurry</td>
<td>Winter/spring</td>
<td>85 days</td>
<td>0% loss of viability</td>
</tr>
<tr>
<td></td>
<td>Fresh chicken manure</td>
<td>20 (68)</td>
<td>2 days</td>
<td>0% loss of viability</td>
</tr>
<tr>
<td></td>
<td>Fresh chicken manure</td>
<td>20 (68)</td>
<td>6 days</td>
<td>2 log reduction in viability</td>
</tr>
<tr>
<td></td>
<td>Cattle manure</td>
<td>20 to 37 (68 to 98.6)</td>
<td>1 to 3 weeks</td>
<td>1 log reduction in viability</td>
</tr>
<tr>
<td></td>
<td>Manure slurry</td>
<td>20 to 37 (68 to 98.6)</td>
<td>2 to 35 days</td>
<td>1 log reduction in viability</td>
</tr>
<tr>
<td></td>
<td>Manure</td>
<td>4 (39)</td>
<td>105 days</td>
<td>5 log reduction in viability</td>
</tr>
<tr>
<td></td>
<td>Manure</td>
<td>37 (98.6)</td>
<td>45 days</td>
<td>5 log reduction in viability</td>
</tr>
<tr>
<td>Mixed <em>Salmonella</em> serovars</td>
<td>Loamy sand and clay soils</td>
<td>-18 (-0.4)</td>
<td>10 days</td>
<td>1 decimal reduction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 (39)</td>
<td>20 days</td>
<td>1 decimal reduction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 (77)</td>
<td>12 days</td>
<td>1 decimal reduction</td>
</tr>
<tr>
<td><em>Salmonella</em> Newport</td>
<td>Dairy cow manure</td>
<td>24.5 (76)</td>
<td>184 days</td>
<td>&gt;8 log loss of viability</td>
</tr>
<tr>
<td></td>
<td>Manure-amended nonsterilized soil</td>
<td>24.5 (76)</td>
<td>332 days</td>
<td>&gt;8 log loss of viability</td>
</tr>
<tr>
<td></td>
<td>Manure-amended sterilized soil</td>
<td>24.5 (76)</td>
<td>405 days</td>
<td>&gt;8 log loss of viability</td>
</tr>
</tbody>
</table>

be viable in the pile \(10^{0.066 \times 10} = 0.22\). For another example, assume there are a million fecal coliforms per gram of dairy manure in this pile. How many days would it take for the number of fecal coliforms to drop to 10 per gram if that was the requirement for land application? One will need about 5 logs of reduction to go from a million (or \(10^6\)) to 10 (or \(10^1\)). Since it takes about 15 days for a single log reduction to occur (i.e., \(\frac{1}{0.066} = 15.2\) days; or \(10^{0.066 \times 15} = 0.10\)) and given that we need enough time for 5 logs of reduction (or 0.00001 reduction), then approximately 75 days should do the trick, assuming these original die-off rate constants are accurate.

**Local data vital when estimating survival of pathogens**

Based on the present review, some generalities can be made about survival of pathogens that may be useful in the implementation of practices. For example, higher temperatures or salinities, extremes of pH, and longer exposure to UV radiation all reduce the survival of protozoal and bacterial pathogens. Some conservation practices can take advantage of these chemical, physical, and environmental factors that lead to higher rates of microbial inactivation or reduced survival. For example, turning and aeration of compost piles usually leads to higher internal temperatures and as a consequence, higher rates of pathogen inactivation. However, considerable variability exists regarding survival rates of these waterborne pathogens when comparing manure, soil, and water for the large number of different classes of pathogens. This suggests that agronomists, NRCS staff, water quality regulators, and other land managers may want to generate their own survival estimates for local conditions for the specific pathogens of concern rather than relying on laboratory-derived values or field results generated in different media, under different conditions, and likely from a different geographical region.

<table>
<thead>
<tr>
<th>Material</th>
<th>Organism</th>
<th>pH</th>
<th>Season or temperature °C (°F)</th>
<th>Die-off rate, K/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy manure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pile-uncovered</td>
<td>Fecal coliform</td>
<td>Oct to Feb</td>
<td>0.066</td>
<td></td>
</tr>
<tr>
<td>pile-covered</td>
<td>Fecal coliform</td>
<td>Oct to Feb</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>anaerobic slurry</td>
<td><em>Salmonella</em> Dublin</td>
<td>Feb</td>
<td>0.107-0.428</td>
<td></td>
</tr>
<tr>
<td>anaerobic slurry</td>
<td><em>E. coli</em></td>
<td>Feb</td>
<td>0.102-0.287</td>
<td></td>
</tr>
<tr>
<td>Swine manure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>stored slurry</td>
<td><em>E. coli</em></td>
<td>7.0</td>
<td>4 (39)</td>
<td>0.686</td>
</tr>
<tr>
<td>stored slurry</td>
<td><em>E. coli</em></td>
<td>8.0</td>
<td>4 (39)</td>
<td>0.867</td>
</tr>
<tr>
<td>stored slurry</td>
<td><em>E. coli</em></td>
<td>9.0</td>
<td>4 (39)</td>
<td>0.931</td>
</tr>
<tr>
<td>stored slurry</td>
<td><em>E. coli</em></td>
<td>7.0</td>
<td>20 (68)</td>
<td>0.588</td>
</tr>
<tr>
<td>stored slurry</td>
<td><em>E. coli</em></td>
<td>8.0</td>
<td>20 (68)</td>
<td>0.816</td>
</tr>
<tr>
<td>stored slurry</td>
<td><em>E. coli</em></td>
<td>9.0</td>
<td>20 (68)</td>
<td>1.079</td>
</tr>
<tr>
<td>applied to grass field plots</td>
<td>Fecal coliform</td>
<td>6.4</td>
<td>0-25 (32-77)</td>
<td>0.47</td>
</tr>
<tr>
<td>Poultry manure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>applied to bare soil plots</td>
<td>Fecal coliform</td>
<td>4.5-6.5</td>
<td>25 (77)</td>
<td>0.342</td>
</tr>
<tr>
<td>applied to bare soil plots</td>
<td>Fecal strep</td>
<td></td>
<td></td>
<td>0.093</td>
</tr>
</tbody>
</table>

7.2.3 Viruses

As with other pathogens, the survival of viruses is related to temperature, with greater inactivation at higher temperatures, typically greater than 20 °C (68 °F) (table 16).

**Enterovirus**

Under experimental conditions, enteroviruses are rapidly inactivated in soil. These viruses decline more rapidly during warm and dry fall conditions than during warm and wet summer conditions. In waste water sludge samples, survival of enteric viruses was significantly dependent upon sludge temperature but not percent solid content. In groundwater, factors affecting the survival of enterovirus include temperature, oxygen, nutrient levels, and groundwater microorganisms.

**Viral titer**

Viral titer is the measurement of the amount of virus present. To determine the titer, several sample dilutions are prepared and the lowest concentration of virus that still infects cells is the viral titer.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Media</th>
<th>Temp °C (°F)</th>
<th>Exposure time</th>
<th>Log reduction in viral titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norovirus</td>
<td>Produce</td>
<td>4-8 (33-46)</td>
<td>50 days</td>
<td>&lt;1 log reduction</td>
</tr>
<tr>
<td></td>
<td>22 (72)</td>
<td>9 days</td>
<td>&gt;1 log reduction</td>
<td></td>
</tr>
<tr>
<td>Enterovirus</td>
<td>Soil</td>
<td>23 (73)</td>
<td>26 days</td>
<td>1 log reduction</td>
</tr>
<tr>
<td></td>
<td>2 (36)</td>
<td>180 days</td>
<td>1 log reduction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-70 (-94)</td>
<td>163 days</td>
<td>1 log reduction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lake water</td>
<td>22 (72)</td>
<td>8 weeks</td>
<td>7 log reduction</td>
</tr>
<tr>
<td></td>
<td>1 (34)</td>
<td>12 weeks</td>
<td>4-5 log reduction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-20 (-4)</td>
<td>12 weeks</td>
<td>0.4-0.8 log reduction</td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td>River water</td>
<td>20 (68)</td>
<td>Several days</td>
<td>2 log reduction</td>
</tr>
<tr>
<td></td>
<td>4 (39)</td>
<td>32 days</td>
<td>2 log reduction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lake water</td>
<td>20 (68)</td>
<td>16 days</td>
<td>2 log reduction</td>
</tr>
<tr>
<td></td>
<td>2° effluent</td>
<td>20 (68)</td>
<td>&lt;16 days</td>
<td>2 log reduction</td>
</tr>
<tr>
<td></td>
<td>Groundwater</td>
<td>20 (68)</td>
<td>&lt;16 days</td>
<td>2 log reduction</td>
</tr>
<tr>
<td></td>
<td>Creek water</td>
<td>20 (68)</td>
<td>9 days</td>
<td>2 log reduction</td>
</tr>
<tr>
<td></td>
<td>Tap water</td>
<td>20 (68)</td>
<td>3 days</td>
<td>2 log reduction</td>
</tr>
<tr>
<td>H7N2</td>
<td>Chicken manure</td>
<td>15-20 (59-68)</td>
<td>&lt;1 week</td>
<td>100% reduction in titer</td>
</tr>
<tr>
<td></td>
<td>56 (133)</td>
<td>30 mins</td>
<td>100% reduction in titer</td>
<td></td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>Groundwater</td>
<td>0-10 (32-50)</td>
<td>1 day</td>
<td>0.02 log reduction</td>
</tr>
<tr>
<td></td>
<td>2-30 (68-86)</td>
<td>1 day</td>
<td>0.04 log reduction</td>
<td></td>
</tr>
</tbody>
</table>

Introduction to Waterborne Zoonotic Pathogens in Agricultural Watersheds

**Rotavirus**

Rotavirus contained in manure may survive prolonged periods of time under nonaerated conditions (6 months for 90 percent reduction of virus titer). The virus survives longer in semi-liquid wastes that consist of mixtures of feces, urine, water, and bedding materials (pH < 8.0) than in liquid cattle manure (pH > 8.0).

**7.2.4 Enteric Fungi**

Concerns of waterborne *Enterocytozoon bieneusi* and *Encephalitozoon* spp. have been growing in recent years. There is currently limited knowledge on the survival of these organisms in environmental media, and only a few laboratory investigations have been conducted (table 17). Survival of spores of *Encephalitozoon cuniculi*, *Encephalitozoon hellem* and *Encephalitozoon intestinalis* in water depend on environmental temperatures. Survival time of the three species at different temperatures are presented in table 18. These laboratory findings demonstrated that *Encephalitozoon* spp. is resistant to low temperatures in aqueous media but sensitive to high temperatures. In addition to temperature, factors like UV and gamma radiation and solar disinfection negatively impact the survival of *Encephalitozoon* spp. spores in water.

