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REVIEW OF ZOONOTIC PATHOGENS IN AMBIENT WATERS

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Office of Water
Health and Ecological Criteria Division

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ACRONYMS

AEC	attaching *E. coli*
AIDS	Acquired Immune Deficiency Syndrome
AWQC	ambient water quality criteria
BEACH Act	Beaches Environmental Assessment and Coastal Health Act of 2000
CDC	U.S. Centers for Disease Control and Prevention
CPV	*C. parvum* virus
CWA	Clean Water Act
DAEC	diffuse adherent *E. coli*
DEC	diarrheagenic *E. coli*
EAggEC	enteroaggregative *E. coli*
EEC	effacing *E. coli*
EHEC	enterohemorrhagic *E. coli*
EPA	U.S. Environmental Protection Agency
EPEC	enteropathogenic *E. coli*
ETEC	enterotoxigenic *E. coli*
GI	gastrointestinal
GLV	*Giardia lamblia* virus
GBS	Guillain-Barré Syndrome
HC	hemorrhagic colitis
HEV	hepatitis E virus
HUS	hemolytic uremic syndrome
IPSID	immunoproliferative small intestinal disease
MALT	mucosa-associated lymphoid tissue
NA	not available or not applicable
PCR	polymerase chain reaction
POTW	Publicly Owned Treatment Works
ppt	parts per thousand (salinity)
RFLP	restriction fragment length polymorphism
SPE	serial passage experiment
STEC	Shiga toxin-producing *E. coli*
U.S.	United States
U.K.	United Kingdom
USDA	U.S. Department of Agriculture
UV	ultraviolet (light)
VTEC	verocytotoxin-producing *E. coli*
WHO	World Health Organization (United Nations)

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EXECUTIVE SUMMARY

Introduction

The overall goal of the current Clean Water Act (CWA) §304(a) ambient water quality criteria (AWQC) for bacteria in the United States is to provide public health protection from gastrointestinal (GI) illness (gastroenteritis) associated with exposure to fecal contamination during recreational water contact. Water quality criteria are specified throughout the world in terms of concentrations of fecal indicator organisms because fecal matter can be a major source of pathogens in ambient water and because it is not practical or feasible to monitor for the full spectrum of all pathogens that may occur in water. For decades, these fecal indicator organisms have served as surrogates for potential pathogens and subsequent health risks in both recreational and drinking waters.

The U.S. Environmental Protection Agency (EPA) currently recommends AWQC for recreational water that encompass all fecal sources that contain the relevant indicator species (enterococci and *E. coli*). This approach assumes that animal fecal material is as hazardous as human fecal material. There is limited evidence, however, that recreational water contaminated with animal fecal material is less risky to swimmers than recreational water contaminated with human fecal material.

In order to evaluate the potential risks posed by animal fecal contamination, EPA is interested in understanding what human illnesses are caused by swimming in waters contaminated with animal fecal material ranging from wildlife sources to agricultural inputs. The animal species of most interest are the warm-blooded animals (mammals and birds) whose fecal material is detected by current indicators.

The purpose of this white paper is to provide a summary of information on waterborne zoonotic pathogens that come primarily from warm-blooded animals, and which can be used to conceptualize potential risks from warm-blooded animal feces in ambient (untreated) recreational waters.

Approach

Seventy pathogens from warm-blooded animals were evaluated for their potential to be both waterborne and zoonotic. Twenty of the 70 pathogens evaluated had all 4 of the following attributes:

1. *The pathogen spends part of its lifecycle within one or more warm-blooded animal species.*
2. *Within the lifecycle of the pathogen, it is probable or conceivable that some life stage will enter water.*
3. *Transmission of the pathogen from animal source to human is through a water related route.*
4. *The pathogen causes infection or illness in humans.*

Six of the 20 waterborne, zoonotic pathogens from warm-blooded animals were selected for further discussion based on their relevance in the United States. Five were selected based on their potential for outbreaks in ambient (untreated) recreational water and one (*Salmonella*) was included based on outbreaks in drinking water.

Some well-known waterborne pathogens were excluded from analysis because they are not zoonotic. Excluded pathogens include bacteria that are generally found in the environment, free-living protozoa, viruses, and helminthes that have cold-blooded hosts (e.g., snails, copepods). Some common zoonotic pathogens were also excluded because they do not have well-documented waterborne transmission (i.e., primarily transmitted via soil, food, or drinking water).

Pathogens interact with the ambient environment, other microorganisms, plants, and with their hosts. The behavior of pathogens in ambient waters is often different from the behavior of indicators in ambient water. The most common environmental factors studied for their impact on pathogen survival in water are pH, salinity, light exposure, and temperature. Additional environmental characteristics that may influence pathogen survival, infectivity, and virulence include the following: ultraviolet (UV) light (duration, intensity), rainfall, runoff, dispersal, suspended solids, turbidity, nutrients, organic content, organic foams, water quality, biological community in water column, water depth, stratification, mixing (e.g., wind and waves), presence of aquatic plants, biofilms, and predation.

There is evidence that zoonotic pathogens may change in infectivity, virulence, and the severity of disease caused in humans depending on their previous host environment. There is also evidence that some of these host-factor changes can influence subsequent infection cycles in exposed hosts. The key mechanisms of phenotypic change in pathogens are genetic diversity (coinfection and quasispecies), cryptic genes, mutators, and epigenetic effects.

Six Key Waterborne Zoonotic Pathogens

Pathogenic *E. coli*

E. coli is an important waterborne bacterial zoonosis because many human pathogenic strains occur in livestock and wildlife feces and can survive in ambient waters. In addition, the potential illnesses caused by pathogenic *E. coli* can be severe or fatal. Mortality estimates range from 0.08 to 1.9 percent of *E. coli* O157:H7 infections. Children are more at risk than healthy adults to suffer more severe outcomes. Between 1991 and 2004, 14 outbreaks of *E. coli*-related illness have been associated with ambient recreational waters. *E. coli* O157:H7 can survive for at least several weeks in animal feces and slurries and has been demonstrated to survive at least 500 days at -20 °C in frozen soil.

Campylobacter

Campylobacter is a well-known foodborne bacterial pathogen that is commonly associated with poultry and livestock. There are also wildlife hosts. Although few waterborne outbreaks have been reported, there is potential for *Campylobacter* to cause recreational water-related illness. Normally, infection results in diarrhea that is self-limiting; however, approximately 1 out of 1,000 infections results in Guillain-Barré syndrome, which is a serious nervous system affliction.

Reactive arthritis is also a possible chronic sequela of *Campylobacter* infection. *Campylobacter* has been shown to survive in aquatic environments with low temperatures (4°C) up to 4 months.

Salmonella

Salmonella is also a well-known foodborne bacterial pathogen that is associated with poultry and livestock. There are also wildlife hosts. Elevated levels of *Salmonella* have been observed in major water bodies that receive discharges of meat processing wastes, raw sewage, farming operations, and effluents from ineffective sewage treatment plants. The clinical symptoms of salmonellosis may include diarrhea, abdominal pain, nausea, chills, and fever. Between 1991 and 2002, 3 waterborne outbreaks were attributed to nontyphoid *Salmonella*; *Salmonella* were the etiologic agent in 0.9 percent of 259 recreational waterborne outbreaks occurring from 1971 to 2000. Under suitable environmental conditions, *Salmonella* can survive for weeks in waters or years in soils.

Leptospira

Leptospira is an important waterborne bacterial pathogen for most of the world. Because warmer climates favor its survival in the environment, the highest incidence of leptospirosis in the United States is found in Hawaii. The source of infection in humans is usually either direct or indirect contact with the urine of an infected animal. There are numerous symptoms associated with leptospirosis, but the more severe form is known as Weil's disease. In addition, acute infection during pregnancy has been reported to cause abortion and fetal death. From 1971 to 2000, 16 percent of recreational waterborne disease outbreaks in the United States were attributed to *Leptospira.* In 1998, an outbreak (375 cases) of leptospirosis was reported that was associated with a triathlon in a lake in Illinois.

Cryptosporidium

Cryptosporidium is a well-known waterborne protozoan pathogen. EPA currently regulates it under the Safe Drinking Water Act because the level of *Cryptosporidium* in many ambient source waters is sufficiently high that risks to drinking water must be managed. *Cryptosporidium* infection has been reported in more than 155 mammalian species and numerous reptiles, amphibians, birds, and fish. In watersheds with diverse land-use patterns, it is likely that different sources contribute different proportions to the total contamination load at different times during the year, with the relative contributions depending on a wide range of watershed characteristics. Cryptosporidiosis is primarily characterized by GI symptoms such as profuse, watery diarrhea. Immunocompromised individuals generally experience chronic gastroenteritis, which may last as long as the immune impairment. The largest known outbreak of cryptosporidiosis occurred in 1993 in Milwaukee, Wisconsin and infected 403,000 individuals. Animal fecal contamination of drinking water was indicated in the outbreak. In the United States, from 1991 to 2004, 6 outbreaks of cryptosporidiosis have been associated with untreated recreational waters. Excreted *Cryptosporidium* oocysts can survive for substantial periods in animal wastes and soils. Thus, contaminated runoff can enter ambient water and result in potential human exposures. The majority of oocysts (99 percent) are inactivated by repeated freeze-thaw cycles; therefore, *Cryptosporidium* may be environmentally limited in parts of the United States during winter months. Between 4 and 20° C, there is very little inactivation of oocysts in different types of agricultural soils. Oocyst survival in various water matrices is highly variable, but survival for longer than 30 days has been demonstrated in several studies.

Giardia

Giardia is also a well-known waterborne protozoan pathogen. Although both livestock and humans have been implicated in contaminating water sources with *Giardia*, humans are responsible for the majority of the contaminations. Zoonotic transfer plays only a minor role in the infection cycles of *Giardia*, and animal contact is not a major risk factor. There is a wide spectrum of symptoms associated with giardiasis that ranges from asymptomatic infection and acute self-limiting diarrhea to persistent chronic diarrhea, which sometimes fails to respond to treatment. Asymptomatic infection is very common, with 50 to 75 percent of infected persons reporting no symptoms. In the United States, from 1991 to 2004, 7 reported outbreaks of giardiasis were associated with untreated recreational waters. At 4° C, *Giardia* cysts were infective for 11 weeks in water, 7 weeks in soil, and 1 week in cattle feces.

Summary

Although the most common waterborne recreational illnesses are probably due to nonzoonotic human viruses, which typically cause short-term gastroenteritis, the waterborne zoonotic pathogens discussed in this report have the potential to cause serious health effects—especially in immunocompromised persons and subpopulations. While serious health outcomes are likely to be rare in comparison with self-limiting illnesses as a result of ambient (recreational) water exposure, the adverse health impacts of the rare, but more serious illnesses remain an important public health challenge.

I. BACKGROUND AND INTRODUCTION

I.1 Background: Context and Purpose

Since the U.S. Environmental Protection Agency (hereafter EPA or the Agency) last published recreational water quality criteria in 1986, there have been significant scientific and technical advances, particularly in the areas of molecular biology, microbiology, and analytical chemistry. EPA believes that these advances need to be considered and evaluated for feasibility and applicability in the development of new or revised CWA §304(a) criteria for recreational water. To this end, EPA has been conducting research and assessing relevant information to provide the scientific and technical foundation for the development of new or revised criteria. The enactment of the Beaches Environmental Assessment and Coastal Health (BEACH) Act of 2000 (which amended the CWA) required EPA to conduct new studies and to issue new or revised criteria for Great Lakes and coastal marine waters.

In response to the BEACH Act of 2000, EPA also has engaged a range of stakeholders representing the general public, public interest groups, state and local governments, industry, and municipal wastewater treatment professionals. In March 2007, EPA convened a group of 43 national and international technical, scientific, and implementation experts from academia, numerous state agencies, public interest groups, EPA, and other federal agencies at a formal workshop to discuss the state of the science on recreational water quality research and criteria implementation. Among the input from the individuals attending the workshop were suggestions for incorporating the ability to differentiate sources of fecal contamination and to determine the relative human health risk from these sources into the new or revised criteria.

Based on the feedback from the large group of stakeholders, as well as input and recommendations from the scientific community, the Agency has developed a *Critical Path Science Plan for Development of New or Revised Recreational Water Quality Criteria*. One of the key questions posed in the science plan asks: what is the risk to human health from swimming in water contaminated with human fecal matter as compared to swimming in water contaminated with nonhuman fecal matter? Human and animal feces can both potentially contain pathogens that cause human illness. Some human pathogens are host-specific (i.e., human enteric viruses), while other human pathogens are found in and can be shed by both humans and other animals. Moreover, while all enteric pathogens of humans are infectious to other humans, only a subset of the enteric pathogens of animals is infectious to humans. Understanding which pathogens could be present depending on the source of fecal contamination might allow the Agency to better estimate human health risks from identified sources of fecal matter.

EPA's current recommended recreational water quality criteria for microbes treat human health risks from the various sources of fecal contamination as equivalent (USEPA, 1986). These criteria are based on the risk of illness from swimming in waters influenced by sewage treatment plants effluents. Health risks from other sources (e.g., poorly-treated or untreated human waste, nonhuman sources of fecal contamination, mixed sources such as urban stormwater runoff) were not well understood at the time the 1986 criteria were developed, and EPA's approach was to be protective of human health regardless of the source. EPA recognizes, however, that the health

risk from sources other than sewage treatment plants may be different and that the scientific advances over the intervening years may now allow the Agency to better characterize the relative risks to human health from these various sources of fecal contamination. Specifically, the Agency is interested in understanding which human illnesses can be caused by swimming in waters contaminated with nonhuman fecal matter including both wildlife sources and agricultural inputs. The animal species of most interest are the warm-blooded animals (mammals and birds) for which fecal matter is detectable by current fecal indicators.

The purpose of this white paper is to provide summary information on waterborne zoonotic pathogens that come primarily from warm-blooded animals, and which can be used to conceptualize potential risks to humans from warm-blooded animal feces in recreational waters.

I.2 Introduction

In the development of health protective criteria for recreational waters, pathogen contamination is the central concern, and fecal matter can be a major source of pathogens in ambient water. Current recreational AWQC are designed specifically to protect humans from GI illness (gastroenteritis) associated with exposure to fecal contamination in recreational waters (USEPA, 1986). Because widespread monitoring of recreational waters directly for all disease-causing microorganisms (especially pathogenic bacteria, viruses, and protozoa) remains infeasible, public health and environmental protection agencies have relied on the detection of fecal indicator organisms, which comprise a few groups of nonpathogenic fecal bacteria and some viruses, to indicate the presence and magnitude of fecal material. This approach assumes that waterborne pathogens co-occur with the fecal material. "More specifically, fecal indicator bacteria provide an estimation of the amount of feces, and indirectly, the presence and quantity of fecal pathogens in the water" (NRC, 2004). Therefore, use of bacterial indicators is predicated on the presumption that there are no significant environmental sources of these microorganisms (i.e., nonenteric sources). However, this presumption is not entirely valid; fecal indicator organisms have been demonstrated to have natural reservoirs in the aquatic environment where they can survive for extended periods and even proliferate (e.g., fecal indicator bacteria in U.S. coastal [Yamahara et al., 2007] and Great Lake waters and sand [Whitman et al., 2006]).