### Table 17  *Encephalitozoon cuniculi* survival in different media

<table>
<thead>
<tr>
<th>Media</th>
<th>Temperature °C (°F)</th>
<th>Exposure time</th>
<th>Infectivity or % Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>4 (39)</td>
<td>2 years</td>
<td>Still infective</td>
</tr>
<tr>
<td></td>
<td>60 (140)</td>
<td>5 minutes</td>
<td>100% noninfective</td>
</tr>
<tr>
<td></td>
<td>70 (158)</td>
<td>1 minute</td>
<td>100% noninfective</td>
</tr>
<tr>
<td>Medium 199</td>
<td>-20 (-4)</td>
<td>2 days</td>
<td>0% survival</td>
</tr>
<tr>
<td></td>
<td>4 (39)</td>
<td>98 days</td>
<td>&lt;0.1% survival</td>
</tr>
<tr>
<td></td>
<td>22 (72)</td>
<td>7 days</td>
<td>0% survival</td>
</tr>
<tr>
<td></td>
<td>37 (98.6)</td>
<td>3 days</td>
<td>0% survival</td>
</tr>
</tbody>
</table>


### Table 18  Survival of *Encephalitozoon cuniculi*, *E. hellem*, and *E. intestinalis* in water

<table>
<thead>
<tr>
<th>Temperature °C (°F)</th>
<th>Time of survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. cuniculi</em></td>
</tr>
<tr>
<td>10 (50)</td>
<td>3 months</td>
</tr>
<tr>
<td>15 (59)</td>
<td>2 months</td>
</tr>
<tr>
<td>20 (68)</td>
<td>1 month</td>
</tr>
<tr>
<td>25 (77)</td>
<td>3 weeks</td>
</tr>
<tr>
<td>30 (86)</td>
<td>1 week</td>
</tr>
</tbody>
</table>

1. Adapted from Li et al., 2003, *J. Parasitol.* 89:185-188.
7.3 References and further reading


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8. Waterborne Transport of Pathogens

8.1 Fecal matrix

Most waterborne zoonotic pathogens are shed in the feces compared to urine or other biological excretions

When fecal material from domestic and wild animals is deposited on the terrestrial part of a watershed, microbial release or escapement from the fecal matrix via erosion is a key process determining the risk of waterborne transport to downstream or down-gradient uses. This is because waterborne zoonotic bacteria and protozoa of primary concern, along with many of the viruses and microsporidia listed in table 4 (see section 1.5 beginning on page 7) are excreted from the infected animal primarily in fecal material. Some bacterial pathogens of secondary concern and some of the viruses listed in table 4 are excreted in other fluids such as urine (Leptospira), respiratory secretions (influenza virus), and reproductive tissues and milk (Brucella). Given that waterborne zoonoses of primary concern are excreted in feces, the spatial pattern of fecal deposition from animal hosts in relation to surface water sources is a key predictor and management tool for reducing the risk of waterborne microbial contamination.

The majority of pathogens are retained in the fecal deposit during rainfall conditions. Several studies provide data that either directly or indirectly demonstrate that the majority of bacteria or protozoa in bovine feces are not washed out from the fecal matrix during either simulated or natural rainfall; instead, the majority of these microbes appear to remain within the fecal deposit due to rainfall not eroding the entire amount of feces. One study showed that over 90 percent of C. parvum oocysts and over 85 percent of G. duodenalis cysts were retained in fresh dairy calf feces after 250 minutes of drip irrigation. Increasing droplet size and mixing cow manure with calf feces increased the numbers of C. parvum and G. duodenalis oocysts released from the pat. In studies using simulated rainfall, only 17 percent of fecal coliforms washed out of cow pats and 0.5 to 0.9 percent of Cryptosporidium oocysts and 1.3 to 1.4 percent of E. coli bacteria washed out of cow pats. In another study using cow pats on annual grassland and exposed to natural rainfall, over 95 percent of C. parvum oocysts and E. coli were either retained in the fecal matrix or filtered within the first 10 centime-

ters (cm) of grass immediately downslope of the cow pat. What is unknown is the percentage of microbial pathogens released from fecal material when an animal defecates directly into flowing or standing water (rivers, lakes, ponds) where pathogen release is likely the result of numerous variables, such as the erosive force of water (stream velocity, wave action, etc.) and the fat content of the fecal matrix. In addition, it is unclear how the above estimates would translate for other domestic or wild animal species with grossly different fecal characteristics.

Aged feces tend to release fewer pathogens

As fecal material ages, researchers have found reductions in the concentration of microbial pathogens in runoff below the pats, but a few exceptions do occur. Early work has shown that concentrations of fecal coliforms in runoff from cowpats are reduced by several logs (90 to 99.9 percent) after 30 to 100 days of aging. Increasing days since last grazing lead to several log reductions in the concentration of E. coli in irrigated pasture runoff, either because fecal release or the concentrations of E. coli in the fecal load were reduced as pats aged. Table 19 shows that less than 1 percent of C. parvum oocysts washed out of fresh bovine fecal pats under simulated rainfall, and after 1 day of ambient exposure this value reduced to less than 0.001 percent. One can presume that as E. coli and other waterborne zoonotic pathogens of primary concern eventually decline in fecal material after months of environmental exposure (see chapter 7), concentrations in runoff downslope from these fecal loads will likewise decline if the primary source of these microbial pathogens is the fecal matrix.

When intense rainfall causes pasture or rangeland runoff to occur, pathogens can leach out of the fecal pat and be carried in overland flow and into nearby streams and lakes where they become a waterborne hazard. Some of the overland flow percolates or infiltrates into the soil to be carried within subsurface flow. When this occurs, many of the pathogens become filtered before reaching groundwater or nearby streams. In general, as more and more of overland flow infiltrates into the subsurface before reaching a stream or lake, the less risk these pathogens pose to humans and animals. Conservation practices that encourage surface water to infiltrate into the subsurface tend to reduce the risk of waterborne pathogens from agricultural watersheds compared to letting runoff reach streams and lakes via overland flow (fig. 12).
**Table 19** Runoff and waterborne *Cryptosporidium parvum* oocysts collected over a 2-hour period using 200g bovine fecal pats spiked with $5 \times 10^6$ oocysts and a drip rainfall simulator (15 mm/hr). Three replicate trials per day of age; maximum air temperature ranged from 29 to 38 °C (84 to 100 °F) $^1$

<table>
<thead>
<tr>
<th>Age of fecal pat (days)</th>
<th>Mean runoff collected (ml) +/- s.d.</th>
<th>Total oocysts in runoff ($T_t$)</th>
<th>$T_t / 5 \times 10^6$ oocysts (%)</th>
<th>$T_t / T_0$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>571.7 +/- 78.2</td>
<td>25,498</td>
<td>0.51</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>556.7 +/- 86.2</td>
<td>334</td>
<td>0.007</td>
<td>1.31</td>
</tr>
<tr>
<td>2</td>
<td>530.0 +/- 56.3</td>
<td>106</td>
<td>0.002</td>
<td>0.42</td>
</tr>
<tr>
<td>3</td>
<td>503.3 +/- 58.6</td>
<td>201</td>
<td>0.004</td>
<td>0.79</td>
</tr>
<tr>
<td>4</td>
<td>485.0 +/- 47.7</td>
<td>631</td>
<td>0.013</td>
<td>2.5</td>
</tr>
<tr>
<td>8</td>
<td>485.0 +/- 69.5</td>
<td>194</td>
<td>0.004</td>
<td>0.76</td>
</tr>
</tbody>
</table>


**Figure 12** Pathogens in pasture or rangeland runoff
8.2 Overland flow

Once microbial pathogens of zoonotic concern are eroded or washed out of the fecal matrix and suspended in surface flow, their fate and transport are driven in large part by such factors as the turbulent forces occurring in surface flow, the exchange between surface and subsurface flow, exposure to UV, and attachment to organic or mineral particles that can increase their settling velocity. As long as these microorganisms remain unattached and as individual microbes, their small size and near neutral density (typically 1.05 to 1.1 g/cm³) predicts that they will remain suspended while being transported in overland flow. During this time, pathogens are subject to such processes as predation by free-living protozoa (if present), inactivation through UV exposure, growth (bacteria only), background rates of die-off or senescence, and other such mechanisms. These various processes have little time to act on the pathogen load given the typical short time periods that pathogens are in overland flow or runoff. In some studies, microbial pathogens, such as C. parvum have been shown to readily attach to minerals or other particles. C. parvum oocysts suspended in various aqueous solutions can attach to a range of sediments, resulting in a substantial increase in their settling velocity that could reduce their likelihood of reaching a stream or lake if these estimates are accurate.

8.3 Rivers and streams

Predicting transport of waterborne zoonotic pathogens being carried in rivers and streams is difficult given the large variation in channel morphology, streambed composition, biofilm formation on sediments, variable turbulence and flow regimes, microbial attachment to sediment particles, fluctuating levels of suspended solids, and other such factors that can influence the fate and transport of pathogens in turbulent riverine flow. Transport distances of 21 kilometers (km) have been observed for bacteria (Serratia marcescens) injected into a small stream in southern Ontario, but transport length is likely to vary considerably for different streams given the many processes governing in-stream transport. For example, in-stream transport of E. coli is likely to be quite different for a high-gradient, narrow stream channel dominated by granite boulders and gravel compared to a low-gradient, wide stream channel dominated by fine sands. If bacteria and protozoa that discharge into rivers and streams remain unattached to sediment and dense organic particles as they move with surface flow, they are unlikely to settle by gravitational forces to the streambed given their small size (less than 1 micrometer (µm) to 15 µm) and near neutral density (1.05 to 1.1 g/cubic centimeter (cm³)), which results in slow settling velocities. Surface water that infiltrates into the streambed, referred to as hyporheic exchange, is one key mechanism that can deposit unattached microbes such as C. parvum oocysts into the streambed and potentially remove them as a waterborne hazard. As these waterborne bacteria and protozoa attach to minerals or dense organic particles, their settling velocities are markedly increased, along with their likelihood of gravitational settling and streambed deposition. Settling velocities for unattached C. parvum oocysts have been estimated at 0.76 µm/second (s), but this increased to 7.9 to 53.3 µm/s once the oocysts were attached to mineral or stream sediments. Settling velocities in a salt solution at 23 °C (73 °F) were 0.35 µm/s for unattached C. parvum oocysts and 1.4 µm/s for G. duodenalis cysts, which increased ~80 fold following attachment to sewage particles. In small streams in Ontario, Canada, settling velocities for sediments 45 to 125 µm in size that contained attached E. coli were 20 to 300 µm/s, compared to negligible settling for unattached E. coli.

When water infiltrates into the streambed, microbial pathogens, such as bacteria and protozoal parasites, can be filtered if the streambed is made of small sediment particles, such as sand interspersed with small gravel. When microbes attach to heavier suspended sediments, they tend to settle much faster down through the water column and are more likely to become filtered once they enter the streambed. In con-

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**Do pathogens settle out of pasture runoff, overland flow, or riverine flow?**

Waterborne pathogens such as protozoa and bacteria have a slightly heavier density than water, but because they are so small they are usually kept suspended in the water column due to turbulent forces of surface water. If bacteria or protozoa become attached to soil or organic particles that are substantially denser than water, they tend to settle much more quickly compared to being unattached. Once the surface water infiltrates into the soil or stream bed, a variety of mechanism can occur that filter or remove the pathogen from water flow, such as getting stuck in a very small soil pore or becoming attached to the surface of sediment.
trast, streambeds composed of large cobble or boulders may not be as effective at filtering pathogens due to the larger space between the boulders. Pathogens are also inactivated by UV radiation while being transported downstream. Therefore, rivers that are wide, shallow, with slow current and streambeds composed of sand and gravel will filter pathogens better than rivers that are narrow, deep, fast, and have streambeds composed of cobble and boulders (fig. 13).