Historically, EPA has recommended that recreational water quality criteria encompass all fecal sources of pathogens that contain the relevant indicator microbes. This recommendation is based on the regulatory premise that animal-derived (zoonotic) human pathogens in fecally contaminated waters are as hazardous as their human-derived counterparts (Schaub, 2004). This presumption is supported by current research that confirms that there are many waterborne zoonotic bacteria and protozoa common to both humans and animals, especially mammals (WHO, 2004). Research also suggests, however, that there may be some attenuation of infectivity, virulence, and disease severity to humans from animal-derived human pathogens (see Section III.2). In addition, there are many pathogens that could be zoonotic, fecal, and/or waterborne, but these routes of transmission have not yet been conclusively demonstrated.

Given the scientific advancements in pathogen characterization since EPA's 1986 AWQC were released, it is appropriate to examine the broadest array of currently known and suspected waterborne, fecal, and zoonotic pathogens and their human health impacts. These include

primarily bacteria, viruses, protozoa, and helminths. There are many zoonotic pathogens and many waterborne pathogens; however, there is a more limited subset of pathogens that are both. For this report, the following attributes were used to select the waterborne, zoonotic pathogens of concern (partially adapted from Bolin et al., 2004a):

1. *The pathogen must spend part of its lifecycle within one or more warm-blooded animal species.*
2. *Within the lifecycle of the pathogen, it is probable or conceivable that some life stage will enter water.*
3. *Transmission of the pathogen from animal source to human must be through a water related route.* There are zoonotic pathogens for which waterborne exposure has not been detected as a significant route of cross-species transmission. This does not exclude the possibility that these zoonotic pathogens could be transmitted via water.
4. *The pathogen must cause infection or illness in humans.* There are animal pathogens that have waterborne transmission between animals yet are not known to cause illness in humans.

There are many waterborne zoonotic pathogens that exhibit all four attributes listed above. See Appendix A, Table A-1, for a summary of waterborne pathogens that meet the above criteria and selected pathogens that meet some, but not all, of the above criteria.

The remainder of this paper is organized into two main sections. Section II characterizes six key groups of zoonotic pathogens for which there is evidence of human health risks from recreational exposure via ambient waters. Section III presents an overview of how the key pathogens interact with their environment, which includes both the water environment and the host animals.

II. KEY WATERBORNE ZOONOTIC PATHOGENS OF CONCERN

Based on a review of the literature and other information sources (Appendix B), 6 of the 20 pathogens identified as waterborne and zoonotic from warm-blooded animals stood out as having the most evidence for human health impacts due to recreational exposures in the United States. The six pathogens discussed in this paper in more detail are pathogenic *E. coli*, *Campylobacter*, *Salmonella*, *Leptospira*, *Cryptosporidium*, and *Giardia*. This list correlates well with the top five waterborne pathogens for recreational and drinking waters. The top five waterborne pathogens for recreational water are *E. coli*, *Campylobacter*, *Leptospira*, *Cryptosporidium*, and *Giardia*, while the top five for drinking water are *E. coli*, *Campylobacter*, *Salmonella*, *Cryptosporidium*, and *Giardia* (Craun et al., 2004a). The waterborne zoonosis potential of viruses also is discussed because viruses may be emerging waterborne zoonoses.

For each of the six key pathogens, information on strain variation, known zoonoses, route(s) of exposure, illness symptoms, and disease incidence are discussed in the sections that follow. Because incidental ingestion is the primary route of recreational exposure to pathogens, summary information on incidental ingestion is provided in Appendix C.

II.1 Bacteria

II.1.1 *Escherichia coli*

E. coli is an important waterborne zoonosis because human pathogenic strains occur in livestock and wildlife feces and can survive in ambient water. In addition, the potential illnesses caused by pathogenic *E. coli* can be severe or fatal, especially in immuncompromised persons and subpopulations. Pathogenic *E. coli* is also a well-documented foodborne pathogen (USDA, 2001). Although pathogenic *E. coli* have been found in treated wastewater effluents in the United States (Boczek et al., 2007), waterborne outbreaks are not as prevalent as foodborne cases.

E. coli Strain Variation

Benign strains of *E. coli* are a part of the normal microbial flora present in the colons and feces of all warm-blooded animals. However, multiple disease causing serotypes of *E. coli* have been identified in the past few decades. The most well-known serotype is *E. coli* O157:H7, which frequently occurs in wastewater and feces in developed countries and can result in severe illnesses and death. *E. coli* O157:H7 (or just O157:H7) is the "poster child" for pathogenic *E. coli* and is the serotype most extensively investigated (Chart et al., 2000).

E. coli is a versatile bacterium and multiple subtypes and strains that are pathogenic have been documented. Although the nomenclature for these strains has not yet stabilized in the literature, Mølbak and Scheutz (2004) provided the following helpful summary of the current nomenclature:

- Diarrheagenic *E. coli* (DEC) – includes all the strains on this list;

- Verocytotoxin (Shiga toxin)-producing *E. coli* (VTEC or STEC) – *E. coli* that produce verocytotoxin (Shiga toxin) VT1 and/or VT2;
    - Enterohemorrhagic *E. coli* (EHEC) – originally defined as serotypes that cause a clinical illness similar to *E. coli* O157:H7, now used as a term for VTEC that cause hemorrhagic colitis (HC) in humans;
- Enterotoxigenic *E. coli* (ETEC) – *E. coli* that produce enterotoxins that are heat stable (STh, STp) and/or heat labile (LT);
- Attaching and effacing *E. coli* (A/EEC) – *E. coli* that attach to and efface the microvilli of enterocytes, but do not produce high levels of verocytotoxin;
    - Enteropathogenic *E. coli* (EPEC) – Subtype of A/EEC, usually of particular serotypes that mostly contain an EPEC adherence factor plasmid and often produce bundle-forming pili;
- Enteroaggregative *E. coli* (EAggEC) – *E. coli* that exhibit a pattern of aggregative adherence to tissue culture; and
- Diffuse adherent *E. coli* (DAEC) – *E. coli* that exhibit a pattern of diffuse adherence to tissue culture.

Nataro and Kaper (1998) provide a comprehensive, albeit dated, review of the DEC.

E. coli Zoonotic Potential

E. coli is part of the normal intestinal flora of humans and warm-blooded animals and can readily spread to humans through contaminated food and water (WHO, 2004). Pathogenic *E. coli* have been documented in a wide variety of animal species including cattle (Chapman et al., 1997; Rangel et al., 2005), chickens (Schoeni and Doyle, 1994), sheep (Chapman et al., 1997; Kudva et al., 1996), pigs (Booher et al., 2002; Chapman et al., 1997; Feder et al., 2003), deer (Keene et al., 1997; Rice et al., 1995; Sargeant et al., 1999), horses (Chalmers et al., 1997), and dogs (Hammermueller et al., 1995).

Ruminants, and cattle in particular, are considered one of the most important animal sources of *E. coli* O157:H7 and other VTECs. All of the VTECs including EHEC are capable of causing severe disease in humans, are typically shed by healthy cattle and other species, and have documented cases of transmission via humans and water (Mølbak and Scheutz, 2004). Chapman et al. (1997) determined that the monthly prevalence of *E. coli* O157:H7 in cattle ranged from 4.8 to 36.8 percent, which was higher than the prevalence range in poultry, sheep, and pigs. Michel et al. (1999) examined the relationship between 3,001 cases of VTEC and the livestock density in rural areas of Ontario, Canada. Their research indicated that cattle density had a positive and significant association with the number of reported cases of VTEC in humans, suggesting that living near cattle farms may increase a person's risk of contracting VTEC.

Current data suggest that the prevalence of *E. coli* O157:H7 in poultry is low (Chapman et al., 1997), although contact with chickens has resulted in outbreaks. Schoeni and Doyle (1994) inoculated 1-day-old chicks with strains of serotype O157:H7. The chicks shed serotype O157:H7 up to 11 months after inoculation, and O157:H7 was subsequently recovered from their egg shells, but not from the yolks or whites. In an outbreak in northern Italy, the source of exposure was believed to be chickens in 15 cases of hemolytic uremic syndrome (HUS; see more

below) that were caused by serotype O157 and other EHEC serotypes (Tozzi et al., 1994). In this outbreak, a case-controlled study showed an association between contact with chickens and HUS although VTEC was not isolated from any of the chickens.

In a study by Chapman et al. (1997), O157:H7 was isolated from 2.2 percent of sheep and 4 percent of pigs, respectively. In a Russian study, O157:H7 was found in sheep, and the incidence was highly variable, ranging from 31 percent in June to 0 percent in November (Kudva et al., 1996). Kudva and colleagues also showed that 80 percent of the O157:H7 isolates had at least two of the Shiga-like toxin types I or II or the attaching-effacing lesion genes. Strains that produce Shiga-like toxins have been documented in humans and in animals (Kudva et al., 1996; Mølbak and Scheutz, 2004).

E. coli Route of Exposure

Waterborne transmission of *E. coli* O157:H7 has been reported from both recreational water (Ackman, 1997; CDC, 2002; McCarthy et al., 2001; Samadpour et al., 2002; Yoder et al., 2004) and contaminated drinking water (CDC, 2002; Hrudey et al., 2002, 2003; Olsen et al., 2002; Pond, 2004; Swerdlow et al., 1992; Yarze and Chase, 2000). Most studies examining contamination from recreation immersion have suggested that ingestion was the primary route of exposure (Keene et al., 1994). Because *E. coli* O157:H7 has a relatively low infectious dose, swallowing a small amount of contaminated water may cause illness (Haas et al., 2000; Keene et al., 1994).

Immersion in and ingestion of recreational waters has been the route of exposure for strains of *E. coli* other than EHEC (Yoder et al., 2004). In Connecticut, 11 persons were infected with *E. coli* O121 in an outbreak associated with swimming in a lake (CDC, 2000).

E. coli Illness Symptoms

E. coli can cause a relatively wide range of illness symptoms, depending on the strain and the underlying health of the host (Hunter, 2003; Mølbak and Scheutz, 2004). Incubation periods vary and can be as short as 8 hours for EAEC infections (Nataro et al., 1995), 14 to 50 hours for ETEC (Dupont et al., 1971), and 3 to 4 days for EHEC, with shorter (1 to 2 days) and longer (5 to 8 days) incubations noted in some outbreaks (Nataro and Kaper, 1998). Approximately 82 to 95 percent of all *E. coli* cases result in relatively minor illness symptoms including abdominal cramps, vomiting, diarrhea (often bloody), and sometimes fever (Ostroff et al., 1989; Swerdlow et al., 1992). The duration of these symptoms is 4 to 10 days (CDC, 1993a) although, in the Cincinnati outbreak, infants remained hospitalized for 21 to 120 days (Nataro and Kaper, 1998). Symptoms may be more severe for persons with hemorrhagic colitis (HC) or bloody inflammation of the colon (Griffin and Tauxe, 1991).

Symptoms commonly associated with human illness from the various DEC include the following:

- VTEC (or STEC) – Diarrhea, hemorrhagic colitis, HUS;
    - EHEC – hemorrhagic colitis (HC);

- ETEC – Acute watery diarrhea;
- A/EEC – Acute or persistent diarrhea;
    - EPEC – Acute or persistent diarrhea;
- EAggEC – Acute watery, often protracted diarrhea; and
- DAEC – Acute or persistent diarrhea.

In rare cases, HC can develop into HUS, which is a severe life-threatening disease that can result in kidney failure and neurological complications such as seizures and strokes (Brotman, 1995). Brooks et al. (2005) conducted a study that suggests *E. coli* strains that produce Shiga toxin 2 are much more likely to result in HUS than strains that only produce Shiga toxin 1. Approximately 2 to 7 percent of all *E. coli* O157:H7 infections result in HUS, and HUS is most common among children under 5 years old and the elderly (Griffin and Tauxe, 1991; WHO, 2004). Less than 10 percent of HUS cases turn into a chronic illness such as chronic kidney failure, blindness, or partial paralysis (Tarr, 1995). Approximately 33 percent of persons that contract HUS have abnormal kidney function for several years, sometimes requiring long-term dialysis (WHO, 2004). In very rare cases, HUS can also lead to death. Cases of infection with *E. coli* O157:H7 from swimming-associated outbreaks, compared to other routes of exposure, have the highest rate of HUS. This difference may be due to the higher proportion of young children participating in swimming and becoming infected during such outbreaks and the higher likelihood of young children developing HUS (Rangel et al., 2005). HUS, non-bloody diarrhea, and HC may occur with O157:H7 infections; other complications may include cholecystitis, colonic perforation, intussception, pancreatitis, posthemolytic biliary lithiasis, post-infection colonic stricture, rectal prolapse, appendicitis, hepatitis, hemorrhagic cystitis, pulmonary edema, myocardial dysfunction, and neurological abnormalities (Nataro and Kaper, 1998).

As noted above, infection with *E. coli* O157:H7 can also result in death. The U.S. Centers for Disease Control and Prevention (CDC) estimates a death rate of 0.08 percent, although case studies reveal slightly higher rates. For example, the 1993 fast-food *E. coli* O157:H7 outbreak in Washington, California, Idaho, and Nevada resulted in 4 deaths out of approximately 700 who fell ill, which corresponds to approximately 0.57 percent mortality (Brotman, 1995). Griffin and Tauxe (1991) reviewed 12 outbreaks in the United States between 1982 and 1990 and calculated a mortality rate of 1.9 percent among those with diagnosed *E. coli* O157:H7 infections.

E. coli Illness Incidence

The CDC estimates that there are 73,000 cases of *E. coli* 0157:H7 infections and 61 deaths annually in the United States. Non-O157 Shiga-like toxin serotypes cause approximately 37,000 illnesses per year (Mead et al., 1999).[1] Craun et al. (2004a) estimated that *E. coli* was the etiological agent for 30 percent of the outbreaks from zoonotic contamination in untreated recreational waters from 1971 to 2000.

E. coli O157:H7 infection associated with recreational exposure was first reported in June 1991 in a lake in Oregon (Keene et al., 1994). From 1991 until 2002, 20 additional outbreaks as a result of exposure to contaminated recreational waters have been reported to the CDC (Rangel et

[1] The CDC is currently updating these numbers, but the newest values have not yet been released.

al., 2005). Fourteen of the outbreaks occurred as a result of exposure to contaminated lakes or ponds, while seven occurred from exposure to contaminated swimming pools (Rangel et al., 2005).

CDC's surveillance system probably captures a small proportion of *E. coli* O157:H7 outbreaks that occur because many illnesses are not reported to public health officials or the CDC, are not recognized as *E. coli* infections, or the outbreak is considered of unknown etiology (Cieslak et al., 1997).

From 1991 to 2004, 14 outbreaks of *E. coli* related illness have been associated with untreated recreational waters (Table II.1.1-1).

II.1.2 *Campylobacter*

Campylobacter Strain Variation

Of the 17 species in the genus *Campylobacter*, *C. jejuni*, and *C. coli* are the most important human pathogens. Eight strains of *C. jejuni* have been DNA sequenced. At least two strains of *C. jejuni* have been shown to cause illness in ferrets, mice, rabbits, and rats, and several of the strains have been associated with Guillain-Barré syndrome (GBS) (Nachamkin, 2002).