Pathogen reduction within streams and rivers

Length of stream reach needed for a log reduction (90 percent reduction) for waterborne *C. parvum* oocysts was estimated by Searcy et al. (2006) using parameters generated from flume experiments. Assuming a headwater agricultural stream (mean depth 15 cm, velocity of 15 cm/s, discharge of 0.5 m³/s), a one-log reduction (i.e., 90 percent reduction) for waterborne *C. parvum* oocysts would require a distance of 7.3 km, if sedimentation was the sole process for deposition and oocysts were unattached; a distance of 1.1 km if unattached oocysts were deposited via stream-subsurface exchange; and only 0.37 km if oocysts were attached to sediments and deposited via stream-subsurface exchange. However, these distance estimates need to be revised to incorporate microbial inactivation due to exposure to UV and solar radiation occur during daylight hours (especially for water with low-dissolved-oxygen content), temperature-dependent background senescence, and other such inactivating processes. Presumably, as transport time is increased, especially during the daylight hours, not only are greater numbers of pathogens removed due to filtration in the streambed and gravitational settling, but an increasing proportion of the microbial load is inactivated, so long as the microorganisms are not replicating in-situ (protozoa and viruses cannot replicate outside their host). For example, the estimated mean T90 values (time to inactivate 90 percent microbial load) for exposure to sunlight for *E. coli* obtained from raw sewage diluted in river water (3 percent suspension vol/vol) and held at 14 °C (57 °F) was 3.3 hours in summer and 6.9 hours in winter; for fecal coliforms these values were 3.3 hours in summer and 7.7 hours in winter. Repeating these experiments with waste stabilization pond effluent diluted 10-fold in river water and held at 14 °C (57 °F), the mean T90 values for *Salmonella enterica* in summer and winter sunlight were 4.8 and 26.8 hours, respectively; for *Campylobacter jejuni* the T90 values in summer and winter sunlight were 0.8 and 1.6 hours, respectively; and for *E. coli* the T90 values in summer and winter sunlight were 3.9 and 17.3 hours, respectively. The values shown in table 15 are consistent with these estimates. These inactivation rates and T90 estimates indicate that substantial inactivation may occur for various bacterial pathogens while being transported in longer riverine systems, or during low flow velocities and sunny conditions. What is less clear is to what extent and under what conditions will these injured bacteria resuscitate to become a waterborne hazard. Lower inactivation rates have been observed during nighttime for bacteria in fresh water, with T90 values ranging from 60 to 500 hours, suggesting that pathogen loading occurring after sunset will not be inactivated to any appreciable degree while being transported at night until the next sunlight day occurs and sunlight exposure begins to accrue. Replicating and validating these types of estimates (stream distance for log reduction; solar inactivation rates; microbial attachment to sediments, etc.) for a wide range of agricultural watersheds across different climate patterns, water chemistries, and river flow velocities for the suite of microbial pathogens listed in table 4 (page 7) are needed if we are to develop better predictions regarding the transport of waterborne zoonoses once they reach riverine systems.
8.4 Lakes and ponds

The primary factors influencing the occurrence of pathogens in lakes and ponds is the rate at which pathogens enter from sources such as river and creek inflows, direct fecal inputs from sources such as wildlife, temperature of the water, and UV radiation, especially for highly clear bodies of water. Warmer water increases the die-off rate of pathogens as well as UV radiation in shallow, clear lake water. Cold water and deep lakes can prolong pathogen survival.

Microbial pathogens and bacterial indicators that discharge into lakes and ponds are subjected to many of the same processes as occur in riverine systems. For example, microorganisms are subject to gravitational settling as either free (negligible settling) or attached to mineral or organic particles; advection and dispersion driven by various hydrodynamic processes (e.g., presence of riverine inflows, wind velocity); temperature-dependent background senescence; inactivation via UV or solar radiation, especially for low dissolved organic carbon (DOC) and low turbidity systems; and other such factors. After reviewing the literature, Brookes et al. (2004) concluded that the major processes affecting fate and transport of pathogens in lakes and reservoirs are riverine inflows, pathogen inactivation, and temperature. Riverine inflows influence the advection of waterborne microorganisms and can function as a primary input of waterborne pathogens when compared to within-lake or lake-shore sources; pathogen inactivation, especially for systems of high clarity, are heavily driven by exposure to sunlight and especially UV-B and UV-C radiation; warmer water temperatures have been repeatedly shown to increase rates of pathogen inactivation for almost all matrices that have been studied. Although riverine systems can be a primary source in pathogen intrusion into lakes and reservoirs, beach sand and sediments can be reservoir of *E. coli* that are released during turbulent conditions such that waterborne concentrations are elevated. The assumption that resuspended *E. coli* in freshwater surf zone are from wild or domestic vertebrate sources may not always be accurate for these freshwater-adapted strains if they replicate during summer conditions in sediments and persist overwinter.

8.5 Groundwater

Zoonotic pathogens reaching groundwater

Groundwater basins are underground regions that are permanently saturated. Above this region is a layer of soil that is partially saturated with water, its thickness varies depending on local conditions and a variety of factors. Microbial pathogens that infiltrate into the subsurface can, under some circumstances, be transported through the unsaturated zone and into the groundwater below. The majority of pathogens however, tend to become trapped in narrow soil pores or attached to soil surfaces while moving through the unsaturated zone. The percentage of pathogens capable of reaching groundwater is highly site-specific and a function of numerous factors such as the distance between the soil surface and groundwater, the porosity or size and quantity of the pores of the soil, the presence of small channels or macropores in the subsurface, or fractures in the bedrock that allow water to quickly move deep into the subsurface. The concern regarding pathogens in groundwater is that many privately owned domestic and irrigation wells are not treated for pathogens before being used, so zoonotic pathogens like *Salmonella* or *C. parvum* have the potential to be consumed or applied to irrigated foods directly. Wells that are not properly constructed and are poorly sealed from surface water intrusion can also lead to contamination of groundwater by zoonotic pathogens, especially if large amounts of fecal material are allowed to be deposited near the well head. An excellent review has been published by the EPA regarding the impact of animal feeding operations and their manure management systems on groundwater microbial contamination.

Water obtained from a private well is generally not treated to reduce microbial pathogens. In these circumstances even small numbers of pathogens can cause human illness or contaminate irrigated foods so it is important to protect the area around a wellhead from excessive amounts of pathogens and fecal loading.
8.6 References and further reading


Numerous practices have been proposed to reduce the risk of contamination of water with microbial zoonotic pathogens listed in table 4 (page 7). These practices are also known as beneficial management practices, good agricultural practices (GAPs), or best management practices (BMPs), depending on the agency or organization of origin. This technical report covers practices targeted to impact water supplies prior to municipal treatment and is focused on practices that a landowner can implement. In addition, we have focused on practices that have been shown to be effective through one or more field trials or indirectly through basic research on the underlying mechanisms driving the effectiveness of the suggested practice. An example of indirect evidence would be showing how specific soils could filter bacteria using soil columns as a proxy for subsurface filtration in the zone underneath a vegetated area used for treatment. This technical report does not cover the large body of literature regarding conventional or advanced water treatment technology (municipal systems), the distribution system post treatment, or point-of-use technology, such as home filters or boiling water.

Reducing infection from waterborne pathogens not associated with animals

Not all waterborne pathogens of public health concern are of animal origin. For example, animals do not appear to be the primary environmental reservoir of bacteria such as *Vibrio* sp., *Legionella* sp., *Plesiomonas shigelloides*, and *Pseudomonas aeruginosa*. Practices for reducing human infection with these environmental bacteria tend to focus on reducing human exposure (not swimming with cuts and abrasions), improved food hygiene practices (adequately cooking food), minimizing environmental growth of these bacteria, or maximizing their die-off in treated recreational water systems (e.g., adequate chlorine levels in a hot tub).

Developing practices

The majority of practices that a landowner or farm manager would use to reduce zoonotic pathogens of primary concern from entering a local waterway fall into three basic strategies:

- Reduce pathogen loading from the animal population (e.g., reduce stocking density)
- Minimize transport of the pathogen load from the animal population to surface or groundwater resources (e.g., vegetated treatment areas for pasture runoff)
- Maximize inactivation and reduce the infective pathogen load once it has been excreted by the animal populations (e.g., manure composting)

Most of the waterborne zoonotic pathogens of primary concern are excreted from infected animals in their feces, hence, a common theme for many practices is manure management. For example, one goal for grazing or holding livestock away from streams is to ensure that their fecal material is deposited a greater distance from the edge of critical water resources. In another example, composting procedures for manure solids and animal bedding material are designed in part to inactivate the fecal load of pathogens, similar to aeration of liquid manure storage lagoons. All practices can fail when the water quality or other environmental conditions fall outside of the original design parameters for the conservation practice, which underscores the recommendation for having multiple barriers or practices for reducing the risk of microbial contamination of source-water supplies. For example, utilizing flood irrigation with high flow rates on grazed pasture can result in excessive tail water flows that readily exceed the ability of a downslope vegetated treatment area to retain the waterborne pathogen. Moreover, site-specific conditions typically differ from the scientific trials originally conducted to assess the effectiveness of the conservation practice, leading to site-specific differences in the effectiveness for the practice. For example, vegetated buffer strips of only 1 to 2 meters in length comprised mostly of California annual grassland at slopes of 5 to 25 percent retained 90 to 99.99 percent of *E. coli* and *C. parvum* in bovine fecal pats from discharging in surface runoff. It is quite likely that different estimates for pathogen retention would be generated for such short vegetated buffer strips composed of different plant communities, soil composition, and rainfall patterns located in other regions of the United States.
Multibarrier approach for reducing waterborne pathogens

Developing a multibarrier approach to protect water resources from microbial pathogens helps safeguard against the chance that a single practice fails. In other words, it is best not to rely on a single practice for water protection, but instead have a system of practices placed one after the other in a series to maximize overall effectiveness. A four-tiered multibarrier approach has been developed for managing pathogens from animal agriculture. These barriers consist of—

- preventing pathogens from entering the farm.
- preventing pathogens from multiplying on the farm.
- manure collection, storage, and treatment to reduce pathogen survival.
- preventing pathogens from leaving the farm.

The first barrier involves reducing the potential for pathogens to enter the farm from outside sources. Parasites can come onto the farm through the introduction of infected animals; the transport of infected manure onto the farm on clothing, boots, or equipment; or pets, rodents, and other animals can transport contaminated manure from other farms (external biosecurity). This can be accomplished by carrying out actions such as the following:

- Testing nonchlorinated water supplies that serve the herd for fecal coliform bacteria
- Establish appropriate biosecurity measures including those controlling people, pets, pests and other animals, equipment, or materials that may transport pathogens from other sources
- Maintain good hygiene and minimize herd or flock contact with manure from other animal groups
- Maintain an accurate animal identification system and record all health events

The second barrier is to minimize cross-contamination among animals and amplification of infection on the farm. Parasite movement and multiplication on the farm can be minimized by keeping young animal-raising areas clean and ensuring that all feeds and feeding utensils are clean (internal biosecurity). This can be accomplished by actions such as—

- Keeping animal raising areas clean and dry.
- Proper worker hygiene when moving between facilities or animal groups.