Table II.1.1-1. Outbreaks of *E. coli* Associated with Recreational Waters in the United States

Year	Number of Cases	Number of Outbreaks	Source of Outbreak(s)
1991	80	1	Lake
1992	0	0	NA
1993	0	0	NA
1994	166	1	Lake
1995	28	4	Lakes
1996	24	2	1 pool, 1 lake
1997	8	1	Lake
1998	31	2	1 pool, 1 lake
1999	61	5	1 pool, 3 lakes, 1 ditch water
2000	0	0	NA
2001	49	2	Lakes
2002	9	1	Pool
2003	0	0	NA
2004	0	0	NA

Source: Data from CDC Surveillance Summaries: Morbidity and Mortality Weekly Report (MMWR) Surveillance for Waterborne-Disease Outbreaks - United States: 1991-1992, 1993-1994, 1995-1996, 1997-1998, 1999-2000, 2001-2002, 2003-2004 (CDC, 1993b, 1996, 1998, 2000, 2002, 2004, 2006).

Campylobacter Zoonotic Potential

Because most of the published literature focuses on food contamination from human waste as the primary risk factor for *Campylobacter* infection, some professionals in the health community have concluded that the zoonotic waterborne route is unlikely to be important (Till and McBride, 2004). Some evidence indicates that the human sources of *Campylobacter* may obscure the zoonotic risk factors (McBride, 1993).

McBride et al. (2002) found that 60 percent of all samples collected from 25 freshwater recreational water sites in New Zealand over 15 months contained at least one species of *Campylobacter*. This finding led to an inference that 4 percent of all campylobacteriosis cases in New Zealand were due to water contact recreation (McBride et al., 2002). Donnison and Ross (2003) note that nearly all streams near dairy farms in New Zealand contain *Campylobacter*, and despite generally low concentrations, these streams may help cycle *Campylobacter* in farm animals and indirectly contribute to the high incidence of human infection in New Zealand.

The species and strain of *Campylobacter* contained in a stream is highly influenced by the path of the stream, with surface waters running through beef- and sheep-grazing pastures typically contaminated with *C. coli* and *C. jejuni* (Jones, 2001). Surface waters that have contact with avian species are typically contaminated with *C. jejuni*, *C. lari*, *C. coli*, and avian-sourced urease-positive thermophilic campylobacters (Jones, 2001). The main source of campylobacters for bathing waters is typically assumed to be sewage effluent, though there is evidence that birds may be primarily responsible for contamination (Jones, 2001).

Campylobacter Route of Exposure

The main route of exposure to *Campylobacter* from recreational waters is from incidental ingestion of water during full immersion activities such as swimming. The bacteria may be of anthropogenic or animal sources. From 1991 to 2002, 3 percent of waterborne outbreaks were attributed to *Campylobacter* (7 outbreaks, 360 cases) (Craun et al., 2006). Craun et al. (2005) summarized and discussed 259 waterborne outbreaks occurring in the United States from 1971 to 2000 and associated only with recreational water. *Campylobacter* was the etiologic agent for 0.9 percent of the outbreaks. Although waterborne sources of *Campylobacter* are important, most infections occurred as a result of handling contaminated foods (e.g., raw chicken) and direct person-to-person transmission is rare (Allos, 2001).

Campylobacter Illness Symptoms

Campylobacter infection can result in a number of symptoms, including loose and watery or bloody diarrhea, dysentery, fever, and severe abdominal cramps (Allos, 2001; Fricker, 2006a). Infections of *Campylobacter* are symptomatically indistinguishable from those of other bacterial pathogens such as *Salmonella* (Nachamkin, 2002). Diarrheal symptoms may be frequent at the peak of the illness, typically occurring 8 to 10 times per day. Although the disease usually lasts only one week, some infected individuals experience relapses leading to several weeks of symptoms (Allos, 2001).

Some complications may result from *Campylobacter* infection including cholecystitis, pancreatitis, peritonitis, and massive GI hemorrhage. Some immunocompromised individuals experience bacteremia as a result of campylobacteriosis, and in rare instances, some individuals have experienced meningitis, endocarditis, septic arthritis, osteomyelitis, or neonatal sepsis. Infection rarely results in death, with 1 death per 20,000 infections (Allos, 2001).

Several chronic sequelae have been associated with *Campylobacter* infection including the development of GBS (Fricker, 2006a). GBS is an acute demyelinating disease of the peripheral nervous system that affects 3,000 to 6,000 people in the United States annually, with approximately 1,000 to 2,000 cases preceded by *C. jejuni* infection (Allos, 1998). The risk of developing GBS after *C. jejuni* infection, however, is small (approximately 1 case of GBS per 1,000 infections), and many GBS-related *C. jejuni* infections are asymptomatic (Allos, 2001). Reactive arthritis and depression also have been reported as chronic sequelae following campylobacteriosis (Garg, 2006; Nachamkin, 2002). Recent evidence has associated *C. jejuni* with a rare form of mucosa-associated lymphoid tissue (MALT) lymphoma called immunoproliferative small intestinal disease (IPSID); campylobacteriosis has not yet been shown as causative agent of IPSID (Poly and Guerry, 2008).

In 2000, a municipal water supply in Walkerton Ontario became contaminated with *E. coli* and *C. jejuni*. In the outbreak that resulted from that contamination, persons who showed symptoms of acute bacterial gastroenteritis at the time of the outbreak were more likely to exhibit hypertension and reduced kidney function approximately 4 years after infection than those who were asymptomatic at the time of the outbreak (Garg et al., 2005).

Campylobacteriosis Incidence

Although *Campylobacter* became a reportable illness in the United States in the early 1980s, it is estimated that only 1 in 38 cases of detected infection actually are reported (Mead et al., 1999). Approximately 2.4 million *Campylobacter* infections are estimated to occur in the United States every year (Allos, 2001). Between 1996 and 1999, the incidence of campylobacteriosis in the United States declined by 26 percent, from 23.5 to 17.3 cases per 100,000 people (Allos, 2001). According to reports from 1999, Britain experiences approximately 5 times this infection rate (103.7 cases per 100,000 people), though reporting rates may not be comparable (Gillespie et al., 2002). *C. jejuni* infection is one of the most common causes of gastroenteritis across the world and is frequently responsible for diarrheal illness in travelers (Allos, 2001).

Other than an outbreak in 1999, in Florida, where 6 cases of *C. jejuni* infections resulted from a private swimming pool, no other recreational waterborne outbreaks have been reported to the CDC between 1991 and 2004 (CDC, 2002).

II.1.3 *Salmonella*

Salmonella Strain Variation and Zoonotic Potential

Salmonella are non-encapsulated, Gram-negative bacteria of the Enterobacteriaceae family that infect both animals and humans causing a wide range of illnesses (Cohen, 1986; Lightfoot, 2004;

Ohl, 2001). There are more than 2,500 serovars/serotypes in the genus *Salmonella* (Lightfoot, 2004).

The genus *Salmonella* consists of two species, *S. enterica and S. bongori. S. enterica* is divided into the following six subspecies: *S. e. enterica*; *S. e. salamae*; *S. e. arizonae*; *S. e. diarizonae*; *S. e. houtenae*; and *S. e. indica* (Lightfoot, 2004). The many serovars in the group are closely related to each other by somatic and flagellar antigens, and most strains show diphasic variation of flagellar antigens). Thus, *Salmonella* can be serotyped by means of somatic and flagellar antigens and further subtyped by antibiotic-sensitivity testing, biochemical reactions, phage-typing, and analysis of the plasmids they carry. All *Salmonella* serotypes share the ability to invade the host by inducing their own uptake into cells of the intestinal epithelium (Lightfoot, 2004). Although more than 2,500 *Salmonella* serotypes exist, only 10 account for more than 70 percent of the isolates reported annually in the United States (Cohen, 1986; Lightfoot, 2004). Because the vast majority of *Salmonella* isolates from humans are of the subspecies *S. e. enterica*, the CDC recommends that *Salmonella* strains only be referred to by their genus and serotype (e.g., *S. typhi*).

S. typhimurium and *S. enteritidis* are the most prevalent serotypes found in the United States (CDC, 2006). The other serotypes have much smaller incidences in the United States; *S. enteritidis* accounted for 10 percent in 1984, and *S. newport*, *S. infantis*, and *S. heidelberg* accounted for 4.5, 3.0, and 1.0 percent, respectively.

Some *Salmonella* serotypes are highly host-specific, restricted to a single host species and rarely causing disease in other species (Lightfoot, 2004). *S. typhi* and *S. paratyphi* are exclusively human pathogens, with no known animal reservoirs, while *S. enteriditis* and *S. typhimurium* infect a wide range of animal hosts, including poultry, cattle, and pigs. The serotypes with a wide range of animal hosts can also infect humans, usually via food consumption, and cause self-limited gastroenteritis in humans (WHO, 2004). *S. gallinarum* and *S. pullorum* are almost exclusively pathogens of poultry (Cohen, 1986). Human pathogens *S. heidelberg* and *S. litchfield* have primarily avian and reptilian reservoirs, respectively (Cohen, 1986). *S. abortusovis* is specific to sheep (Lightfoot, 2004). *Salmonella* has also been reported in swine, cattle, rodents, birds, turtles, dogs, and cats (Covert and Meckes, 2006). The CDC estimates that 74,000 cases of salmonellosis per year are associated with exposure to reptiles or amphibians (directly or indirectly) (Lightfoot, 2004).

In 1993, an outbreak of *S. typhimurium* where more than 650 people became ill and that resulted in 7 deaths, was traced to a water-storage tower that allowed access to birds (Covert and Meckes, 2006). Although this outbreak was from drinking water rather than recreational water, it illustrates the potential for waterborne exposure due to birds.

Salmonella Route of Exposure

Salmonella infections begin with the ingestion of organisms in contaminated food or water (Lightfoot, 2004; Ohl, 2001). Although ingestion or exposure to infected water from recreational swimming is less common, it has been reported worldwide (Cohen, 1986; Lightfoot, 2004). Researchers have observed a reduction in the infectious dose of *Salmonella* under conditions where the gastric pH is elevated suggesting that gastric acidity may create an initial barrier to infection (Lightfoot, 2004). *Salmonella* also exhibit an adaptive, acid-tolerance response in low pH conditions thereby allowing them to live in acidic host environments like the stomach (Ohl, 2001).

Elevated levels of *Salmonella* also have been observed in major water bodies that receive discharges of meat processing wastes, raw sewage, and effluents from ineffective sewage treatment plants (Geldreich, 1996). Farming operations with cattle and poultry result in large quantities of fecal products in relatively small areas due to the dense population of animals. Thus, if the animal waste is not discharged into a lagoon or landfill, the stormwater runoff over the animal feedlots will transport massive loads of fecal pollution to the receiving waters of the drainage basin (Lightfoot, 2004).

Salmonella Illness Symptoms

Illnesses caused by *Salmonella* range from asymptomatic colonization and mild gastroenteritis to the more serious enteric fever (typhoid), meningitis, and osteomyelitis (Cohen, 1986). Enteric fever and gastroenteritis are the key clinical syndromes associated with a *Salmonella* infection (Lightfoot, 2004).

Different *Salmonella* serovars cause different clinical symptoms. *S. typhi* causes enteric fever (typhoid) in humans, and *S. typhimurium* causes diarrhea in humans and other animal species but a typhoid-like syndrome in mice (Lightfoot, 2004). *S. abortusovis* is responsible for abortion in ewes. Most *Salmonella* serovars cause an acute and mild enteritis, but *S. blegdam*, *S. bredeney*, *S. choleraesuis*, *S. dublin*, *S. enteritidis*, *S. panama*, *S. typhimurium*, and *S. virchow* may also be invasive and cause pyemic infections localizing in the viscera, meninges, bones, joints, and serous cavities (Lightfoot, 2004; Covert and Meckes, 2006). *S. dublin* is also particularly associated with different extraintestinal infections in persons with acquired immunodeficiency syndrome (AIDS) (Lightfoot, 2004).

Infection with *S. typhi* or *S. paratyphi*, which are exclusively human pathogens, results in enteric fever (Ohl, 2001). Clinical symptoms of enteric fever include diarrhea, abdominal pain, fever, and sometimes a maculopapular rash. The pathological sign of enteric fever is mononuclear cell infiltration and hypertrophy of the reticuloendothelial system (Ohl, 2001).

Salmonellosis is caused by ingestion of nontyphoidal salmonellae (e.g., *S. enteriditis* and *S. typhimurium*) with an incubation period of 8 to 72 hours (Lightfoot, 2004). The estimated inoculum size of nontyphoidal *Salmonella* required to cause symptomatic disease in healthy adult volunteers is 10^5 to 10^{10} organisms (Lightfoot, 2004). The infectious dose varies depending on the age and health of the person, strain differences, and the vector. Most

salmonellosis cases are self-limiting, and the affected persons recover without treatment (CDC, 2006; Cohen, 1986). Some infections are more severe, however, particularly in the young, elderly, and people with weakened immune systems, and such infections may become invasive (APHA, 2004; Lightfoot, 2004).

The clinical symptoms of salmonellosis may include diarrhea, abdominal pain, nausea, chills, fever, and prostration with the duration of illness ranging approximately 2 to 7 days (APHA, 2004; WHO, 2004). Vomiting may occur as well, but it is rare and usually a sign of invasive disease (APHA, 2004). Organisms that leave the GI tract and invade the rest of the body can cause bacteremia and septicemia, thus spreading the salmonellae to many organs in the body and possibly leading to abscesses, septic arthritis, cholecystitis, endocarditis, meningitis, pericarditis, pneumonia, pyodrema, or pyelonephritis (APHA, 2004).

Diarrhea from *Salmonella* infection is usually self-limiting and does not require treatment unless severe. Overall, there is an estimated 22.1 percent hospitalization rate, and an estimated 0.8 percent fatality rate (USDA, 2005). Mortality from *S. typhi* and *S. paratyphi* is estimated to be between 10 to 15 percent without treatment (Ohl, 2001). In severe cases, fluid and electrolyte replacement may be needed. Antibiotics are not recommended to treat *Salmonella* infections, except where there is evidence of invasion and septicemia, because they do not alleviate the symptoms or reduce the duration of the illness (Kanarat, 2004). Antibiotics may even prolong excretion of *Salmonella* in the feces.

Salmonellosis Incidence

Foodborne *Salmonella* are the estimated cause of approximately 1.4 million foodborne-related illnesses, 15,600 foodborne illness-related hospitalizations, and 550 foodborne-related deaths each year in the United States (Mead et al., 1999). In 2005, 45,322 salmonellosis cases were reported to the CDC through the Public Health Laboratory Information System (CDC, 2007a). Community surveys during outbreaks suggest that the proportion of infections reported is between 1 in 10 and 1 in 100 (Cohen, 1986).

Between 1991 and 2002, 3 waterborne outbreaks (833 cases) were attributed to nontyphoid *Salmonella* (Craun et al., 2006). Craun et al. (2005) reported that *Salmonella* were the etiologic agent in 0.9 percent of 259 recreational waterborne outbreaks occurring from 1971 to 2000.