- Ensuring that all feeds are stored and handled properly, and feeding utensils are clean, specifically avoiding manure contamination of feed.
- Implementing rodent and pest control programs.
- Separating pre-weaned animals to prevent direct contact with another young animal and with adult manure.
- Isolating infected animals until they are no longer infectious.
- Identifying the order in which animals should be fed, (i.e., youngest to oldest, etc.) depending upon the pathogen of concern.

The third barrier is to collect, handle, and treat manure and wastes appropriately to reduce the survival of the pathogens. There are a variety of storage and treatment methods that can reduce the survivability of pathogens which will reduce the risk of contamination as the manure is recycled to the land or utilized as by-products in other operations. This can be accomplished by utilizing NRCS conservation practice standards (CPS) including—

- Composting Facility (317).
- Waste Storage (313), extension of time to take advantage of pathogen die-off.
- Anaerobic Digester (366).
- Waste Treatment Lagoon (359).
- Constructed Wetland (656).

The fourth barrier is to restrict movement of contaminated feces into watercourses by preventing runoff from calf housing, exercise lots, and manure storage areas, and avoiding application of manure to areas prone to excessive runoff. This can be accomplished by NRCS CPSs including—

- Diversion (362) to divert clean water away from livestock facilities.
- Nutrient Management (590) for spreading manure uniformly and at proper rates.
- Use Exclusion (472) to keep animals away from water bodies, such as streams, creeks, rivers, and lakes.
- Fence (382) to keep animals away from water bodies such as streams, creeks, rivers, and lakes.
• Filter Strips (393) for protecting downslope water bodies and other sensitive areas from manure runoff.

• Vegetated Treatment Areas (635) to treat confinement area runoff.

9.1 Further reading and references


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10. Practices to Reduce Pathogen Loading from Animal Populations

10.1 Exportation of animal manure

Practices that reduce the amount of pathogens deposited on a watershed by animals include:

- **Remove manure from the watershed.**—This practice is often used by facilities such as equestrian stables or a pack stock station. Costs can be reduced by removing only the manure and bedding from high-pathogen-risk animals, such as young stock or animals in the hospital pen. For the remaining manure, pathogen reduction techniques, such as composting, can be used to treat the manure prior to disposal.

- **Reduce animal populations of concern.**—Livestock and wildlife may congregate near water supplies and leave unacceptable amounts of feces in riparian zones or seasonal creek channels. Fencing, herding, and rotational grazing of livestock are common techniques. Costs can be reduced by focusing on high-pathogen-risk animals, such as locating calving pastures away from surface water sources. Maintaining lower population densities of wildlife and livestock has been associated with lower infection levels of some pathogens.

- **Antibiotics, vaccines, probiotics, and other oral therapies.**—For some pathogens there are products that reduce the infection level among livestock or other domestic animals. For example, vaccines are under development for cattle that reduce fecal shedding of *E. coli* O157. Feeding antibiotics is not considered an effective way to reduce pathogen loading and can lead to antibiotic resistant bacteria in livestock.

- **Farm sanitation, biosecurity, and herd management.**—Improving sanitation and biosecurity among confined animals, maintaining animal health, and implementing livestock quality assurance programs may reduce the amount of pathogens excreted by animals.

- **Nutrition.**—There is considerable debate regarding the role that nutrition plays in elevating or lowering the levels of animal infection for such pathogens as *E. coli* O157. This is an area of active research that is still being developed as a conservation practice.

Exporting animal manure out of critical watersheds will reduce pathogen loading from the animal population of concern, though any manure removed from the watershed needs to be properly disposed of in an environmentally safe manner. While such practices can benefit microbial water quality when fecal loads are too close to source-water supplies, the task of collecting the manure solids or liquid effluent typically requires a manure management system. The requirement of a manure management system typically limits the utility of exportation to confined animal feeding operations (dairy, feedlots, swine, poultry, etc.) where waste solids are routinely collected and handled for stacking or composting. Urban and suburban equestrian facilities, pack stock stations on public land (e.g., national parks), and biomedical facilities using laboratory animals are often required to export their manure and animal bedding to a handling facility for further processing, such as composting. Such practices can be expensive due to labor demands, maintenance of infrastructure, and transportation costs. One strategy to minimize handling costs for an animal operation is to focus exclusively on those animal age groups that exhibit higher prevalence of infection or shed the highest concentrations of pathogens. For example, young calves compared to adult cattle often exhibit a higher prevalence of infection with the protozoal parasites, *C. parvum* and *G. duodenalis*. Moreover, the concentration of parasites in calf feces is often orders of magnitude higher compared to adult cattle despite adults generating much more fecal material per day.

10.2 Reduce animal host populations

On some smaller watersheds, the primary animal source for a specific pathogen may be known. In cases where this animal source is causing water quality impairment, reducing the animal density will reduce the pathogen loading rate and potentially improve microbial water quality as a consequence. Reduced animal densities have also been found to be associated with a lower prevalence of infection within a group of animals. For example, beef cattle herds with lower stocking densities and populations of feral pigs with lower population densities were associated with reduced levels of infection with *Cryptosporidium*. Lower densities of pack stock have been associated with a lower proportion of stock infected with *G. duodenalis*. Despite these potential reductions in the environmental loading rate of zoonotic pathogens, permanent reductions in the size of livestock herds is often resisted by farmers and ranchers resulting in low cooperation. In
some circumstances removal or reduction of one host population results in an increase in another cohabitating species that may locate in close proximity to water resources or be more heavily infected with microbial pathogens such that microbial water quality eventually worsens. For example, reductions in livestock may result in increases in one or more wildlife species in riparian zones, with potentially unpredictable consequences to water quality if these wildlife are infected with zoonotic pathogens.

A common mistake that is made when deciding which animal is responsible for contaminating a watershed with too many pathogens is to blame the animal with the highest prevalence of infection. The prevalence is the percentage of animals shedding a pathogen at any point in time (infected animals/total animals sampled). This is an incorrect measure to compare the rate of environmental contamination from different animal species because it does not take into account the concentration of pathogen per gram of feces (defined as intensity of infection), how much an animal defecates per day, nor the density of animals per acre. A rare animal with a high prevalence but low intensity of infection does not load a watershed with as many pathogens as a common animal with a moderate prevalence and high intensity of infection. The first step in comparing the ability of two different animal species to contaminate or load a watershed with pathogens is to calculate the environmental loading rate, defined below, for each animal species of concern. Moreover, it is often not the adult animals that load a watershed with the highest amounts of pathogens; the young stock are often responsible for the highest loading rates given the typical high concentration of pathogens per gram feces (see table 20).

<table>
<thead>
<tr>
<th>Species</th>
<th>Oocysts/Kg feces</th>
<th>Kg feces/day/animal</th>
<th>Oocysts excreted/day</th>
<th>Oocysts/Kg feces</th>
<th>Kg feces/day/animal</th>
<th>Oocysts excreted/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>San Joaquin dairy cattle - Holstein (Bos taurus)</td>
<td>67</td>
<td>60</td>
<td>4,000</td>
<td>3,000,000,000</td>
<td>1</td>
<td>3,000,000,000</td>
</tr>
<tr>
<td>California beef cattle - mixed breeds (Bos taurus)</td>
<td>150</td>
<td>40</td>
<td>6,000</td>
<td>150,000</td>
<td>4</td>
<td>600,000</td>
</tr>
<tr>
<td>Striped skunks (Mephitis mephitis)</td>
<td>2,800,000</td>
<td>0.05</td>
<td>140,000</td>
<td>4,400,000</td>
<td>0.02</td>
<td>88,000</td>
</tr>
<tr>
<td>California ground squirrels (Spermophilus beecheyi)</td>
<td>6,500,000</td>
<td>0.012</td>
<td>78,000</td>
<td>10,300,000</td>
<td>0.004</td>
<td>41,200</td>
</tr>
<tr>
<td>Coyotes (Canis latrans)</td>
<td>205,000</td>
<td>0.2</td>
<td>41,000</td>
<td>505,000</td>
<td>0.07</td>
<td>35,000</td>
</tr>
<tr>
<td>Yellow-bellied marmots (Marmota flaviventris)</td>
<td>10,400,000</td>
<td>0.02</td>
<td>208,000</td>
<td>Not done</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reducing winter use of lots and loafing areas can improve water quality

With respect to animal feeding operations (AFO) and other livestock production facilities, reducing or eliminating winter cattle use of dry lots and other high use areas that are typically poorly vegetated or located on impervious surfaces like concrete can reduce the number of bacteria and protozoa discharging from these facilities. While reducing the use of winter lots and heavy use areas is one option to reduce bacterial loading, simply placing these facilities further from water bodies or surrounding them with vegetative areas can greatly improve local water quality. In some cases the use of winter feeding facilities can be beneficial, particularly if the manure is scraped and stored on a regular basis.

Spreading excessive amounts of manure onto frozen fields can result in large amounts of fecal pathogens discharging off the field once the snow melts as the hardened ground can act like an impervious surface. The estimated rates of Cryptosporidium oocyst loading per animal shown in table 18 indicate that careful management of calf manure will likely have a much bigger impact on reducing discharges of this waterborne parasite compared to management of adult cattle manure. An excellent review by the EPA has been published on additional AFO manure management practices designed to reduce groundwater microbial contamination. (See section 11.4)

10.3 Antibiotics, vaccines, probiotics, and other oral therapies

Various prophylactic or therapeutic products have been investigated for their ability to reduce the likelihood of gastrointestinal infection in livestock species (prevalence) or the intensity of pathogen shedding (pathogens/g feces). Products range from vaccines, antibiotics, and parasiticides, to probiotics, with a wide range of efficacy reported for these various products. Vaccines against various serovars of S. enterica subspecies enterica have shown some efficacy in poultry and swine, with promising trials recently conducted in cattle. Much of this research has focused on reduction in clinical severity of the infection and not on the effect on the environmental loading rate of the pathogen, but it stands to reason that if vaccination reduces the prevalence or percentage of livestock infected with the pathogen, that will lead to a reduction in the environmental loading rate.

Vaccination of cattle with a product containing type III secreted proteins of E. coli O157:H7 was associated with a reduction in the probability of shedding among vaccinated cattle, with vaccination also reducing the prevalence among unvaccinated pen mates due presumably to elevated herd immunity against this strain of E. coli. Assuming this vaccine would work for cattle on extensively grazed systems such as rangeland, this may be an effective method to help reduce environmental loading of E. coli O157:H7 by cattle in watersheds used for drinking-water supplies, irrigation water for produce and livestock feeds, and human recreation. A more thorough review of vaccine trials in post-weaned cattle showed mixed results for reducing the odds of infection. A variety of antibiotics, probiotics, and other compounds have been evaluated for reducing the prevalence of fecal shedding of E. coli O157:H7 in cattle using either observational studies, randomized trials, or challenge studies. Antibiotics were mostly unsuccessful in providing a positive benefit, but oral ingestion of combinations of Lactobacillus acidophilus and Propionibacterium freudenreichii and of sodium chlorate were associated with a reduction in infection levels for numerous randomized clinical trials. A vaccine for Giardia duodenalis has been shown to be effective in dogs and cats, but not for cattle.