II.1.4 *Leptospira*

Leptospira Strain Variation and Zoonotic Potential

Leptospira is an aerobic, motile spirochaete, 6 to 20 µm long and 0.1 µm wide. *Leptospira* occurs worldwide and has become an important recreational zoonosis due to its prolonged survival in water (Pond, 2005). The genotypic classification of *Leptospira* into two species (*L. interrogans* and *L. biflexa*) has been replaced by a phenotypic classification system in which 13 genomospecies are currently defined (*L. interrogans*, *L. noguchii*, *L. santarosai*, *L. meyeri*, *L. wolbachiic*, *L. biflexac*, *L. fainei*, *L. borgpetersenii*, *L. kirschneri*, *L. weilii*, *L. inadai*, *L. parvac*, and *L. alexanderi*) (Levett, 2001). Of the 28 serovars, several occur in more than one

genomospecies. Leptospirosis is probably the most widespread zoonosis in the world (Levett, 2001; Meites et al., 2004). Zoonotic reservoirs include livestock (pigs and cattle), domestic pets (dogs), and wild or feral animals (rats, voles, and mice) (Kanarat, 2004; Levett, 2001).

Complete genome sequences of *L. interrogans* serovars Copenhageni and Lai reveal that despite overall genetic similarity there are significant structural differences in their genomes (Nascimento et al., 2004). Nascimento and colleagues analyzed the genomic sequences to gain insight into genes that influence motility, chemotaxis, pathogenicity, and colonization of the pathogen.

Leptospira Route of Exposure

The source of infection in humans is usually either direct or indirect contact with the urine of an infected animal (Levett, 2001; WHO, 2003). Workers in direct contact with animal reservoirs are at increased risk (e.g., cattle, pig, and dairy farmers, slaughterhouse workers, and veterinarians) (Meites et al., 2004). Recreational exposure from swimming or boating in freshwater lakes is also possible (Levett, 2004; Meites et al., 2004). Outbreaks are often associated with unusual rainfall events or flooding (Bolin et al., 2004b).

Leptospira Illness Symptoms

Leptospirosis was first described by Adolf Weil in 1886; thus, the more serious form of leptospirosis is still known as Weil's disease (WHO, 2003). Leptospirosis is biphasic with a week-long acute stage followed by approximately 2 weeks of convalescence (Levett, 2001). During the acute stage, antibodies are low and the pathogen is detected mainly in the blood and cerebrospinal fluid, whereas the convalescent stage corresponds with the appearance of antibodies and the presence of pathogens in the urine (Levett, 2001).

Anicteric leptospirosis can be mild or acute. The majority of infections are either subclinical or mild, and patients usually do not seek medical attention (Levett, 2001). Icteric leptospirosis is a much more severe disease than anicteric leptospirosis. Icteric leptospirosis accounts for most of the high mortality rate, which ranges between 5 and 15 percent. Between 5 and 10 percent of all patients with leptospirosis have the icteric form of the disease (Levett, 2001).

Symptoms associated with leptospirosis include the following: jaundice, anorexia, headache, conjunctival suffusion, chills, vomiting, myalgia, abdominal pain, nausea, cough, hemoptysis, hepatomegaly, lymphadenopathy, diarrhea, rash (usually lasting less than 24 hours), and fever, which can be biphasic and reoccur after 3 to 4 days of remission (Levett, 2001).

In some cases, acute infection in pregnancy has been reported to cause abortion and fetal death (Levett, 2001). Uveitis (ocular complications) is recognized as a chronic sequela of leptospirosis in humans and horses. Chronic visual disturbance lasting 20 years or more has been reported (Levett, 2001). In 1994, CDC removed leptospirosis from the notifiable diseases list; however, the Hawaii Department of Health still requires reporting (Katz, 2001; Levett, 2001).

Leptospirosis Incidence

Levett (2001) summarized information for 28 waterborne outbreaks of leptospirosis worldwide, 22 of which were associated with swimming, 1 with kayaking, and 1 with rafting. Within the United States, the highest incidence of leptospirosis is found in Hawaii (Katz, 2001). Between 1971 and 2000, 16 percent of recreational waterborne disease outbreaks were attributed to *Leptospira* (Craun et al., 2004a). In 1998, in Illinois, there was an outbreak (375 cases) of leptospirosis associated with a triathalon in a lake (CDC, 2000).

In a prospective, population-based study of patients presenting with acute febrile illness, the geographic distribution of human *Leptospira* isolates mirrored the distribution of *Leptospira* 16S ribosomal gene sequences in urban and rural water sources (Ganoza et al., 2006).

II.2 Protozoa

II.2.1 *Cryptosporidium*

Cryptosporidium is a small protozoan parasite that infects the microvillous region of epithelial cells in the digestive and respiratory tract of humans and other mammals, birds, reptiles, and fish. *Cryptosporidium* does not replicate outside of a host. Environmentally robust oocysts are shed by infected hosts into the environment and can survive in environmental conditions for long periods of time (up to months) until ingested by a new host. In the new host, the life cycle starts again, and multiplication occurs using the biological resources of the host (WHO, 2006). *Cryptosporidium* exists in the natural environment in the oocyst form and which are resistant to conventional drinking water treatment measures such as chlorination. *Cryptosporidium* is recognized as a widespread pathogen for the general population, including both immunocompromised and immunocompetent persons (WHO, 2006).

Cryptosporidium Life Cycle and Strain Variation

Cryptosporidium has a complex life cycle. Each oocyst, which has an environmentally resistant wall, holds four sporozoites. Oocysts enter the environment by passing with the feces of an infected host organism (Fayer and Ungar, 1986; Fayer et al., 1997). Oocysts are immediately infectious and may remain in the environment for very long periods without losing their infectivity. Oocysts are resistant to environmental conditions and natural decay and can travel passively through the environment until they are ingested by a new host organism. In the GI tract of the new host, 4 sporozoites exit each oocyst (excyst) and may form an infection in the epithelial cells of the small intestine of the host (USEPA, 2001a). The sporozoites transform through several life stages including an asexual and a sexual reproduction cycle. Oocysts are the result of the sexual reproduction cycle. Oocysts of the two species of *Cryptosporidium* responsible for most human infections—*C. hominis* and *C. parvum*—are spherical with a diameter of 4 to 6 µm. Thin-walled oocysts may excyst within the same host and start a new life cycle (autoinfection), whereas thick-walled oocysts generally are shed by the host. Autoinfection may lead to a heavily infected epithelium of the small intestine resulting in secretory diarrhea (WHO, 2006).

Cryptosporidium is part of phylum Apicomplexa, family Cryptosporidiidae, and has been classified as a member of the group of eimeriid coccidian—a diverse group of parasitic protozoa (WHO, 2006). There are currently 16 species of *Cryptosporidium* identified in the literature (Table II.2.1-1). However, this taxonomy is likely to change as molecular methods continue to characterize isolates and potential new species. Fayer (2004a) and Olson et al. (2003) updated the list of *Cryptosporidium* species that have been reported to infect humans to include *C. baileyi*, *C. canis*, *C. felis*, *C. hominis*, *C. meleagridis*, *C. muris*, and *C. parvum*. It is important to note that the human form of *C. parvum* (formerly referred to as H-type or genotype 1) was recently reclassified as a new species, *C. hominis* (Morgan-Ryan et al., 2002). The cattle form of *C. parvum* (formerly referred to as C-type or genotype 2) maintains the designation *C. parvum*. Thus, studies published prior to 2002 should be interpreted carefully keeping in mind that authors who refer to *C. parvum* may be referring to *C. parvum*, *C. hominis*, or both. Of the current 16 species of *Cryptosporidium*, *C. parvum* and *C. hominis* most commonly cause GI illness in humans.

Studies with volunteers have demonstrated that a low dose of *C. parvum* (e.g., 10 oocysts) is sufficient to cause infection in healthy adults although some strains may be more infectious than others (Chappell et al., 1999; DuPont et al., 1995; Okhuysen et al., 2002). The relationship

Table II.2.1-1. *Cryptosporidium* Species

***Cryptosporidium* Species**	**Initially Described Host Species**
C. andersoni	*Bos taurus* (domestic cattle)
C. baileyi	*Gallus gallus* (domestic chicken)
C. bovis	*Bos taurus* (domestic cattle)
C. canis	*Canis familiaris* (dogs)
C. felis	*Felis catis* (domestic cat)
C. galli	*Gallus gallus* (domestic chicken)
C. hominis	*Homo sapiens* (humans, formerly *C. parvum* genotype 1)
C. meleagridis	*Meleagris gallopavo* (turkey)
C. molnari	*Dicentrarchus* labrax (fish)
C. muris	*Mus musculus* (house mouse)
C. nasorum	*Naso lituratus* (fish)
C. parvum	*Mus musculus* (house mouse) (formerly genotype 2)
C. scopthalmi	*Scopthalmi maximus* (turbot)
C. serpentis	*Elaphe guttata* (corn snake) *E. subocularis* (rat snake) *Sanzinia madagascarensus* (Madagascar boa)
C. suis	*Sus scrofa* (pig)
C. varanii	*Varanus prasinus* (emerald monitor lizard)
C. wrairi	*Cavia porcellus* (guinea pig)

Source: Adapted from Cacciò, 2005; Fayer, 2003, 2004a; Fayer et al., 1997, 2000; Fayer and Xiao, 2007; Morgan-Ryan et al., 2002; Ryan et al., 2003; and Xiao and Ryan, 2004.

between the number of oocysts humans are exposed to and the probability of infection is discussed in detail in the subsequent section. Studies of immunosuppressed adult mice have demonstrated that a single viable oocyst can induce *C. parvum* infections (Okhuysen et al., 2002; Yang et al., 2000).

Genetic and molecular studies of *C. parvum* (including *C. hominis*) indicate that the species is genetically heterogeneous among isolated strains found in different host species (Xiao and Ryan, 2004). There also is evidence that *C. parvum* and *C. hominis* experience recombination and that polymorphisms exist in the *C. hominis* species (Widmer et al., 1998). Okhuysen et al. (1999) showed that different isolates of *C. parvum* (including *C. hominis*) have different levels of infectivity for humans, and therefore the heterogeneity of the species may influence the risk posed to public health. Furthermore, distinct transmission cycles are evident among different genotypes of *C. parvum*. Multiple genotypes have been shown to circulate among different host species, and mixed infections with genotypically distinct populations have been reported (Widmer et al., 1998).

Cryptosporidium Zoonotic Potential

Human cases of cryptosporidiosis typically have been associated with different kinds of animal contact, which has led to the widespread belief that the host-range of *Cryptosporidium* is very broad and many animals can serve as reservoirs for *Cryptosporidium*. *Cryptosporidium* infection has been reported in more than 155 mammalian species (Fayer, 2004a) as well as numerous reptiles, amphibians, birds, and fish (O'Donoghue, 1995).

Several lines of evidence indicate that livestock, primarily cattle and sheep, are a major source of *Cryptosporidium* contamination of drinking water sources. These include the detection of *C. parvum* (presumably from animals) in many source waters and elevated levels of *Cryptosporidium* in watersheds with extensive agricultural activity (Fayer, 2004a; WHO, 2006). Also, direct zoonotic transmission of *Cryptosporidium* infection from livestock to humans has been repeatedly demonstrated (WHO, 2006). During outbreaks of cryptosporidiosis, frequent detection of *Cryptosporidium* in human stool samples suggests that human sources can also add significantly to the occurrence of oocysts in source waters (Fayer, 2004a).

Based on data from the western United States, depending on climate and feedlot management systems, the average animal in a cattle feedlot excretes between 28,000 and 140,000 oocysts per day (Atwill et al., 2006). Furthermore, 91 percent of dairy farms studied by Sischo et al. (2000) had *Cryptosporidium* at their locations, with 15 percent of infant dairy calves shedding oocysts. Nine percent of farm-associated streams contained *C. parvum*. Therefore, cattle represent a significant reservoir and potential environmental source of *C. parvum*. Tate et al. (2000) demonstrated that oocysts can be carried by runoff during rain events.

Most outbreaks of cryptosporidiosis are caused by *C. hominis*, which is only transmitted by human hosts. Outbreaks represent only 10 percent of domestic cryptosporidiosis cases, however, and at least one study (Feltus et al., 2006) has shown that the zoonotic *C. parvum* may be responsible for the majority of sporadic (endemic) cases. Atwill et al. (1997) reported that feral

pigs may serve as an environmental reservoir for *C. parvum* and may represent a potential source of *Cryptosporidium* contamination of ambient waters.

Although it is clear that livestock may be a major contributor to drinking water source contamination, there are few data to support a quantitative estimate of the proportion of this contribution. *Cryptosporidium* levels in source water are known to vary seasonally (USEPA, 2005a, 2005b), and short-term levels in surface water can be strongly associated with storm events or other weather variables (Fayer, 2004a; Naumova et al., 2005; USEPA, 2005a; WHO, 2006). In watersheds with diverse land-use patterns, it is likely that different sources contribute different proportions to the total contamination load at different times during the year, with the relative contributions depending on a wide range of watershed characteristics.

Besides animal contamination, the other major source of *Cryptosporidium* in ambient water is human fecal wastes. Several mechanisms can be responsible for the contamination of source waters and include malfunctioning septic systems, routine or "upset" releases from municipal wastewater treatment facilities, combined sewer system overflows, or human recreational uses (e.g., swimming, camping, and hiking). Due to the large numbers of oocysts excreted by infected individuals (Okhuysen et al., 1999) and oocyst resistance to many conventional wastewater treatment processes, even small releases can be significant. The World Health Organization (WHO) found reports of raw sewage samples containing up to 14,000 oocysts/L (average was up to 5,300 oocysts/L) and treatment plant effluents containing between 17 and 250 oocysts/L (WHO, 2006). Clearly, in water bodies where treatment effluents comprise an appreciable proportion of total flow, their contribution to the total *Cryptosporidium* load may be substantial.

LeChevallier et al. (2003) reported the results of *Cryptosporidium* monitoring for approximately 600 samples from 6 watersheds located in the United States and Canada. They found that the two watersheds with the highest proportion of agricultural land use had the highest average *Cryptosporidium* levels[2] and that approximately 90 percent of the samples exhibited the bovine (cattle) genotype (*C. parvum*). These findings implicate livestock, at least in these watersheds, as the major contributor to overall *Cryptosporidium* levels. This finding is consistent with studies indicating that the prevalence of *Cryptosporidium* infection in young livestock (i.e., calves and lambs) is very high (Fayer, 2004a) and that the oocyst counts in feces of young infected animals ranges from 10^6 to 10^8 oocysts per gram (WHO, 2006).

McCuin and Clancy (2006) conducted a 15-month occurrence study of *Cryptosporidium* occurrence in 10 wastewater facilities across the United Sates. Indigenous oocysts were detected in 30 percent of raw influents, 46 percent of primary effluents, 58 percent of secondary effluents and 19 percent of tertiary effluents in the 289 analyzed samples. Zhou et al. (2003) analyzed 179 wastewater treatment plant effluent samples from Milwaukee, Wisconsin using polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) methods to characterize genotypes of detected *Cryptosporidium*. In contrast with the results observed for source waters by LeChevallier et al. (2003), *C. hominis* (13.4 percent of samples) was detected more frequently than *C. parvum* (2.8 percent). This finding suggests that the distribution of *Cryptosporidium* in

[2] These results were obtained using EPA Method 1623 (USEPA, 2001b), for which the authors reported an average recovery rate of 72±22 percent.

Milwaukee's population was not heavily influenced by agricultural sources, though the relative low levels of *C. parvum* in wastewater may also have been due to differences in infectivity, excretion, or both of this species relative to *C. hominis*. As is the case for livestock, the extent to which human wastes contribute to overall *Cryptosporidium* contamination is highly site-specific, seasonal, and variable.