Impediments to using oral treatments for water quality

There are several impediments to using these products as a routine part of a water quality program. Prophylactic use of antibiotics in livestock or companion animals to reduce fecal shedding of bacterial pathogens like Salmonella enterica is likely to be controversial given public health concerns over multidrug antibiotic resistant strains of zoonotic bacteria in our domestic animal populations. Routine use of vaccines and probiotics can be expensive for livestock owners, so unless the zoonotic pathogen causes clinical or other production-related impacts to the farmer or rancher, the economic costs for using these products may be a financial disincentive. For example, much of the interest over developing vaccines for E. coli O157:H7 infection in cattle is with respect to meat food safety and not water quality. This serotype of E. coli generally does not cause clinical problems in cattle; hence, some form of subsidy may be needed to motivate cow-calf or stocker owners to vaccinate their cattle if the goal is to safeguard water quality for other downstream users. Research on the development and clinical efficacy of vaccines, immunomodulators, probiotics, antibiotics, and feed additives is
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highly dynamic, with new products being proposed and evaluated on a constant basis. Interested readers are encouraged to conduct their own literature review on the host species and pathogen of interest to get the latest information on new products.

10.4 Farm sanitation, biosecurity, and herd management

General recommendations exist regarding farm sanitation, biosecurity, and herd health management with the goal of minimizing the introduction, persistence, and dissemination of various bacterial and protozoal pathogens. These pathogen reduction or quality assurance (QA) programs are typically commodity specific and sometimes pathogen specific. For example, the egg quality assurance programs for reducing *Salmonella* Enteritidis in egg-laying flocks have been successful in reducing this pathogen in poultry production systems. These programs often target biosecurity of feed stocks and water supplies, litter or bedding disposal, quarantine of new animals, pest control, waste management, and other such factors. Many States with sizable animal agricultural industries will develop and promote their own programs, with national programs often headed by commodity organizations also available. A few of the many examples include:

- Beef Quality Assurance Program (http://www.bqa.org)
- California Egg Quality Assurance Program (http://www.pacificegg.org/ceqap.html)
- California Dairy Quality Assurance Program (http://www.cdqa.org)
- Pork Quality Assurance (http://www.pork.org)

Some of the recommendations from these programs have been validated through prior scientific studies or by comparing animal infection prevalence before and after implementation of the QA program. However, many recommendations rely on what is perceived to be common sense for reducing animal infection but which may or may not work at the desired level of effectiveness when subjected to well-designed field trials.

10.5 Nutritional management

There is considerable interest in the possibility that manipulating the diet of domestic ruminants will reduce the risk of infection and fecal shedding of *E. coli* O157:H7. Much research has been conducted on the association of different concentrates and feeding forages as opposed to grains to livestock as a means to reduce fecal shedding of *E. coli* O157:H7. Earlier research showed that feeding forages compared to grain-based diets was associated with higher levels of *E. coli* O157:H7 in cattle and sheep. Feeding of steam-flaked grains compared to dry-rolled grains was also associated with higher infection levels of *E. coli* O157:H7. Various competing theories have been advanced, such as feeding cattle less digestible compared to more digestible grains (e.g., dry-rolled corn compared to steam-flaked corn; corn diets compared to barley diets) results in less starch digestion in the rumen, which leads to more starch bypass into the small and large intestine, leading to more secondary fermentation and volatile fatty acid (VFA) production in the large intestine, leading to an increase of VFA in feces thereby decreasing fecal pH that collectively reduce the occurrence of *E. coli* O157:H7 in feces. Yet, studies find conflicting results from this assertion, where researchers have found no correlation between fecal shedding of *E. coli* O157:H7 and fecal pH or fecal starch concentrations.

Much of the motivation for this field of research is to reduce the likelihood of carcass contamination during the slaughtering process and not an improvement of microbial water quality. Motivating owners of confined animal feeding operations to intentionally manipulate their animal's diets for water quality benefits may be challenging given the livestock owner's focus on economic factors such as feed conversion efficiency, milk production, and average daily gain. Moreover, extensive livestock production systems such as cow-calf and range sheep typically graze native forages or irrigated pastures where dietary manipulation such as feeding grains is uncommon or limited to specific age groups (calves) or during lactation.
10.6 Further reading and references


11. Practices to Reduce Pathogen Transport

A variety of practices exist that have the potential to substantially reduce the concentration or load of microbial pathogens and bacterial indicators while these microorganisms are being transported from host populations to receiving bodies of water. Compared to the expected performance of many practices that measure success as a reduction in the percent of animals infected with the pathogen (i.e., reducing infection from 30 to 5 percent via vaccination, nutritional manipulation, improved sanitation, or improved biosecurity), practices that reduce waterborne transport can readily generate 50- to 99.99-percent reductions for waterborne microorganisms from a variety of livestock production systems. These transport practices typically rely on one or more processes to function effectively, for example, adequate infiltration of surface flows into the soil, adequate filtration of waterborne microorganisms once they enter the soil, and adequate retention times for settling basins and waste lagoons so that the various processes that inactivate or reduce zoonotic microorganisms have time to function. Failure of practices described below is often the result of surface flows exceeding the design capacity of the conservation practice rather than evidence that the underlying basic process (e.g., straining, physiochemical attachment, gravitational settling, predation) does not function to remove microorganisms. For example, a 100-meter grassed waterway conveying tail water flows from an irrigated field to a sediment basin may fail to adequately reduce concentrations of waterborne E. coli as a result of poorly matching tail water flows with ditch channel morphology, leading to excessive flow velocities and little exchange between surface water and the bed of the waterway.

11.1 Redistribution of fecal loading away from source water supplies

A variety of range management practices are available that redistribute livestock away from surface water resources and adjoining riparian areas to higher elevations. Common practices involve physically relocating livestock (i.e., herding), controlled placement of feed attractants like salt or low-moisture blocks, fencing, off-stream water developments, and seasonal timing and duration of grazing. By redistributing cattle away from water, the goal is to reduce the amount of fecal material and associated zoonotic pathogens and bacterial indicators that are deposited in close proximity to water. In general, increasing the distance between pathogen loads and water resources reduces the likelihood that pathogens entrained in surface and shallow subsurface flow will reach critical surface water resources due to such processes as infiltration, straining, sedimentation, and attachment. Most studies regarding redistribution of cattle measure effectiveness by measuring changes in time budgets, stubble height, and forage utilization, and not reductions in fecal loading per unit area or improvements in microbial water quality. Significantly higher fecal accumulation rates can occur around livestock concentration areas compared to riparian and other locations, suggesting that strategic placement of these concentration areas (supplemental feed, salt, water trough) could pull fecal loading away from water resources. Bailey et al. (2008) found that herding a group of 42 to 59 first-calf cow-calf pairs with 2 bulls away from riparian upland areas resulted in a reduction of about 54 kilogram (kg) dry-weight fecal mass per hectare compared to not herding. This translates to about 360 kg fecal wet weight (15 percent total solids) reduction, or about 7 kg fecal wet weight per animal unit (AU), or possibly 7 billion fewer commensal E. coli deposited near streams per AU assuming 1 million E. coli per gram feces. Given that methods to measure spatial and temporal shifts in fecal loading of extensively grazed landscapes have been developed, hopefully more of these practices can be evaluated for their ability to reduce fecal loading in riparian or other hydrologically sensitive sites.

Practices that reduce pathogen transport:

- **Increase the distance between fecal loading areas and nearby waterways.**—A variety of methods are available to redistribute livestock away from surface water sources and wellhead locations, such as herding, fencing, relocating livestock drinking water equipment and supplemental feeding. For confined animal populations, proper placement of manure and bedding piles away from waterways, constructing a short berm around the pile, and placing manure piles under a roof to protect it from rainfall are all helpful.

- **Vegetated areas used for treatment.**—Placing vegetated treatment areas (see tables 21 and 22) between livestock and surface waterways or routing runoff from confined animal feeding operations through these vegetated systems can dramatically reduce the amount of waterborne pathogens from livestock. Maintaining vegetated filter strips or buffers at the base of fields on which manure is applied may also reduce water-
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borne pathogens if overland flow rates are not excessive.

- **Infiltration or settling basins, natural or constructed wetlands.**—Routing runoff from irrigated pastures, irrigated row crops, flood irrigated orchards, or runoff from confined animal feeding operations through a system of basins or wetlands can reduce waterborne pathogens if residence time is sufficiently long and runoff rates properly matched to the storage capacity of the system.

- **Manure storage and treatment lagoons.**—Proper lining and sufficient freeboard of a manure storage lagoon helps reduce groundwater seepage and catastrophic overflow. These manure storage systems will reduce pathogen concentrations if residence time is sufficiently long, especially for aerated systems. Guidance documents are available for the design, construction, and management of these systems.

An excellent review by the EPA has been published on additional AFO manure management practices designed to reduce groundwater microbial contamination. (See section 11.4.)

### 11.2 Vegetation

**Vegetated treatment areas**

A variety of designs for vegetative treatment areas (VTAs) that function either alone or in combination with other practices (e.g., settling basins, constructed wetlands) (tables 21 and 22) have been evaluated for a variety of extensive and intensive livestock production systems. Evaluations of the efficacy of VTAs have utilized bench scale and soil box simulated VTA systems, field trials at various spatial scales, or observational studies of smaller catchments. The general consensus from these evaluations is that when VTAs are properly designed and maintained, they can reduce the concentration and instantaneous load (total flow multiplied by microbial concentration (QxC)) of a variety of waterborne zoonotic microbial pathogens of primary concern and various bacterial indicators by 50 percent to well over 99.9 percent. Predicting exact performance of a newly installed VTA is generally not possible given site-specific changes in such factors as vegetation composition, rainfall patterns, macropore alterations (vertebrate burrows), animal density, and livestock infection levels. Instead, general observations can be made from these numerous studies and reviews,

- Addition of a settling basin or infiltration basin can improve VTA performance.
- VTAs that receive sheet-like flow rather than channelized flow are in general, more effective at reducing contaminants per unit length.
- Maintaining the infiltration capacity of the VTA improves performance.
- Higher density vegetated cover are generally more effective at removing microbial contaminants compared to low density cover or bare ground.
- Excessive surface flows generated from high intensity rainfall or high irrigation rates are one of the primary reasons for failure of VTAs to adequately remove microbial contaminants from surface and shallow subsurface flow.