Cryptosporidium Route of Exposure

The main route of exposure to illness-causing microorganisms in recreational waters is through accidental ingestion of contaminated water while engaging in full immersion activities such as swimming or bathing. Secondary contact or partial body contact recreation such as wading, canoeing, motor boating, and fishing, in which ingestion is unlikely due to lack of direct water contact with the ears, eyes, mouth, or nose, is not considered to result in significant exposure (USEPA, 2002). A recent pilot study of anglers in the Baltimore area by Roberts et al. (2007), however, suggested that between 1 and 8 of 10 urban anglers could become infected with *Cryptosporidium*. This small study (56 anglers; a total of 46 fish/hand wash samples) included quantitative risk modeling. No data were reported on microbial water quality at the sampling sites, nor was there any attempt to obtain either health effects data or clinical samples to evaluate infection rates in the study population.

The potential for person-to-person secondary transmission is high for *Cryptosporidium* infections. Based on an analysis of data from the 1993 Milwaukee outbreak, Eisenberg et al. (2005) suggested that 10 percent (95 percent confidence interval: 6 to 21 percent) of the cases of disease were due to person-to-person transmission. There is considerable evidence that direct person-to-person transmission, as well as indirect transmission through contact with contaminated objects, can be a significant route of infection, especially where human population densities are high or personal contact is frequent (USEPA, 2001a). Direct transmission is affected by behavioral factors (e.g., frequent travel) and ethnic and dietary differences (USEPA, 2001a). Using data from the Milwaukee outbreak, approximately 3 to 5 percent of infected individuals transmit the disease to others (Mackenzie et al., 1995; Osewe et al., 1996). Among children and their caretakers, however, the transmission rate is considerably higher (12 to 22 percent) (Osewe et al., 1996).

Cryptosporidium Illness Symptoms

Cryptosporidiosis is primarily characterized by GI symptoms such as profuse watery diarrhea; however, diarrheal symptoms are generally not distinguishable from those caused by other common enteric pathogens. Other symptoms reported by individuals afflicted with cryptosporidiosis include dehydration, fever, anorexia, weight loss, weakness, abdominal cramps, vomiting, lethargy, general malaise, and progressive loss of overall condition (Hunter et al., 2004). The incubation period (time from ingestion to appearance of symptoms) has been reported to range from 2 to 10 days (Arrowood, 1997).

Some infections may be asymptomatic. In other words, not all infections will result in illness and observable symptoms. Asymptomatic hosts may still shed oocysts, however. Asymptomatic carriage, as determined by stool surveys, generally occurs at very low rates (less than 1 percent)

in industrialized countries (Current and Garcia, 1991), though higher rates have been reported in day care centers. Routine bile endoscopy suggests a higher asymptomatic prevalence; for example, 13 percent of nondiarrheic patients were shown to carry *Cryptosporidium* oocysts (Roberts et al., 1989). High rates of asymptomatic infection (between 10 and 30 percent) are common in nonindustrialized countries (Current and Garcia, 1991).

In more severe illnesses, the parasite may be found in the stomach, colon, liver, or lungs with associated symptoms corresponding to infections in those tissues. However, the presence of the parasite in tissues other than the small intestine does not necessarily indicate infection of host cells in those organs (O'Donoghue, 1995).

The level of immunocompetence of the infected person directly relates to the symptoms experienced. Age, concurrent illness/medical treatment, genetic background, pregnancy, and nutritional status all contribute to immune status. Symptoms may be more severe in immunocompromised persons (Carey et al., 2004; Frisby et al., 1997). Such persons include those with AIDS, certain cancer patients undergoing chemotherapy, organ transplant recipients treated with drugs that suppress the immune system, and patients with autoimmune disorders (e.g., lupus). In AIDS patients, *Cryptosporidium* has been found in the lungs, ears, stomach, bile duct, and pancreas in addition to the small intestine (Farthing, 2000). Clifford et al. (1990) found that cryptosporidiosis affected 10 to 15 percent of the AIDS patients, resulting in death in 50 percent of those cases. Besides the immunocompromised, children and the elderly may also be at higher risk from *Cryptosporidium* than the general population; however, specific data are not currently available to document the degree to which these individuals are subject to elevated risk. However, previous exposure to *Cryptosporidium* has been shown to confer some amount of immunity (Chappell et al., 1999).

Symptoms of cryptosporidiosis typically last from several days to 2 weeks although, in a small percentage of cases, the symptoms may persist for months or longer. Individuals with either compromised or healthy immune systems may experience illness for long periods. Illness from *Cryptosporidium* is usually self-limiting, with a median duration of 6 days and a mean duration of 9 days (Dupont et al., 1995; Palmer et al., 1990), although longer durations (mean 19 to 22 days, maximum 100 to 120 days) were reported in a recent Australian survey by Robertson et al. (2002). Relapses were common, with 1 to 5 additional episodes in 40 to 70 percent of patients. Shedding of oocysts may continue after the cessation of the disease symptoms.

Both individuals with compromised and with healthy immune systems have been shown to exhibit chronic sequelae. Immunocompromised individuals generally experience chronic gastroenteritis, which may last as long as the immune impairment. Immunocompromised populations include patients undergoing chemotherapy for treatment of neoplasms, persons undergoing immune suppression treatment to prevent rejection of skin or organ transplants, malnourished individuals, persons with concurrent infectious diseases (e.g., measles), the elderly, and persons with AIDS. Chronic illness may manifest itself as a series of intermittent episodes or may be persistent. Individuals with CD4+ cell counts (a key measure of the health of the immune system) less than 100 cells per mm^3 of blood are at increased risk of illness from *Cryptosporidium*, while individuals with less than 50 cells per mm^3 are at the greatest risk for

severe disease and prolonged carriage of *Cryptosporidium* (Hunter and Nichols, 2002; Roefer et al., 1996).

As noted above, chronic sequelae in immunocompetent patients also have been documented. After resolution of the acute phase in the 2 months following their initial diagnosis, 40.9 percent of patients in one case study reported recurrence of intestinal symptoms (includes both *C. hominis* and *C. parvum*) (Hunter et al., 2004). In addition, in individuals infected with *C. hominis*, other sequelae such as joint pain, eye pain, recurrent headache, dizzy spells, and fatigue were significantly more common than in control subjects. Both *C. parvum* and *C. hominis* infections have sometimes resulted in recurrence of GI symptoms, but only *C. hominis* infections have been related to the other sequelae noted previously.

Cryptosporidiosis Incidence

Limited information is available on the endemic incidence of cryptosporidiosis in the United States. Mead et al. (1999) estimated that there are approximately 15 million physician visits annually for diarrhea and that approximately 2 percent of these, or 300,000 cases, are due to cryptosporidiosis. Mead and colleagues also estimated that of these 300,000 cases, only about 10 percent are attributable to foodborne transmission, with the remainder due to the consumption of contaminated water (from drinking or recreational exposure) or person-to-person contact. Mead et al. (1999) estimated that there are approximately 211 million episodes of gastroenteritis (GI illness) in the United States each year, of which only about 38 million are attributable to known pathogens.[3]

Prior to 1982, when the CDC implemented routine reporting of *Cryptosporidium* among AIDS patients, only 13 cases of cryptosporidiosis had been documented (Ungar, 1990). Subsequently, between 1982 and 1997, more than 1,000 cases of the disease were reported worldwide (Fayer et al., 1997). Cases reported by CDC between 1999 and 2005 (for all routes of exposure) are shown in Figure II.2.2-1; however, documented cases underestimate actual *Cryptosporidium* infection rates because most cases go unreported.

Worldwide, infection is widespread, exceeding several million according to Casemore et al. (1997). As noted previously, the largest known outbreak of the disease occurred in 1993 in Milwaukee, Wisconsin (MacKenzie et al., 1994) and infected 403,000 individuals (CDC, 1996). The most recent data from CDC (2007b) for the year 2005 reported 8,269 cases of *Cryptosporidium* infection nationally, and reported significant fluctuations in incidence between summer and other seasons, peaking in late July and early August. According to CDC (2007b), *Cryptosporidium* is the leading cause of diarrheal illness outbreaks in recreational (chlorinated and nonchlorinated) water. Although 8,269 cases were reported in the United States in 2005, CDC (2007b) estimates that the total incidence of domestic cases of *Cryptosporidium* infection exceeds 300,000 each year.

[3] Mead et al. (1999) based the estimates on reported cases and estimates for degree of under reporting. For example, in the 1993 cryptosporidiosis outbreak in Milwaukee, Wisconsin, medical care was sought in only 12 percent of cases (Corso et al., 2003).

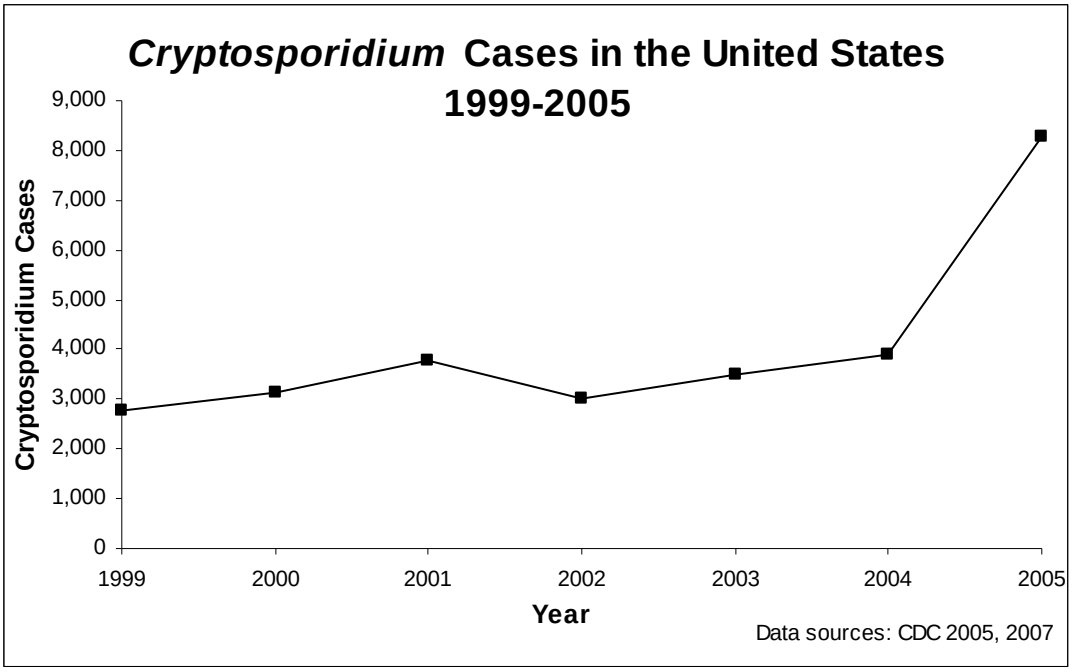

Figure II.2.1-1. Reported *Cryptosporidium* Infections in the United States, 1999 to 2005

The large increase in the number of cases reported from 2003 to 2005 might have resulted from outbreak-related case reporting (CDC, 2007b); however, that factor is unlikely to account for all of the increase. It is not clear how much, if any, of the increase may be due to changes in reporting patterns and diagnostic testing practices or to a real change in infection and disease (CDC, 2007b).

From 1991 to 2004, six outbreaks of cryptosporidiosis have been associated with untreated recreational waters (Table II.2.1-2).

II.2.2 *Giardia*

Giardia Life Cycle and Strain Variation

Organisms in the genus *Giardia* are binucleate, flagellated protozoan parasites that exist in trophozoite and cyst forms and are an important cause of waterborne illness worldwide. Both humans and some animal species can carry and transmit *Giardia lamblia* (also known as *G. intestinalis* or *G. duodenalis*), which causes the GI illness giardiasis. *G. lamblia* is highly infectious and has been shown to cause giardiasis with exposure to as few as 10 cysts (Rendtorff, 1954).

Over the course of the *Giardia* life cycle, the parasite lives both as a trophozoite and as a cyst form. Inside a vertebrate host, the *Giardia* trophozoites divide by binary fission, attach to the brush border of the small intestinal epithelium, detach, then become rounded and form a cyst wall. The environmentally resistant cyst is excreted with the feces, where it moves passively

Table II.2.1-2. Outbreaks of Cryptosporidiosis Associated with Recreational Waters in the United States

Year	Number of Cases	Number of Outbreaks	Source of Outbreak(s)
1991	0	0	NA
1992	526	2	Waterslide and wave pool
1993	174	4	Pools
1994	519	2	1 pool, 1 lake
1995	5,487	3	1 pool, 2 waterparks
1996	3,025	3	2 pools, 1 lake
1997	369	1	Fountain
1998	169	8	7 pools, 1 lake
1999	64	4	3 pools, 1 fountain
2000	1,368	13	12 pools, 1 lake
2001	538	4	3 pools, 1 hot spring
2002	936	7	6 pools, 1 lake
2003	702	5	4 pools,* 1 lake
2004	567	7	Pools

* In one pool outbreak, both *Cryptosporidium* and *Giardia* were detected in water samples, so that outbreak of 63 cases is counted in both the *Cryptosporidium* and the *Giardia* data.

Source: Data from CDC Surveillance Summaries: Morbidity and Mortality Weekly Report (MMWR) Surveillance for Waterborne-Disease Outbreaks - United States: 1991-1992, 1993-1994, 1995-1996, 1997-1998, 1999-2000, 2001-2002, 2003-2004 (CDC, 1993b, 1996, 1998, 2000, 2002, 2004, 2006).

through the environment and may be ingested by another host organism. Inside the digestive track of a new host, active cysts release trophozoites, and repeat their lifecycle (USEPA, 1998). Cysts can be excreted in the stool intermittently for weeks or months, resulting in a protracted period of communicability (CDC, 2007b). Furthermore, cysts can remain viable under typical environmental conditions for periods up to 77 days (Bingham et al., 1979, as cited in USEPA, 1998).

Currently, there are five recognized species of *Giardia* and six generally recognized assemblages of *G. lamblia*. Each assemblage has a varying degree of host specificity (see Table II.2.2-1). Some assemblages (i.e., Assemblages A and B) act as zoonotic assemblages that can infect most species of mammal, while others (i.e., Assemblages C through F) are more adapted to a particular host species (Appelbee et al., 2005). For example, Assemblage A has been found in human, beaver, cat, lemur, sheep, calf, dog, fox, chinchilla, alpaca, horse, pig, and cow. The role of these animals as a source of human infection, however, remains unclear. Assemblages C and D seem to primarily infect dogs, while Assemblage E infects livestock, and Assemblage F infects only cats (Appelbee et al., 2005).

Table II.2.2-1. *Giardia* Taxonomy

Species	Assemblage	Host(s)
Giardia lamblia (syn. *G. duodenalis*, *G. intestinalis*)	A1	Human, beaver, cat, lemur, sheep, calf, dog, fox, chinchilla, alpaca, horse, pig, cow
G. lamblia	A2	Human, beaver
G. lamblia	B (*G. enterica**)	Human, beaver, guinea pig, dog, monkey, horse (B IV)
G. lamblia	C and D (*G. canis**)	Dog, coyote, mouse
G. lamblia	E (*G. bovis**)	Cow, sheep, alpaca, goat, pig
G. lamblia	F (*G. cati**)	Cat
G. lamblia	G (*G. simoni**)	Domestic rat
G. lamblia	Novel I	Marsupial (Quenda – bandicoot, mouse, sheep)
G. lamblia	Novel II	Marsupial II (Tasmanian devil)
G. muris		Rodents (mice)
G. microti		Vole and muskrat
G. psittaci		Birds (budgerigars)
G. ardeae		Birds (heron and ibis)
G. agilis		Amphibians (frogs)

* Denotes recently proposed new species names (Hunter and Thompson, 2005; Thompson and Monis, 2004).