An overriding assumption for utilizing a VTA to improve a watershed's microbial water quality is that infiltration of microbial-contaminated surface water into the soil or groundwater environment is preferred to the alternative of allowing pasture and rangeland runoff or manure effluents from livestock production facilities to discharge into surface water or attempting to catch all runoff and effluent into a storage facility for treatment (e.g., chemical, thermal, solar radiation). In other words, microbial-laden surface water has to go somewhere in the environment, either before or after treatment with one or more practices. The preference for partitioning microbial-laden surface water into the subsurface is based on a large body of literature demonstrating the ability of soil in either the vadose (unsaturated) and groundwater (saturated) zone to remove large amounts of microbial contaminants per meter length via such processes as straining, attachment, and inactivation relative to the same physical lengths of most VTAs. Exceptions occur with highly fractured or highly unconsolidated, coarse-grained formations where viral, bacterial, and protozoal contaminants may travel considerable distances to become drinking and irrigation water hazards.
Table 21  Efficacy of vegetative treatment areas on commercial or research livestock facilities. Reductions in bacterial pollutants are either in concentration or mass for each study as indicated in the last column 1

<table>
<thead>
<tr>
<th>Study description</th>
<th>VTA information</th>
<th>Percent reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study period</td>
<td>Pollutant source</td>
</tr>
<tr>
<td>Settling basin and VTA were placed below two cattle feedlots and monitored for seven storm events</td>
<td>1995 to 1996</td>
<td>200-head capacity lot (35 cattle during test)</td>
</tr>
<tr>
<td></td>
<td>225-head feedlot</td>
<td>Yes</td>
</tr>
<tr>
<td>300-head heifer feedlot with runoff directed to settling basin (1st stage) and VTA (2nd stage)</td>
<td>May 1 to 2</td>
<td>300-head dairy heifer feedlot</td>
</tr>
<tr>
<td></td>
<td>May 1998</td>
<td>350-head beef feedlot</td>
</tr>
<tr>
<td></td>
<td>Nov. 1998</td>
<td>300-head beef feedlot</td>
</tr>
<tr>
<td>*Same study, different VTA location and study design Nov. 1998 for all sites</td>
<td>Nov. 1998</td>
<td>300-head beef feedlot</td>
</tr>
<tr>
<td>*Same study, different VTA location Nov. 1998 for all sites</td>
<td>200-head beef feedlot</td>
<td>Yes</td>
</tr>
</tbody>
</table>

1. This table was originally developed by Ikenberry and Mankin. 2000. Presented at ASAE Mid-Central Conference, St. Joseph, MO. ASAE, St. Joseph, MI., updated by Koelsch et al. 2006, and included in this review with permission from R. Koelsch.
2. AR=area ratio= (VTA Area)/(Feedlot Drainage Area)
3. m = reductions calculated on a mass basis.
4. c = reduction calculated on a concentration basis
### Efficacy of vegetative treatment areas under simulated conditions

<table>
<thead>
<tr>
<th>Study description</th>
<th>VTA information</th>
<th>Percent reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 VTA plots placed after poultry manure amended pasture area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study description</td>
<td>VTA information</td>
<td>Percent reduction</td>
</tr>
<tr>
<td>VTA information</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensity</td>
<td>Length (m)</td>
<td>AR</td>
</tr>
<tr>
<td>64 mm/hr</td>
<td>4.5</td>
<td>0.25</td>
</tr>
<tr>
<td>9</td>
<td>0.66</td>
<td>9</td>
</tr>
<tr>
<td>A dairy slurry and water mix was applied to upper end of three lengths of VTA and three vegetative covers were tested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (m)</td>
<td>Width = 1.2m</td>
<td>AR</td>
</tr>
<tr>
<td>1.2L/s applied to upper end of filter strip</td>
<td>5</td>
<td>1.2</td>
</tr>
<tr>
<td>10</td>
<td>0.5</td>
<td>3.3</td>
</tr>
<tr>
<td>5</td>
<td>0.66</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>0.75</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>0.25</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>0.75</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>0.25</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>0.75</td>
<td>3</td>
</tr>
<tr>
<td>Simulated pasture and rainfall</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensity</td>
<td>Length (m)</td>
<td>AR</td>
</tr>
<tr>
<td>10 cm/hr</td>
<td>6.1</td>
<td>0.50</td>
</tr>
<tr>
<td>12.2</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>18.3</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>VTA test plots, natural rainfall, swine lagoon effluent pumped to VTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rainfall simulator applied 25-year, 24-hour storm to VTA plots over 2 year period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensity</td>
<td>Length (m)</td>
<td>AR</td>
</tr>
<tr>
<td>6.35 cm/hr for 71 minutes</td>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>27</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
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<td>4</td>
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<td>----</td>
<td>----</td>
<td>---</td>
</tr>
<tr>
<td>21</td>
<td>1.6</td>
<td>4</td>
</tr>
<tr>
<td>21</td>
<td>1.6</td>
<td>4</td>
</tr>
</tbody>
</table>

1. This table was originally developed by Koelsch et al. 2006, and included in this review with permission from R. Koelsch. Simulated conditions refer to studies conducted under simulated rainfall conditions and/or studies where the VTA is a bench scale or small plot-scale facility.
2. AR=Area Ratio: (VTA Area)/(Feedlot Drainage Area)
3. m = reductions calculated on a mass basis, c = reductions calculated on a concentration basis.
4. Percent reductions in overall volume of runoff
5. TC= total coliforms
11.3 Extensively grazed animal agricultural systems

On extensively grazed animal agricultural systems, vegetated treatment areas can be designed in a variety of formats. Many incorporate some form of fencing or barrier that will exclude the livestock from an area wide enough to incorporate part or all of the riparian area of the channel, creek, river, pond, or lake of concern. The aim is to keep livestock from defecating close to water bodies. This is a low-cost, minimal disturbance approach to maintaining vegetated treatment areas on existing grazed or wildlife sites that have reasonable amounts of percent vegetative cover and established plant communities. Another common application of buffers is to filter runoff from grazed locations that generate surface flow following rainfall, such as swales, upslope of ephemeral stream channels, or at the bottom of a hill slope. These sites may generate surface flow later in the rainfall season or subsequent to intense rainfall when rainfall intensity exceeds the infiltration capacity of the site. The concern is that when these locations are grazed during the rainfall season, animal fecal deposits might become a source of pathogens when surface flow occurs. If surface flow is capable of reaching a seasonal or perennial creek prior to infiltrating into the soil subsurface, then these nonriparian locations can contribute waterborne contaminants to downstream locations of concern.

Land managers may prefer to install a permanent fence on the upslope side of the buffer, with the water body of concern marking the downslope border. If cattle or other grazers are to be permanently excluded from the buffer, alteration of the plant community within the buffer may occur over time that will either benefit or harm the ability of the buffer to remove or store various contaminants. Several research projects conducted at the University of California Sierra Foothill Research and Extension Center found that when none of the vegetation from a buffer was removed on some annual basis, the buffer can become a source of waterborne contaminants and exhibit poor retention of microbes such as *E. coli*. Optimal vegetation management practices within a buffer that will maximize pathogen retention are not fully understood at this time. Land managers considering a permanently installed buffer that will not be managed for vegetation are encouraged to consult with a qualified expert on how rangeland plant communities will respond to a cessation to grazing. Such experts might include certified range managers, NRCS personnel, natural resource advisors, cooperative extension personnel, or the knowledgeable staff of a local resource conservation district.

Practices to reduce waterborne pathogens on irrigated pasture and grazed rangeland

Numerous practices can be used to reduce waterborne pathogens, such as vegetated filter strips, constructed wetlands, tail-water ponds, excluding cattle prior to irrigation, and when possible, reducing irrigation rates so that no water runs off the irrigation site.

An alternative to a permanently fenced buffer would be to use temporary fencing only during the rainfall season to insure that livestock cannot defecate within the waterway or in proximity to the water’s edge. Temporary fencing may facilitate the land manager's decision to graze the buffer during times of year in which no surface flow is present, and then seasonally exclude the cattle in the fall or early winter as the rainfall season commences, the exact time depending on the location’s traditional rainfall patterns. When to exclude cattle from the buffer prior to the onset of winter rains is in part a function of how quickly the load (i.e., total amount) of pathogen or bacterial indicator is either inactivated (killed) by such processes as heat or trapped within the drying fecal pat. A variety of studies have found that resting a pasture or aging manure prior to application to a VTA can reduce the amount of bacteria or protozoa in the fecal matrix or in the runoff. Use of temporary or seasonal buffer is one strategy that would allow microbial water quality benefits to be generated for our grazed rangelands and yet allow ranchers and other land managers to utilize the forages being grown within the buffer during late spring through early fall for regions where rainfall is limited to winter. While this does elevate the risk of waterborne microbial contamination from livestock grazing, these risks may be offset by such benefits as reduced fire hazard (where applicable) and reduced feed costs for landowners, leading to higher compliance in the ranching and farming community, compared to a permanently installed buffer that excludes cattle on a year-round basis.

Numerous studies using vegetated filter strips have shown that buffers as short as 3 to 6 feet can reduce waterborne pathogens and indicators like *E. coli*, *Enterococcus*, *Giardia duodenalis*, and *Cryptosporidium parvum* by 90 to 99.9 percent during rainfall runoff conditions. What is less clear is whether vegetated buffers are equally effective at reducing viruses which are much smaller in diameter than bacteria or protozoa.
Effluents and runoff from livestock production facilities

VTAs on AFOs and other livestock production facilities are often combined into an integrated vegetated treatment system that allows effluent high in solids to settle out prior to application to the VTA. An excellent review is provided by Koelsch, Lorimar, and Mankin (2006) regarding numerous simulated and on-farm vegetated treatment systems that were found to reduce concentrations or loads (mass) of various bacterial indicators in runoff from open lots on a livestock production system (tables 21 and 22). Other reviews provide various recommendations on how to handle and process manure effluent and strategies to reduce runoff. Gravity-fed or passive treatment systems have been advocated as a low-cost method to reduce both runoff and nutrients, and more recently have been validated as an effective strategy for reducing zoonotic pathogens and bacterial indicators in feedlot runoff. Studies have demonstrated that application of straw mulching and strategic seeding with annual ryegrass (25 pounds of seed per acre) and annual barley (100 pounds of seed per acre) on coastal California dairy dry lots can result in a 10-fold reduction in the concentration of fecal coliforms discharging from these sites during rainfall. Restricting winter use is also highly beneficial in fecal coliform reduction. At these same California coastal dairies, grassed waterways and mulching with straw were effective practices at reducing the concentration or instantaneous load of *Giardia* cysts, *Cryptosporidium* oocysts, and fecal coliforms in dairy runoff from natural rainfall events. If the pathogens of concern are protozoal parasites (*Cryptosporidium parvum* and *Giardia duodenalis*), dairy VTAs should be focused on runoff from calves and young stocks given the high infection levels in these populations compared to older animals. Regarding grassed waterways, factors such as the cross-sectional morphology, channel roughness, and the gradient of the side slopes were shown to determine the effectiveness in reducing sediment delivery and runoff volumes at the terminus of the waterway. Maintaining a standing height of 13 to 15 cm (compared to 5 to 7 cm) on cropped mixed-grass hay land was found to reduce runoff concentrations of *E. coli* from fields receiving 90-day-old dairy manure. When the rainfall occurred 3 days after manure application, no difference was observed between these two stubble heights when fresh manure was applied or when rainfall occurred 1 day after manure application.