Adapted from: Adam, 2001; Appelbee et al., 2005; Hamnes et al., 2007; Olson et al., 2004; and Traub et al., 2005.

Giardia Zoonotic Potential

Cross-species transmission of *Giardia* is known to occur, and there are many known species and variants (or assemblages) of the *Giardia* parasite. Of all of the animal host species suspected of being a significant zoonotic source of human giardiasis by waterborne transmission, the evidence presently available suggests that the beaver (Dykes et al., 1980) and muskrat (USEPA, 1998) are the most likely candidates. The role of these animals as a source of human infection, however, remains controversial. Both of these aquatic mammals can be infected with isolates of *Giardia* from humans, but each has also been shown to harbor strains of *Giardia* that are phenotypically distinct from those found in humans. Thus, it is possible that the beaver harbors two types of *Giardia*. One type may be highly adapted to this animal and rarely, if ever, transmitted to humans. The other type may be one acquired by the beaver from human sources, which can multiply in the beaver and in turn be transmitted via water back to humans (USEPA, 1998).

The role that livestock play as zoonotic reservoirs of *Giardia* infection also remains controversial. *G. lamblia* may be maintained independently through transmission cycles involving wildlife and livestock, though it is unclear how these cycles may interact in zoonotic transfer (Hunter and Thompson, 2005). While both livestock and humans have been implicated

in contaminating water sources with *Giardia*, humans are responsible for the majority of the contaminations. Hunter and Thompson (2005) examined case studies for the zoonotic potential of *Giardia* and found only one case having a significant association with animal contact. They concluded that zoonotic transfer plays only a minor role in the infection cycles of *Giardia* and that animal contact is not a major risk factor. They authors did not, however, rule out the importance of zoonotic transfer indirectly through water sources. Both Traub et al. (2004) and Inpankaew (2007) have shown that, in some communities with inadequate sanitation, zoonotic transfer is evident between humans and dogs; however, it is unclear which species is the primary reservoir.

Thompson (2007) suggested that data gaps regarding zoonotic transfer for *Giardia* can be filled using molecular epidemiological studies. Molecular genotyping of parasite isolates from susceptible hosts in localized foci of transmission or longitudinal surveillance with genotyping might help address such data gaps on zoonotic transfer of *Giardia*.

Giardia Routes of Exposure

Giardia is transmitted via fecal-oral exposure and causes both endemic and epidemic cases of giardiasis. It is frequently spread person-to-person, especially among children or among persons with poor access to or practice of sanitation. The main route of exposure to *Giardia* from recreational immersion in water is from incidental ingestion of water during full immersion activities such as swimming.

Although inhalation of aerosolized water that contains cysts is theoretically possible, cysts are not known to be infectious in lung tissue. Dermal absorption is not known to be a route of exposure to *Giardia* cysts in environmental waters.

Giardia Illness Symptoms

Giardia is responsible for a number of health effects including acute symptoms that occur during and after the infection. However, *Giardia* has also been implicated in a number of chronic sequelae. There are a wide variety of symptoms associated with giardiasis that range from asymptomatic infection and acute self-limiting diarrhea to persistent chronic diarrhea, which sometimes fails to respond to treatment.

Giardia produces a broad spectrum of GI symptoms including one or more of the following symptoms: diarrhea, bloating, weight loss, malabsorption, steatorrhea (fatty stool), pale greasy and malodorous stools, flatulence, abdominal cramps, nausea and vomiting, fatigue, anorexia, and chills (CDC, 2000; Hellard et al., 2000; Hopkins and Juranek, 1991; Thompson, 2000). Fever may occur at the beginning of the infection (Ortega and Adam, 1997). Lactose intolerance is frequently present during infection and may persist even after *Giardia* has cleared from the stool (Wolfe, 1992). Chronic giardiasis appears to be infrequent, but when it occurs, may persist for years (USEPA, 1998). Case reports also indicate that giardiasis can be associated with the development of reactive arthritis (Tupchong et al., 1999).
Illness durations vary, lasting only 3 to 4 days for some individuals and several months for others. Most infections resolve spontaneously, and the acute stage lasts from 1 to 4 weeks

(USEPA, 1998), but individuals with compromised immune systems may have more serious and prolonged infection (APHA, 2004). Immunodeficiency with varying degrees of hypogammaglobulinemia or agammaglobulinemia is the most commonly reported form of immunodeficiency associated with chronic giardiasis (Farthing, 1996). However, giardiasis is one of the few potentially treatable causes of diarrhea in persons with AIDS, and chronic giardiasis does not appear to be a major clinical problem in persons with HIV infections or AIDS (Farthing, 1996; USEPA, 1999).

Asymptomatic infection is very common, with 50 to 75 percent of infected persons reporting no symptoms (Mintz, 1993)—especially in children and in persons with prior infections (CDC, 2007b). In a study at the Swiss Tropical Institute, only 27 percent of 158 patients who had *Giardia* cysts in their feces exhibited symptoms (Degremont et al., 1981). Although persons with asymptomatic *Giardia* infection are not likely to seek medical treatment and be diagnosed, they can serve as carriers of infection.

Hospitalizations and deaths due to giardiasis are relatively rare. The CDC estimates that giardiasis causes approximately 10 deaths and 5,000 hospitalizations annually in the United States (Mead et al., 1999). Blood volume depletion or dehydration is the most frequently listed codiagnosis on hospital admission. Among children under 5 years of age who had severe giardiasis, almost 19 percent also were diagnosed with failure to thrive (Lengerich et al., 1994). Additionally, in the United States and Scotland, more severe cases of giardiasis (i.e., hospitalized patients) seem to occur primarily in children under the age of 5 (Lengerich et al., 1994; Robertson, 1996). Age has been shown to significantly affect recovery time; in Scotland, the median length of stay in the hospital for giardiasis was significantly longer for persons older than 70 years than for other age groups (11 days compared to 3 days) (Robertson, 1996). Infants and young children may have increased susceptibility to giardiasis due to immunological factors that increase sensitivity and behavioral factors that increase exposure.

Chronic giardiasis patients often experience recurrent, persistent, brief episodes of loose, foul smelling stools that may be yellowish and frothy in appearance and frequently accompanied by distension of the bowel, foul flatus, anorexia, nausea, and uneasiness in the epigastrium (Wolfe, 1979). In some cases, these symptoms may persist for years; however, in the majority of cases, the parasite and symptoms disappear spontaneously. Among 65 cases of giardiasis encountered in an urban private practice outpatient setting, the mean duration of symptoms was reported to be 1.9 years, and in 38 patients (58 percent) who exhibited chronic symptoms for 6 months or longer, the mean duration of symptoms was 3.3 years (USEPA, 1998).

Corsi et al. (1998) evaluated ocular manifestations in 141 Italian children with current and past giardiasis and 300 children without giardiasis. Retinal changes were diagnosed in 20 percent of the children with giardiasis (mean age was 4.7 years) and in none of the children without giardiasis. These findings suggest that asymptomatic, nonprogressive retinal lesions may occur in young children with giardiasis. The risk of retinal lesions did not seem to be related to the severity of infection, its duration, or use of metronidazole to treat the infection, and may reflect a genetic predisposition to retinal lesions (Corsi et al., 1998).

There is usually no extra-intestinal invasion when *Giardia* trophozoites infect the small intestine, but reactive arthritis may occur, and in severe giardiasis, duodenum and jejunal mucosal cells may be damaged (APHA, 2004).

Giardiasis Incidence

Giardia lamblia is the most common intestinal parasite identified by public health laboratories in the United States (Kappus et al., 1994; Rose et al., 1991). CDC estimates that there are approximately 2 million illnesses annually in the United States due to *Giardia* (Mead et al., 1999). As noted previously, while all age groups are affected by giardiasis, the highest incidence is in children (USEPA, 1998). High risk groups for giardiasis include infants and young children, travelers to developing countries, the immunocompromised, and persons who consume untreated water from lakes, streams, and shallow wells (USEPA, 1998).

Communities with unfiltered surface water systems experienced a waterborne outbreak rate that was 8 times greater than communities where surface water was both filtered and disinfected (USEPA, 1998). Data therefore indicate that filtering water to remove microorganisms substantially reduces risk of giardiasis.

As with all pathogens, underreporting limits estimates of the true incidence of giardiasis. The ratio of reported cases of giardiasis to actual cases is not known. Mead et al. (1999) used a 38-fold multiplier to estimate incidence for nonbloody diarrhea outcomes (based on *Salmonella* data) and a 20-fold multiplier for bloody diarrhea outcomes (based on *E. coli* data). Applying the 38-fold multiplier to the 20,075 giardiasis cases that were reported in 2005 (CDC, 2007b) results in an estimated incidence of approximately 762,800 total cases in 2005. However, broader estimates are also supported. For example, an estimated 1 to 5 percent of cases of salmonellosis are reported to CDC through passive surveillance (Chalker and Blaser, 1988). If the 1 to 5 percent reported cases is applied to the giardiasis data, then the giardiasis disease burden in the United States in 2006 could have been 401,500 to 2,007,500 cases (135.4 to 677.0 cases per 100,000 population).[4] The true burden of giardiasis in the United States is likely to fall between these two estimates (CDC, 2007b).

Using data from 1992 to 1997, the number of states reporting occurrence of giardiasis increased from 23 to 43, while the annual count of giardiasis cases rose from 12,793 to 27,778, nationally (CDC, 2000). Between 1996 and 2001, however, the number of reported cases of *Giardia* infection in the United States (50 states plus Washington, DC) decreased gradually from 27,778 to 19,659 (CDC, 2007b). The cause of this decrease is unknown. Giardiasis became a nationally notifiable disease in 2002, and the number of cases increased from 2001 to 2002, then stabilized, averaging approximately 20,200 cases per year (CDC, 2007b). See Figure II.2.2-1 and Table II.2.2-2 for reported cases of *Giardia* infection in the United States from 1992 to 2005.

[4] Based on U.S. Census data for 2006, the U.S. population was estimated to be 296,528,800.

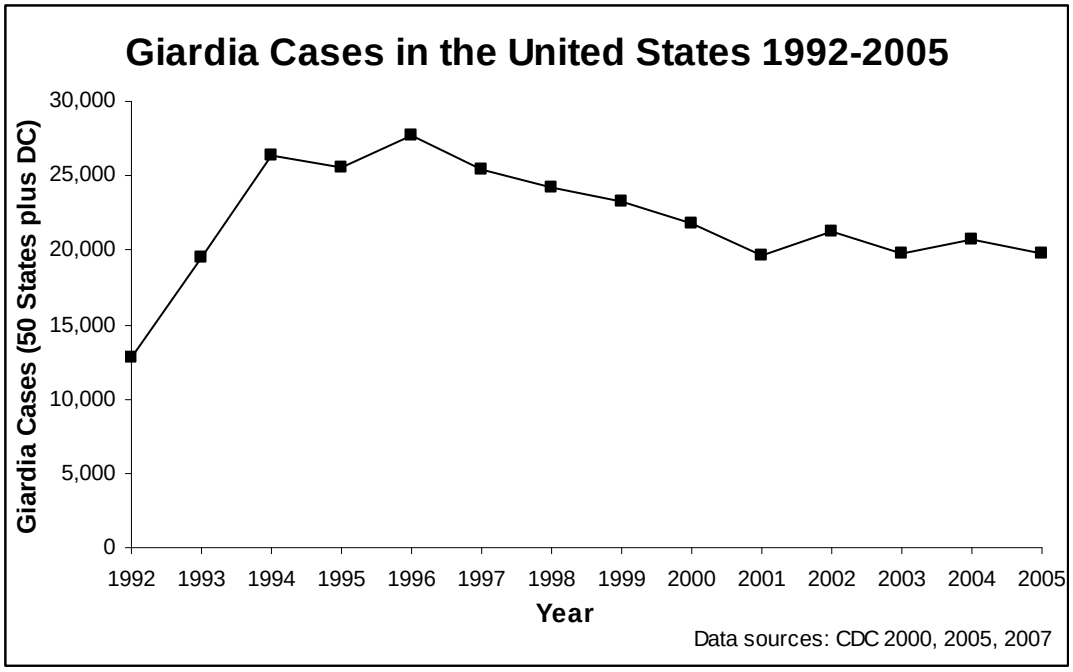

Figure II.2.2-1. Reported *Giardia* Cases in the 50 States plus Washington DC, 1992 to 2005

The increase in *Giardia* cases observed for 2002 might reflect increased reporting after the designation of giardiasis as a nationally notifiable disease starting in 2002. Outbreak-related cases made up 1.6 to 11.6 percent of the total number of cases reported annually for 1999 to 2002. Although the number of states reporting cases increased from 42 to 46 during that time, the number of states reporting more than 15 cases per 100,000 population decreased from 10 in 1998 to 5 in 2002. Transmission of giardiasis occurs throughout the United States, with increased diagnosis or reporting of cases per 100,000 population occurring in northern states than

Table II.2.2-2. *Giardia* Infection Occurrence in U.S. Populations

Population Description	Occurrence	Reference(s)
General	2-5% infection	USEPA, 1998
	Prevalence in stool samples (infection) 4-12% depending on year and state	USEPA, 1998
	30% of the population has seropositivity for *Giardia* (indicates current or past infection)	Frost and Craun, 1998
	In national survey, 7.2% (of 216,675) stool specimens were positive for *Giardia* in 1987 and 5.6 percent (of 178,786) were positive in 1991 (infection)	USEPA, 1999
Homosexual males (in New York)	18% found positive for *Giardia* (infection) (compared with 2% among other patients, but giardiasis is not a major clinical problem in persons with HIV or AIDS)	Faubert et al., 2000; Kean, 1979; USEPA, 1999
Small children	7% are asymptomatically infected with *Giardia*	Frost and Craun, 1998

in southern states (CDC, 2005). For 2002, among states reporting cases, the incidence of giardiasis ranged from less than 0.1 cases (Texas) to 23.5 cases (Vermont) per 100,000 population. Vermont reported the greatest number of cases per 100,000 population for each of the 5 years of the reporting period.

Giardiasis occurs most frequently in the early summer through early fall, with increases in transmission during the summer (CDC, 2007b). This increased transmission coincides with the summer recreation season, which includes increased use of recreational (including community) swimming facilities. Given that a single person can shed millions of cysts and yet remain asymptomatic, transmission in these facilities is likely to be an important mechanism for increased incidence during the summertime (CDC, 2005). People at recreational beaches have been shown to disturb sediment, leading to resuspension of cysts and an increase in exposure to *Giardia*, with higher densities of bathers leading to higher turbidity and correspondingly higher cyst concentrations (Graczyk et al., 2007; Sunderland et al., 2007).

Giardiasis is found most frequently in children 9 years old and younger and in adults aged 35 to 44 years. These groups correspond to young children and their caretakers, who are at increased risk of infection (CDC, 2007b). In 2005, 54.3 percent of reported cases occurred in males compared to only 43.9 percent in females, while 1.8 percent of reports did not record gender (CDC, 2007b). According to CDC (2007b), the discrepancy between cases in males versus females might be attributable in part to increased risk of infection during sexual contact between men although the discrepancy was found in nearly every age group.