11.4 Infiltration or settling basins, wetlands, manure storage lagoons

Capturing surface runoff with infiltration or settling basins, natural and constructed wetlands, and manure storage lagoons have been shown to reduce the volume of runoff or effluent and, in general, to substantially reduce but not eliminate bacterial, protozoal, or viral contaminants from a variety of influent sources. Addition of these vegetated or bare catchments is typically considered part of an overall runoff water quality system such that nutrients, solids, microbes, and other contaminants are reduced prior to discharge into the VTA or other water quality practice. Optimization for one class of contaminants (microbes) may not fully optimize removal for other classes of contaminants (nutrients, pesticides), so prioritization of contaminant class will need to occur. Depending on the type and design of the system (wetland, infiltration basin, storage lagoon); the quality and concentration of microbes of the influent water, flow rates through the system, vegetation composition; proportion of pathogen load attached to sediment or organic particles; and other such factors, the observed reductions of enteric viruses, bacteria, and protozoal parasites ranged from 30 to over 90 percent in many cases. Such factors as longer hydraulic residence time, adequate vegetation, higher temperatures, higher levels of aeration and aerobic digestion, and including one or more finishing lagoons were generally associated with higher removal levels for microbial contaminants. There are a few examples where microbial contaminants increase in concentration while transiting a constructed wetland.

Interested readers should consult the numerous reviews, technical manuals, and guidance documents that cover the design and operation of the various natural and constructed systems (http://www.nrcs.usda.gov/wps/portal/nrcs/main/national/technical/cp/ncps). The EPA has published guiding principles for citing, design, construction, operation, maintenance, and monitoring of constructed treatment wetlands which is available online at http://www.epa.gov/owow/wetlands/constructed. Additionally, land grant universities that have cooperative extension programs typically have free-access Web sites for distributing information on the design, construction, operation, maintenance, and monitoring of animal manure storage or treatment lagoons.
Impact of tile drains

The use of tile drains in fields that receive livestock manure as a soil amendment can lead to discharges of pathogens. Coarse soils, high application volumes of manure, or shallow tile drains can all increase the chance that manure pathogens leach down into the drain field and then be transported with the drain effluent. In essence, tile drains allow pathogens to bypass much of the potential subsurface filtration that can occur when waterborne pathogens infiltrate into the soil.

11.5 Further reading and references


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12. Practices to Maximize Inactivation of Pathogens

12.1 Overview

Waterborne zoonotic pathogens of primary concern, along with many bacterial indicators, are typically excreted in feces by the infected hosts, with a few exceptions among pathogens of secondary concern (table 4). Given that feces are the principal source of waterborne zoonotic pathogens of primary concern and of bacterial indicators like commensal E. coli, practices that maximize the inactivation of these protozoa and bacteria tend to focus on manure management. Once these microorganisms are excreted into the environment, a key management tool is to allow sufficient residence time of the fecal matrices (solids, effluent, etc.) in the AFO manure management system (e.g., anaerobic storage lagoon, stacked bedding material) so that sufficient inactivation of the microbial load occurs. Strategic use of time can be employed in rotational grazing systems or seasonal exclusion of riparian pastures from grazing by beef cattle and range sheep. Chapter 7, "Survival of Pathogens in the Environment," provides a variety of die-off coefficients and survival estimates for a wide range of pathogens listed in table 4 for manure slurries, water, and in some cases, fecal pats. Passive mesophilic processes can require weeks, several months, or sometimes longer to inactivate a sufficient percentage of the pathogen load in the manure matrix. Consequently, a large amount of expensive storage space may be necessary to hold the high volume of manure generated by AFOs and other livestock production facilities. If the rate of microbial inactivation is not sufficiently high using mesophilic and other low-input systems for the water quality goals of the operation, there are manure handling and processing practices that are proven to substantially increase the rate of microbial inactivation. These practices typically function by either encouraging thermophilic processes to occur within the manure matrix (e.g., adding bulking agents and stacking of manure solids, aeration and turning of compost piles), increasing the amount of aerobic digestion via aeration of manure effluent storage lagoons, adding chemical treatments such as lime, ozone, or chlorine that inactivate a variety of microorganisms, or using energy intensive methods such as ultraviolet radiation. Placing two or more of these practices for pathogen inactivation in serial can generate substantial log-reductions of the pathogen load and also provides for a multibarrier approach to pathogen-reduction goals in case one or more components of the manure management system fail to operate as expected. An example of a serial system would be a settling basin followed by VTA for feedlot runoff or a solids separator followed by composting of manure solids and thermophilic digestion of manure effluents.

12.2 Manure solids and other animal agricultural waste

There are numerous methods to increase the proportion of microbial pathogens and bacterial indicators that are inactivated in manure solids and slurries. One of the most common procedures is to actively compost the manure solids via aerated windrows, in-vessel composting, aerobic and thermophilic digesters, or to just passively stack manure and other solids (soiled bedding, old feed, etc.), such that the thermophilic processes occur. There is widespread scientific evidence that the waterborne zoonotic pathogens of primary concern and also bacterial indicators are substantially reduced during thermophilic processes involving stacked manure, aerated composting, and aerobic/aerated digesters that generate internal temperatures of at least 50 to 55 °C (122 to 131 °F) for 3 or more days’ exposure. Longer exposures, such as 15 days, can typically lead to further microbial reductions if these elevated temperatures can be maintained. Although properly designed and operated aerated compost piles and aerobic digesters can substantially reduce many pathogens contained in manure solids, stacked manure piles that are neither turned nor aerated and have excess moisture content will likely have a variety of residual microbial pathogens and bacterial indicators left in the pile, especially for cooler locations and during cold wet seasons. Equipment failure, inadequate thermal monitoring, cold weather, and poor compliance with maintaining high temperatures for adequate amounts of time for the entire mass of manure solids are cited as reasons why composting procedures may fail to inactivate nearly all of the pathogens. Using berms or similar such methods is important to capture and control the rainfall or snowmelt runoff from stacked manure piles which may contain high levels of bacterial indicators that have yet to be completely inactivated. Other options include covering the pile with a waterproof tarp or locating such piles under a roof, where possible.
Factors that increase pathogen inactivation when composting and stacking manure solids:

- aeration and turning the pile
- proper balance between wet manure and bulky material
- internal temperatures exceed 50 to 55 °C (122 to 131 °F) for multiple days
- using thermophilic digesters

It may seem an unreasonable goal to expect complete sterilization (100 percent inactivation) of manure solids for many of our low-intensity, low-input manure management systems present on smaller AFOs or livestock production facilities throughout the United States, but the benefit of using these low-input systems would be higher producer acceptance and higher compliance due to the lower cost of such systems and therefore a greater reduction in pathogens overall. For example, storing dairy manure for 30 days prior to field application compared to no storage (e.g., using fresh manure) resulted in a 98.9-percent reduction of *E. coli* counts in aged manure, which then lead to a significant reduction in the amount of *E. coli* in the runoff from land application sites. Storing manure for 90 days instead of 30 days resulted in a 99.6 percent reduction of *E. coli* counts—basically the same as 30 days storage, though the amount contained in the runoff from the 90-day-old application site was lower compared to sites receiving 30-day-old manure. Depending on the water quality objectives of the dairy operation, 30 days of storage may be sufficient and could be more cost effective than 90 days of storage, resulting in improved producer acceptance and higher compliance. The producer may decide it is more economical to limit where they dispose of their treated manure solids rather than spend the resources on developing a manure management system that generates multiple log reductions for their manure solids. Manure management systems that rely on mesophilic processes to treat the manure solids are only moderately effective at reducing microbial zoonotic pathogens and bacterial indicators. These mesophilic processes (35 °C/95 °F) typically require much more time to achieve similar levels of inactivation compared to thermophilic processes (50 °C/122 °F).

Interested readers should consult the numerous guidance manuals, design criteria, monitoring procedures, and other related information that is widely available from a variety of sources, much of it at minimal cost. Every AFO manure management system is slightly different in design and understanding the basic principles driving mesophilic and thermophilic digestion is essential for proper planning and development of a system that generates the expected level of performance. Much of the published information is targeted toward municipal waste, but many of the same principles and design criteria apply to inactivation of animal manure solids.

### 12.3 Liquid manure and effluents

Many of the bacterial waterborne zoonotic pathogens listed in table 4 and bacterial indicators are moderately reduced (0.5- to 2-log reduction) while being stored 30 to 90 days in mesophilic, anaerobic manure storage lagoons. Increased lagoon temperatures (as might occur in summer), addition of aeration to mesophilic manure storage lagoons, and using mesophilic and especially thermophilic digesters generally increases the rate of microbial inactivation compared to the mesophilic, anaerobic, nonaerated manure storage lagoons (low-input systems). For liquid swine manure a multistage treatment system where solids and liquid are separated with polymer, followed by biological nitrogen removal and phosphorus chemical extraction can result in reductions of >6.5 log for total coliforms, >5.9 log for fecal coliform, >5.4 log for *Enterococci*, and >3.6 log for *Salmonella*. Batch reactors generating anaerobic digestion of swine manure slurry at 20 °C (68 °F) for 20 days reduced total coliforms by 98 to nearly 100 percent, reduced *E. coli* by 99.7 to about 100 percent, and generated undetectable levels of *Salmonella*, Cryptosporidium, and Giardia. Chlorine, ultraviolet light, and ozone were effective in reducing various microbial species in swine effluent, but the cost of using such treatment methodologies is likely to be cost prohibitive for many producers.

Factors that increase pathogen inactivation in manure storage lagoons:

- longer residence in the lagoon
- warmer months leading to warmer effluent in lagoon
- aeration of lagoon effluent

### 12.4 Rotational grazing, cattle exclusion, and field application of aged manure solids

A variety of studies have found that resting a pasture prior to irrigation or aging manure prior to application to a VTA or cropland can reduce the amount of
bacteria or protozoa both in the fecal matrix and in the runoff following irrigation or rainfall. Such procedures are often inexpensive to implement unless sufficient manure storage is unavailable, or there is a lack of acreage for grazing or insufficient fencing to establish a rotational grazing system. As little as 9 days of cattle exclusion prior to flood irrigation on California range-land during summer has been shown to reduce the amount of commensal *E. coli* discharged from irrigated pastures by 20 to 25 percent compared to resting the pasture only 1 day prior to irrigation. Storing dairy manure for 30 days prior to field application compared to using fresh manure resulted in a ~99 percent reduction of *E. coli* counts in aged manure and a significant reduction in the amount of *E. coli* in the runoff from land application sites. Excluding sheep from grazing a hill slope for 40 to 70 days prior to sprinkler irrigation can generate a two- to three-log reduction in both the concentration and load of *E. coli* in runoff compared to no rest.

Additionally, excluding dairy cattle from using loafing areas during the winter significantly reduced the amount of fecal coliforms discharging from these sites following rainfall events. This information is supported by work conducted on die-off of *E. coli* in fecal pats, where the rate of inactivation was strongly correlated with age of pat and exposure to solar radiation.