A review of the data presented in Table II.2.2-2 clearly indicates that *Giardia* infection in the United States is common and widespread.

From 1991 to 2004, seven outbreaks of giardiasis have been associated with untreated recreational waters (Table II.2.2-3).

II.3 Viruses Zoonotic Potential

A great deal of public and animal health policy is based on the premise of the host specificity of viruses. That is, it has long been assumed that each virus has a distinct and limited range of host species that it can infect.[5] Viruses also are restricted to particular tissues of the host's body that they can infect (i.e., tropism), which affects both the mode of transmission and the disease that the virus infection may cause (Cliver and Moe, 2004). Most waterborne viruses are thought to be transmitted by a fecal-oral route (i.e., the virus is shed via the intestines and infects upon ingestion) that requires a tropism that includes the lining of the GI tract. Viruses that infect via the intestine and cause GI illness (e.g., enteroviruses) may also have secondary tropisms in other

[5] In this case, a distinction is being made between the important and much studied hypothesis that viral evolution accelerates when viruses "jump species" in the case of emerging diseases and the regular maintenance of multi-host adaptation that is recognized in other pathogens. Although rapid viral evolution facilitated by cross-species transmission is a recognized public health concern for viruses that have airborne transmission (e.g. influenza and severe acute respiratory syndrome [SARS]-associated coronavirus), cross-species transmission has not been a traditional concern for waterborne, fecally transmitted viruses.

Table II.2.2-3. Outbreaks of Giardiasis Associated with Recreational Waters in the United States

Year	Number of Cases	Number of Outbreaks	Source of Outbreak(s)
1991	34	4	3 pools, 1 lake
1992	0	0	NA
1993	61	3	2 lakes, 1 river
1994	80	1	Pool
1995	0	0	NA
1996	77	1	Pool
1997	0	0	NA
1998	0	0	NA
1999	18	1	Pond
2000	0	0	NA
2001	0	0	NA
2002	2	1	River
2003	212	2	Pools*
2004	9	1	Lake

* In one pool outbreak both *Cryptosporidium* and *Giardia* were detected in water samples, so that outbreak of 63 cases is counted in both the *Cryptosporidium* and the *Giardia* data.

Source: Data from CDC Surveillance Summaries: Morbidity and Mortality Weekly Report (MMWR) Surveillance for Waterborne-Disease Outbreaks - United States: 1991-1992, 1993-1994, 1995-1996, 1997-1998, 1999-2000, 2001-2002, 2003-2004 (CDC, 1993b, 1996, 1998, 2000, 2002, 2004, 2006).

tissues (e.g., neurological tissues). While much virus infectivity research has been conducted *in vitro* using cultured animal cells that at least partially reflect the host specificity (but not the tropisms) of viruses, many important enteric waterborne viruses of humans (e.g., noroviruses) remain difficult to detect or quantify in cultured cells (NRC, 2004; Straub et al., 2007). For this reason, it remains unclear whether *in vitro* infectivity is relevant to the *in vivo* host ranges of viruses. Although no confirmed examples of waterborne viral zoonoses have been reported, several viruses (e.g., swine hepatitis E virus [HEV]) are potentially transmissible between species, and water may serve as a vehicle for their transmission under some circumstances. Thus, assessment of the prospect of waterborne viral zoonoses is ongoing (Cliver and Moe, 2004).

Cliver and Moe (2004) consider the criteria for determining whether a virus can function as a waterborne zoonosis to include the following:

1. *Animal reservoir:* Does the agent regularly infect at least one animal species, independent of exposure to humans?
2. *Transmission to humans:* Are humans who are in contact with the alleged animal reservoir more frequently infected with this virus than people who are not?

3. *Shedding:* Is the candidate virus shed by the reservoir animal species in ways that might lead to contamination of water?
4. *Stability:* Is the candidate virus stable enough in the water vehicle to permit transmission by this pathway?

Despite the potential for rapid evolution in viruses, ongoing monitoring and research activities are important for public health protection, even though viruses are not currently known to have the attributes outlined in the introduction of this paper. An overview of information related to zoonotic potential for rotavirus, HEV, and adenovirus follows.

Rotaviruses

Rotaviruses (groups A, B, and C) have been documented in humans and animals, and interspecies transmission including human infection by a bovine strain has been reported (Abbaszadegan, 2006). The primary route of exposure for rotavirus is the fecal-oral route although exposure through other routes also has been reported to a lesser extent. Rotavirus is stable in the environment, and its transmission can occur through ingestion of contaminated water or food. Rotavirus illness typically results in vomiting and watery diarrhea for 3 to 8 days. Fever and abdominal pain occur frequently as well. According to the CDC, this virus is the most common cause of severe diarrhea in children. Symptoms tend to be less severe for adults. Approximately 70,000 children in the United States are treated in the hospital for rotavirus each year (Glass, 2006). A community waterborne outbreak of rotavirus gastroenteritis occurred in Colorado in 1981 (Hopkins et al., 1984). The outbreak was attributed to sewage contamination of the water supply and a failure of chlorination treatment.

Hepatitis E

HEV may be zoonotic (Cliver and Moe, 2004; USEPA, 1999). In pigs and rats, this virus is very similar to human HEV. Experimental studies have indicated that human strains can infect pigs, and porcine strains can infect primates (Cliver and Moe, 2004). In developing countries, the seroprevalence of HEV infection can be as high as 60 percent. The most common route of exposure for HEV is ingestion of contaminated food or water. Transmission via person-to-person contact is less common. Typical symptoms of HEV illness may include jaundice, fatigue, abdominal pain, loss of appetite, nausea, and vomiting. Pregnant women who contract hepatitis E are at high risk of severe illness and death. For this sensitive subpopulation, mortality can be as high as 20 percent in developing countries, but mortality is rare in developed countries (Craun et al., 2004a). HEV is uncommon in the United States and the CDC does not track incidence rates. In an EPA study of sporadic human HEV, nearly 50 percent of the infected persons had traveled to endemic areas in other countries or received blood transfusions (USEPA, 1999).

Adenovirus

Adenovirus may be zoonotic (Mwenda et al., 2005). Mwenda and colleagues identified enteric adenovirus in captive olive baboons, vervet monkeys, and the yellow baboons in Kenya. These findings suggest there may be a possibility of zoonotic transmission of adenoviruses from nonhuman primates to humans in Kenya. Adenovirus can enter a susceptible host by the nose, mouth, or eye membranes. Water may play a meaningful role in the transmission for many human adenovirus serotypes, including the enteric adenovirus that is transmitted via the fecal-oral route (Heerden et al., 2005). Heerden et al. (2005) detected human adenovirus in 4 of 51 (7.8 percent) samples of river water and 9 of 51 (17.7 percent) samples of dam water.

Human adenoviruses may cause of a wide spectrum of acute and chronic diseases, including keratoconjunctivitis, upper respiratory tract infections, pneumonia, gastroenteritis, cystitis, and encephalitis (Gray et al., 2005). Molecular studies have recently shown adenoviruses to be associated with bronchopulmonary dysplasia (Couroucli et al., 2000) and chronic obstructive pulmonary disease (Hogg, 2001).

Immunocompromised persons, including people with AIDS, bone marrow transplant patients, pregnant women, and children are more susceptible to adenovirus infections (Baldwin et al., 2000; Crawford-Miksza and Schnurr, 1996). Infections in these populations may result in severe illness and death (Chakrabarti et al., 2002; Runde et al., 2001). U.S. surveillance for adenovirus is relatively incomplete (Gray et al., 2005) and well-documented incidence rates are not available, especially for waterborne outbreaks.

III. PATHOGEN INTERACTIONS WITH THEIR ENVIRONMENT

Pathogens interact with the ambient environment, other microorganisms, plants, and with their hosts. This section provides a summary of how pathogens respond to various environmental parameters. Information on interactions with other microorganisms and animal manure are briefly covered in this overview followed by sections that describe how the water environment affects pathogen survivability and phenotype and how host animals can influence pathogen characteristics and mechanisms of rapid evolution. The behavior of pathogens in ambient waters is often different from the behavior of indicators in ambient water.

Although there are important waterborne amoebic pathogens, they are not associated with animal fecal material. However, important bacterial pathogens that are associated with fecal material (e.g., *Salmonella, Campylobacter*) interact with free-living amoebae in ways that could impact recreational water quality. The most notable, *Legionella,* infects and replicates in free-living amoebae and is considered more of a risk in drinking water than in recreational waters (Borella et al., 2004; Marrie et al., 2001). Some human bacterial pathogens are even thought to have evolved in association with amoebae (Berk et al., 2006). Tezcan-Merdol et al. (2004) investigated the uptake and replication of salmonellae in amoebae. Three different serovars of *Salmonella enterica* (Dublin, Enteritidis, and Typhimurium) were evaluated for internalization by 5 different isolates of axenic *Acanthamoeba* species. The Dublin serovar was internalized more efficiently than the other two serovars, and the *Acanthamoeba rhysodes* isolate was more efficient than the other four isolates. The researchers concluded that *Acanthamoeba* species can differentiate *Salmonella* serovars and that internalization of the bacteria produces cytotoxic effects mediated by defined bacterial virulence loci. Axelsson-Olsson et al. (2005) studied the infection of *Acanthamoeba polyphaga* by four different *Campylobacter jejuni* strains. The infecting bacterial cells were observed to be actively moving in amebic vacuoles and survived longer when cocultured with amoebae than when cultured alone. They indicated that free-living amoebae may serve as a nonvertebrate reservoir for *Campylobacter jejuni*.

Guan and Holley (2003) examined *E. coli* O157:H7, *Salmonella, Campylobacter*, *Yersinia*, *Cryptosporidium,* and *Giardia* in animal manure. Of those pathogens considered, *E. coli* O157:H7 was the most persistent in cattle manure regardless of the temperature and manure form (solid or slurry) while *Campylobacter* and *Giardia* were weakest survivors in manure. The authors concluded that holding manure at 25° C for 90 days will render it free from the pathogens considered. This has indirect implications for ambient water quality because the conditions experienced by pathogens after excretion but before introduction into ambient water contribute to the overall likelihood the pathogen will be infectious by the time it potentially can reach a human host through recreational contact.

III.1 Water Environment Affects Pathogen Survivability and Phenotype

The most common environmental factors studied for their impact on pathogen survival in water are pH, salinity, light exposure, and temperature. Additional environmental characteristics that may influence pathogen survival, infectivity, and virulence include: UV light (duration, intensity), rainfall, runoff, dispersal, suspended solids, turbidity, nutrients, organic content,

organic foams, water quality, biological community in water column, water depth, stratification, mixing (e.g., wind and waves), presence of aquatic plants, biofilms, and predation.

Thomas et al. (2006) examined extreme rainfall and spring snowmelt in association with 92 Canadian waterborne disease outbreaks between 1975 and 2001. Accumulated rainfall, air temperature, and peak stream flow were used to determine the relationship between high impact weather events and the occurrence of waterborne disease outbreaks. For rainfall events greater than the 93rd percentile, there was a greater than 2-fold increase in the odds of an outbreak compared to rainfall events less than the 93rd percentile. For each degree-day above 0° C, the relative odds of an outbreak increased by a factor of 1.007. The odds ratio is small on a per degree-day scale, but is notable over longer timeframes. For example, over a 42-day period, a 5° C increase in the maximum daily air temperature would result in over a 4-fold increase ($1.007^{(5\times42)} = 4.33$) in the relative odds of an outbreak. Stream flow and stream flow peaks did not show a difference between cases (outbreaks) and controls (no outbreaks), but there was a considerable lack of data on stream flow.

An overview of the available information for the six key waterborne zoonotic pathogens follows. Information concerning these factors is limited for most waterborne pathogens. Thus, in order to develop effective control strategies, additional research may be necessary.

III.1.1 Pathogenic *E. coli* Survival in the Environment

Pathogenic *E. coli* can be expected to survive outside of a host animal anywhere from a few days to several weeks, depending upon environmental conditions. Pathogenic *E. coli* seem to interact with the environment in a similar fashion to nonpathogenic *E. coli* and do not lose their virulence during prolonged survival in the environment. Survival of *E. coli* O157:H7 in surface waters was found to be longer at lower temperatures (Czajkowska et al., 2005) and was 2 to 3 times longer in river and lake sediments at the same temperatures (see Table III.1.1-1).

Table III.1.1-1. Survival of *E. coli* O157:H7 in Ambient Waters

Water matrix	°C	Days to Decrease		Reference
		99%	99.99%	
Surface waters (lakes and rivers)	6	4-11	8-22	Czajkowska et al., 2005
Surface waters (lakes and rivers)	24	2-8	5-10	
Sediments (same lakes and rivers)	6	10-20	25-39	
Sediments (same lakes and rivers)	24	5-8	10-30	
River water with feces	15	7.5	14.5	McGee et al., 2002
Unfiltered lake water	8	91	NA	Wang and Doyle, 1998
Filtered tap water	15	>91	NA	
Unfiltered lake water	25	14-28	NA	

Although direct contamination of ambient water due to domestic animals or livestock wading in water is possible, most contamination of water is due to runoff from pastures and fields after land application of manure. *E. coli* O157:H7 can survive for at least several weeks in animal feces and slurries (Avery et al., 2005) and has been demonstrated to survive at least 500 days at -20° C in frozen soil (Gagliardi and Karns, 2002).

III.1.2 *Campylobacter* Survival in the Environment

Campylobacter has been shown to survive in aquatic environments with low temperatures (4° C) between 8 days (Buswell et al., 1998) and 4 months (Rollins and Colwell, 1986).

Buswell et al. (1998) found that survival times of *Campylobacter* isolates differed by 2- to 4-fold depending on the combination of temperature and oxygenation tested. The mean survival times in sterile microcosms were 202 hours at 4° C, 176 hours at 10° C, 43 hours at 22° C, and 22 hours at 37° C. The survival times were considerably longer in the presence of the autochthonous water microflora (two strains tested survived 700 and 360 hours at 4° C). Aerobic conditions decreased the survival of one strain 30 percent and increased the persistence of another strain by more than 3-fold. Within biofilms, the pathogen persisted up to the termination of the experiments after 28 and 42 days of incubation at 30 and 4° C, respectively.

Rollins and Colwell (1986) found that *Campylobacter* incubated in filter-sterilized stream water was recoverable after 4 months at 4°C. Incubation at 25°C resulted in a decline to the nonculturable state within 28 days, and at 37°C, the nonculturable state was reached in 10 days. Direct counting methods indicate that the nonculturable but viable state of *Campylobacter* is significant.

III.1.3 *Salmonella* Survival in the Environment

Salmonella are also found frequently in sewage, soil, and various surface waters. The greatest source of the bacteria is fecal contamination. Under suitable environmental conditions, *Salmonella* can survive for weeks in waters or years in soils (Lightfoot, 2004). *Salmonella* grow at temperatures ranging from 10 to 43º C, but some serovars have suppressed growth at temperatures above 40º C (Covert and Meckes, 2006). *Salmonella* can grow at pH 4–8 and at water activities above 0.93. Under some conditions, *Salmonella* may proliferate below 4 º C and survive below pH 4 (Lightfoot, 2004).