To the extent possible, land managers can use the generalized die-off coefficients for pathogens in manure matrices described in Chapter 7, “Survival of Pathogens in the Environment,” to roughly estimate the amount of days needed to generate sufficient pathogen load reductions prior to anticipated hydrological events such as flood irrigation or the seasonal onset of rainfall. These policies of seasonal exclusion to allow sufficient time for *C. parvum* inactivation have been recommended and in some cases implemented by drinking water districts that prefer to graze their watersheds for fire hazard and other resource goals resulting from cattle herbivory, but also want to insure that bovine fecal-borne pathogens are inactivated prior to runoff occurring during the winter rainfall season.

### 12.5 Further reading and references


13. Conclusion

Although a variety of microbial pathogens can be excreted by domestic and wild animals that are of waterborne public health concern, NRCS has developed numerous conservation practices that can be used by landowners, agricultural managers, and water quality specialists to substantially reduce the risk of waterborne contamination from pathogens. These can be accessed on the NRCS Web site at http://www.nrcs.usda.gov/wps/portal/nrcs/main/national/technical/cp/ncps. Many of these practices have a decade or more of research supporting their efficacy, with projects extending from basic mechanisms of microbial attachment and inactivation, to replicated field trials on their performance. Despite this large body of work, uncertainties exist in our ability to accurately predict conservation practice performance for novel agricultural systems, complex landscapes, dynamic aquatic systems, and extreme climate scenarios. Nonetheless, straightforward procedures such as simply increasing the distance between fecal loads and surface water supplies or aging manure prior to field application, when guided by common sense and consistently implemented, can generate substantial water quality benefits and reduce the risk of waterborne pathogen contamination from livestock.

Our challenge is to continue to develop the number of practices that land managers, growers, farmers, and others can use to reduce the risk of waterborne microbial contamination and to support efforts to implement these practices on our agricultural watersheds. If we succeed in this challenge, we will better reap the many economic, nutritional, and cultural benefits generated by our Nation's agricultural community while helping minimize the potential public and environmental health risks associated with waterborne pathogens.
Glossary

Aerobic.—Oxygen requiring; usually refers to a habitat, organism, or process that relies on the presence of oxygen for continued existence, growth, or function.

Amnesic shellfish poisoning (ASP).—Impairment or lack of memory caused by the ingestion of shellfish contaminated with the diatom pseudo-nitzschia sp. that has produced the toxin domoic acid. ASP can be a life-threatening syndrome.

Amoeba.—Single-celled organism that has no definite form and consists of a mass of protoplasm containing one or more nuclei surrounded by a flexible outer membrane.

Anaerobe.—Used to describe a biological habitat or an organism that exists and grows with oxygen.

Antibody.—Any of various proteins produced in the blood in response to the presence of an antigen, which it neutralizes, thus producing an immune response.

Antigen.—Any substance that is introduced to the body that causes the production of antibodies (e.g. bacteria, chemicals or donated tissue).

Assemblage.—A particular strain of pathogenic organism (e.g., Giardia) characterized by a specific genetic code.

Asymptomatic.—Carrying a particular disease, but not showing any symptoms.

Autoinfection.—An infection caused by a disease agent that is already present in the body.

Bacteriophages.—A virus capable of infecting and destroying bacterial cells.

Campylobacteriosis.—An infection of the intestines caused by bacteria of the Campylobacter genus. Symptoms include mild to severe diarrhea (often bloody), stomach pain, fever, nausea, and vomiting.

Chitinous.—Possessing a tough, protective, shell composed primarily of a nitrogen-containing polysaccharide, forming the principal component of arthropod exoskeletons and the cell walls of certain fungi.

Ciguatara fish poisoning (CFP).—Gastrointestinal, neurological, and cardiovascular symptoms associated the ingestion of fish contaminated with toxic dinoflagellates. Paralysis and death have been documented, but symptoms are usually less severe although debilitating.

Cilia.—Tiny, hair-like projections from a cell.

Ciliated.—A microscopic single-celled organism that has hair-like projections (cilia) on its surface used for locomotion.

Coccus.—Spherical in shape, such as the bacterium Enterococcus.

Coliphage.—A bacterial virus (phage) that uses coliform bacteria as a host.

Colony forming unit (CFU).—Measure of viable bacterial cells or clumps of bacterial cells capable of reproducing to form a visible colony.

Cryptosporidiosis.—An illness of varying severity caused by the microscopic intestinal parasitic protozoan, Cryptosporidium. It is a common cause of diarrhea worldwide and is common in AIDS patients.

Cyst.—A resting stage of an organism that has a tough outer coating.

Dinoflagellate.—Microscopic, (usually) unicellular, flagellated protists, commonly regarded as "algae" (photosynthetic varieties), or protozoans when exhibiting predation and parasitism (e.g., Pfisteria).

E. coli O157:H7.—A specific serotype of E. coli that is responsible for enterohemorrhagic diarrhea.

Encyst.—The process a cell undergoes to produce a cyst.

Endospore.—An asexual spore formed within a bacterial cell.

Endocarditis.—Inflammation of the inside lining of the heart chambers and heart valves (endocardium). Symptoms include fever, chills, fatigue, muscle/joint pain and unexplained weight loss.

Enteric.—Of or relating to the small intestine.

Epidemiology.—The study of populations to determine the frequency and distribution of disease and to measure risks.

Eukaryotic.—A type of higher order cell possessing a nucleus and other membrane-bound organelles such as mitochondria, chloroplasts and the Golgi apparatus.
**Excystation.**—Escape from a cyst by an encysted organism.

**Fecal coliform.**—A grouping of coliform bacteria that lives in the intestines of warm-blooded animals. Elevated measurements of these bacteria in surface water may indicate the presence of human or animal waste.

**Fecal erosion.**—The wearing down and weathering of feces by water and wind, leading to transport of fecal material from the original site of defecation.

**Flagellum.**—A long, thin, hair-like projection from a cell used for movement.

**Flagellate(d).**—A microscopic, single-celled organism possessing one or more long, hair-like projections (flagellum) used for locomotion.

**Gastroenteritis.**—Inflammation of the mucous membrane of the stomach and intestine causing diarrhea, nausea and vomiting; often referred to as "stomach flu."

**Geometric mean.**—A measure of the central tendency of a data set that minimizes the effects of extreme values; calculated by multiplying a series of numbers and taking the nth root where n is the number of numbers in the series.

**Giardiasis.**—An infection of the small intestine caused by a microscopic organism (protozoa), *Giardia lamblia*. Symptoms include abdominal pain, diarrhea, nausea and vomiting; often referred to as "stomach flu."

**Harmful algal blooms (HAB).**—An explosive increase in the density of phytoplankton within an area, specifically those involving toxic or otherwise harmful phytoplankton (dinoflagellates).

**Groundwater.**—Water beneath the earth’s surface, often between saturated soil and rock that supplies wells and springs. The upper surface of groundwater is the water table.

**Hemolytic uremic syndrome.**—A disorder that usually occurs when an infection in the digestive system produces toxic substances that destroy red blood cells, may lead to kidney failure. Most common in children and the elderly.

**Hemorrhagic colitis.**—A clinical syndrome manifested by bloody diarrhea and inflammation of the colon, typically the result of infection by hemorrhagic *E. coli* O157:H7.

**Hyporheic exchange.**—The subsurface exchange of water between streams/lakes and groundwater through the hyporheic zone.

**Hyporheic zone.**—A subsurface volume of sediment and porous space adjacent to a stream or lake through which water readily exchanges; a mixing zone between surface water and groundwater.

**Immunocompetent.**—The opposite of immunodeficient; capable of developing an immune response able to recognize antigens and react by producing antibodies.

**Inactivate(d).**—To cause (as an infective agent) to lose disease-producing capacity.

**Infectious dose.**—The amount of pathogen (measured in number of organisms) required to cause infection in the host.

**Intracellular.**—Existing, occurring, or functioning within a cell (such as intracellular parasites).

**Isolate(s).**—An organism that is isolated from a single source, usually by culturing.

**Leptospirosis.**—Bacterial disease that affects humans and animals caused by bacteria of the genus *Leptospira*. Symptoms include fever, chills, muscle pain, and vomiting. Can lead to kidney failure if left untreated.

**Mesophilic.**—Requiring a warm temperature in which to develop.

**Microsporidiosis.**—Opportunistic disease occurring mainly, but not exclusively, in severely immunocompromised patients. Symptoms are very diverse, varying according to the causal species with diarrhea being the most common.

**Motile.**—Capable of self-propulsion and spontaneous movement.

**Nonmotile.**—Not capable of movement.

**Obligate.**—Biologically essential for survival.

**Oocyst.**—The environmentally resistant stages of protozoan, such as *Cryptosporidium*.

**Organelle.**—A structurally discrete component of a cell, analogous to organs, including mitochondria and chloroplasts.
Paralytic shellfish poisoning (PSP).—A life-threatening syndrome caused by consumption of contaminated shellfish. The toxins produced are called saxitoxins.

Parasite.—An organism that lives in or on and takes its nourishment from another organism.

Pathogen.—An agent of disease; including bacteria, protozoans, and parasites.

Pathogen of primary concern.—As used in this review/module, are microorganisms infectious to humans during clinical trials and real-world scenarios and are known to be shed by livestock or transmitted by a waterborne route.

Pathogen of secondary concern.—As used in this review/module, are infrequently shed by animals or have rarely been the cause of a waterborne outbreak.

Primary amebic menigoencephalitis.—A brain infection that leads to the destruction of brain tissue. Early stages may be similar to bacterial meningitis with rapid onset of fever, nausea, vomiting and a stiff neck. Most infections are fatal.

Pseudopod.—Temporary outgrowth used by some microorganisms as an organ of feeding or locomotion.

Salmonellosis.—An infection of the intestines caused by the Salmonella bacteria, which causes severe diarrhea and death in some cases.

Sepsis.—A severe illness in which the bloodstream is overwhelmed by bacteria causing clotting and blockage of blood flow; can lead to tissue and organ death.

Serotype.—A group of microorganisms, viruses, or cells classified together based on their cell surface antigens.

Shedding.—The releasing of organisms from the host, usually in feces.

Shigella.—A group of bacteria that normally inhabits the intestinal tract and causes infantile gastroenteritis, summer diarrhea of childhood, and various forms of dysentery.

Spiillum.—A fairly rigid, helically twisted bacterial cell.

Spirochete.—Bacteria that appear worm-like, spiral-shaped, and wiggle vigorously when viewed under a microscope.

Sporozoites.—A stage in the development oocyst of Cryptosporidium that infects intestinal cells.

Symbiont.—An organism in a symbiotic relationship; a close and usually obligatory association of two organisms of different species that live together, often to their mutual benefit.

Thermophilic.—Requiring high temperature in which to develop.

Trophozoite.—The motile feeding stage of a protozoan.

Toxoplasmosis.—Disease caused by the protozoan parasite Toxoplasma gondii; considered to be the third leading cause of death due to foodborne illness in the United States. Symptoms generally mild in nonimmunocompromised persons.

Zoonotic.—A disease caused by pathogens that are transmitted among animals and humans.