III.1.4 *Leptospira* Survival in the Environment

Leptospira survive longer in the environment in warm, humid conditions. Leptospirosis is seasonal, which relates to the pathogen's survival in the environment. In temperate regions, there is a peak during summer or fall, whereas in warm-climate regions, the rainy season is associated with peaks because dessication decreases pathogen survival (Levett, 2001).

Ganoza et al. (2006) compared levels of *Leptospira* in urban and rural environmental surface waters in the Peruvian Amazon region of Iquitos. The concentration of pathogenic *Leptospira* was higher in urban than rural water sources and rats were the indicated zoonosis.

III.1.5 *Cryptosporidium* Survival in the Environment

As noted previously, because *Cryptosporidium* oocysts are extremely resistant to environmental or engineered degradation, the survival of *Cryptosporidium* under a variety of environmental and drinking water treatment conditions has been evaluated by many investigators. While the majority of these studies have considered the effects of physical antagonism (e.g., freezing, heating, UV exposure), studies have also been conducted to consider the role of microbial antagonists (microbial predation), chemical antagonists (such as disinfection), and aging. This section focuses primarily on aspects of physical antagonists in the environment because they are most pertinent to the topic of this paper.

Robertson et al. (1992) evaluated the sensitivity of *C. parvum* oocysts to a variety of environmental pressures such as freezing, dessication, and water treatment processes, as well as in physical environments commonly associated with oocysts. Approximately 97 percent of the test oocysts were inactivated after 18 days at 22° C, suggesting that the levels of viable oocysts in surface waters might be influenced by seasonal temperature variations. After 2 hours of drying oocysts at room temperature, only 3 percent of oocysts were still viable, and after 4 hours, no oocysts were viable. When stored at 4° C, the percentage of oocysts remaining viable in stool samples decreased steadily with time. (In the study, the relationship between oocyst viability and time varied with individual.) After 176 days in tap water, river water, or cow feces, there was a statistically significant increase in the proportion of dead oocysts in test samples. Seawater was even more lethal to oocysts, with a statistically significant increase in dead oocysts by 35 days of exposure to the test conditions. *C. parvum* oocyst viability is sensitive to a wide range of typical environmental conditions while remaining relatively insensitive to some water treatment processes. Robertson et al. (1992) also emphasized that oocyst viability depends on the amount of time to which oocysts are exposed to a physical or chemical stress in the environment.

Temperature has a significant effect on oocyst survival, with (unfrozen) colder waters promoting the highest survival rates (USEPA, 2001a). Warm and boiling water completely neutralizes oocysts, and the temperature of the water determines the time required for the treatment to become effective (Anderson, 1985). Cryopreservation studies conducted by Fayer et al. (1991, 1997) indicate that oocyst survival depends on the temperature and duration of freezing conditions, implying that *C. parvum* oocysts are not necessarily rendered noninfectious by being frozen *per se*. In another study, Fayer and Nerad (1996) demonstrated that the infectivity of *C. parvum* oocysts after freezing is dependent on the temperature and duration of freezing. In general, shorter freezing times are required to neutralize infectivity when lower freezing temperatures are employed (e.g., 1 hour at -70° C versus 168 hours at -15° C to completely neutralize infectivity) (Fayer and Nerad, 1996).

Temperature stability studies also were conducted by Sattar et al. (1999) who evaluated the freeze/thaw susceptibility of various preparations of oocysts including highly purified preparations as well as infected calf feces. The results of this study indicated that oocyst stability under freezing conditions is at least partially dependent upon the surrounding matrix, with fecal material conferring a cryopreservative effect on oocysts. In the absence of freezing conditions, colder water temperatures tended to promote the survival of most microorganisms. In water, *C.*

parvum may survive outside of mammalian hosts for several months or more depending upon water temperature (Straub et al., 1994).

Fayer et al. (1998) investigated the effect of water temperatures ranging from -10 to 35° C and a few higher temperatures on oocyst infectivity. As water temperature was increased from -10 to 20° C, oocysts remained infectious for longer exposure times. For example, oocysts retained their infectivity for only 1 week when suspended in water held at -10°C but remained infectious for up to 24 weeks in 20° C water. As water temperatures were increased above 20° C, oocysts retained their infectivity for shorter exposure times (Fayer et al., 1998). Under conditions of high water temperatures, higher than typically found in surface waters, Fayer (1994) indicated that all evidence of *C. parvum* infectivity was lost within 60 seconds when temperatures exceeded 72° C or when temperatures of at least 64° C were maintained for 2 minutes.

Holding oocysts to 45°C for 5 to 20 minutes was effective in completely neutralizing infectivity (Anderson, 1985). Anderson (1986) examined the infectivity (determined in infant mice) of oocysts from calf fecal samples that had been dried in a barn (< 60 percent humidity) in either winter or summer months. In summer temperatures (i.e., 18 to 29° C), oocysts completely lost infectivity in 1 to 4 days. Experiments conducted in winter, with air temperatures ranging from -1 to 10° C, demonstrated a complete loss of infectivity within 2 to 4 days. Control samples kept moist and refrigerated retained infectivity for up to 2 to 3 weeks.

Limited studies have been conducted on the effects of physical shear on oocyst viability; these studies have attempted to assess the potentially abrasive effects of oocyst contact with sand and gravel particles or through fast-flowing waters. Parker and Smith (1993) found that after shaking with sand for 5 minutes, 90 minutes, and 2 hours, the number of non-viable oocysts increased significantly to 50 percent, 99.7 percent, and 100 percent, respectively. When chlorination followed 5 minutes of sand shaking, the observed non-viable oocysts increased to 68 percent. When oocysts were on an orbital shaker at 60 and 120 rpm, oocyst viability declined linearly over the course of an hour, with approximately 50 percent loss of viability noted at 20 minutes (Sattar et al., 1999). These authors also showed that oocysts subjected to 2000 psi (13.9 Mpa) for 1 minute had little reduction in viability.

Sattar et al. (1999) also evaluated the effects of microbial predation on oocyst survival. They observed that oocysts incubated in dialysis cassettes that were suspended in natural waters exhibited significantly longer survival times when bacterial populations were excluded from the suspension water. The observation implies that microbial predation may play an important role in reducing oocyst survival in ambient (natural) waters.

Nasser et al. (2007) examined the effect of sunlight and salinity on the die-off of *C. parvum*. Experiments were carried out for 7 days in tap and seawater and sunlight and dark conditions. Oocyst die-off was greatest when exposed to seawater and sunlight (0.44 log/day); oocysts in tap water in the dark, exposed to sunlight, and oocysts in seawater in the dark had die-off rates of 0.1, 0.22, and 0.19 log, respectively. At the end of the 7-day study period, a 3 log reduction in infectivity was measured in the sunlight- and seawater-exposed oocysts. *Cryptosporidium* oocysts retain substantial infectivity for several months at salinity levels corresponding to estuarine coastal waters (Fayer, 2004a) and for several weeks in seawater (WHO, 2006). These

studies indicate that *Cryptosporidium* can thus pose a serious health hazard to humans by direct and indirect contact in recreational waters.

King et al. (2005) measured oocyst inactivation rates in reagent-grade and environmental waters over a range of temperatures. Oocysts incubated at 4 and 15° C remained infective over a 12-week holding period. A 4 $\log_{10}$ reduction in infectivity was observed for both 20 and 25°C incubation treatments at 12 and 8 weeks, respectively, for all water types examined. This is a faster rate of inactivation for oocysts than had been previously reported. Inactivation at higher temperatures is likely a function of increased oocyst metabolic activity.

Li et al. (2005) measured the inactivation rate of bovine *C. parvum* oocysts subjected to temperature regimes designed to mimic the diurnal oscillations of ambient temperature in bovine feces exposed to sunlight in commercial cattle operations in California. No infectious oocysts were observed after 1- to 5-day cycles of 40, 50, 60, and 70° C. The loss of infectivity was primarily due to partial or complete *in vitro* excystation during the first 24-hour diurnal cycle and secondarily to thermal inactivation of the remaining intact or partial oocysts. The results suggest that as ambient conditions generate internal fecal temperatures greater than or equal to 40° C, rapid inactivation occurs at a rate equal to or greater than 3.27 $\log_{10}$ reduction per day for *C. parvum* oocysts deposited in the feces of cattle.

Méndez-Hermida et al. (2005) reported on batch-process solar disinfection of .*C. parvum* oocysts in water. Oocyst suspensions were exposed to simulated sunlight (830 W/m^2) at 40° C. Viability assays and infectivity tests indicated that exposures of 6 and 12 hours reduced oocyst infectivity from 100 percent to 7.5 percent and 0 percent, respectively.

The behavior of lake inflows is important in determining pathogen transport and distribution. Inflows that are warmer than the lake water will move over the surface of the lake, whereas inflows that are colder than the lake will sink beneath the surface layer where they will flow along the bottom towards the deepest point (Brookes et al., 2004). The fate of pathogens in lakes is determined by factors such as settling and inactivation by temperature, UV, and predation by other microorganisms. Brookes and colleagues found that inactivation of *Cryptosporidium* by UV light can be rapid or slow, depending on the depth of the oocysts in the water column and the extinction coefficient for UV light.

Pokorny et al. (2002) investigated the effects of temperature on oocysts stored in the dark in filter-sterilized and nonfilter-sterilized river water. They reported that as the temperature was increased from 4 to 23° C, the infectivity of oocysts decreased; no infectious oocysts were detected after 1 week at -20° C.

Excreted *Cryptosporidium* oocysts can survive for substantial periods in animal wastes and soils. Thus, contaminated runoff can enter ambient water and result in potential human exposures. The numbers of oocysts excreted by infected young animals may be especially large, between 1 and 10 million oocysts per gram of feces (WHO, 2006). Oocyst survival for 4 weeks or more has been documented in concentrated animal wastes, particularly at low temperatures (4° C) (WHO, 2006). In the environment, the vast majority of oocysts (99 percent) are inactivated by repeated freeze-thaw cycles independent of the number of times the soil was frozen (Fayer, 2004a).

Therefore, *Cryptosporidium* may be environmentally limited in parts of the United States during the winter season. There is evidence that in warmer ambient temperatures, between 4 and 20° C, very little inactivation of oocysts occurs in different types of agricultural soils (Jenkins et al., 2002; WHO, 2006). Factors that are known to reduce oocyst survival in soils include drying and basic pH (Fayer, 2004a). Oocyst survival in various water matrices is highly variable, but survival for longer than 30 days has been demonstrated in several studies. Approximately 4 to 5 percent of oocysts in tap water and river water samples survived after approximately 6 months, with approximately 37 percent inactivation after 2 days exposure to tap water (USEPA, 2005a, 2005b).

Walker et al. (2001) tested oocyst degradation (as indicated by microscopic examination) in response to the combined stresses of water potential (sodium chloride solute potential), above-freezing temperatures (4 and 30° C), and a subfreezing temperature (-14° C) for different freeze thaw cycles (-14 to 10° C). The degradation coefficients were estimated using multiplicative error and exponential decay models. Increased water potential increased the rate of *C. parvum* population degradation for all temperature conditions investigated. The effects of water potential were roughly four times those noted for freezing alone. The difference between the effects of freeze-thaw cycling and simple freezing may be caused by mechanical damage to the oocyst wall. The authors conclude that water potential conditions encountered under field conditions are likely to lead to more rapid degradation of oocyst populations than might be expected from studies of degradation in calf feces, distilled water with antibiotics, and reverse osmosis water at low temperatures.

III.1.6 *Giardia* Survival in the Environment

Studies of the effect of temperature and other environmental factors on *Giardia* cyst survival were summarized in EPA's *Giardia* Human Health Criteria Document (USEPA, 1998). Survival was determined based on dye inclusion/exclusion, excystation, or animal infectivity studies. Some studies used *Giardia muris* cysts, whereas others used *Giardia lamblia* cysts. Overall, the studies on the effect of temperature indicated that survivability decreased as temperature increased and that while some cysts could survive a single freeze-thaw cycle, repeated freeze-thaws as might be expected in the environment would likely inactivate cysts (USEPA, 1998). Johnson et al. (1997) investigated the survival of cysts in marine waters and determined that viability was reduced 99.9 percent in 3 hours in marine waters exposed to sunlight (as reported in USEPA, 1998). They also found that 77 hours were required to get 99.9 percent inactivation in the dark and that cysts survived longer at a salinity of 28 mg/L than at 35 mg/L. Because different waters were used in the experiments, however, these investigators could not rule out the effect of factors other than salinity.

Olson et al. (1999) found that *Giardia* cysts were noninfective in water, feces, and soil following 1 week of freezing to -4° C and within 2 weeks at 25° C. At 4° C, *Giardia* cysts were infective for 11 weeks in water, 7 weeks in soil, and 1 week in cattle feces. Robertson and Gjerde (2006) tested survival of *Giardia* cysts (as well as *Cryptosporidium* oocysts) during winter in an aquatic environment (approximately 1 to 7° C) in Norway. Three conditions were compared, distilled water, river water, and submersion of a filter chamber containing cysts into the river. The rate of decline in viability was similar under all three conditions, and no *Giardia* cysts with apparently

viable morphology could be detected after 1 month. Boiling *Giardia* for 1 minute reduces viability (as determined by flurogenic dyes) to less than 1 percent and renders them noninfectious (as determined by animal infectivity) (El Mansoury et al., 2004). Storage at 4° C and -4° C for up to 7 days preserves *Giardia* cyst viability and infectivity. Storage at 30 ppt (parts per thousand) salinity for 4 weeks decreased viability to 30 percent, but all animals were infected. Storage at 50 ppt salinity for 4 weeks decreased viability to 5 percent and 80 percent of animals were infected. Storage at 50 ppt salinity for 4 weeks resulted in zero viability (El Mansoury et al., 2004).

III.1.7 Virus Survival in the Environment

Viruses are more stable at lower temperatures; however, different viruses have different stabilities under similar environmental conditions. For example, astroviruses survive longer than poliovirus and adenovirus, but shorter than rotavirus and hepatitis A virus (Sobsey, 2006). For poliovirus and parvovirus, 90 percent inactivation was observed after 1 to 3 days at 28° C, and 90 percent inactivation was observed after 10 days at 6° C (Griffin et al., 2003). Parvoviruses are the most heat stable enteric viruses known (Gerba, 2006a). Enterovirus nucleic acid is detectable for 60 or more days, whereas infectious virus is detectable for 51 days or less (Griffin et al., 2003). Below 5° C, enteroviruses can survive for years (Gerba, 2006b). Limited data suggests that adenoviruses survive longer in water than enteroviruses and hepatitis A virus (Enriquez and Thurston-Enriquez, 2006).

Pesaro et al., (1995) evaluated the persistence of five animal viruses, representing picorna-, rota-, parvo-, adeno-, and herpesviruses, and the coliphage f2, using a filter sandwich technique that mimics the environment in various states of manure. Depending on ambient temperature, pH, and type of animal waste, 90 percent reduction of virus titer varied, ranging from less than 1 week for herpesvirus to more than 6 months for rotavirus. Virus inactivation was faster in liquid cattle manure, a mixture of urine and water (pH > 8.0), than in semiliquid wastes that consisted of mixtures of feces, urine, water, and bedding materials (pH < 8.0). The authors conclude that viruses contained in manure may persist for prolonged periods of time if stored under nonaerated conditions and this may lead to environmental contamination with viruses.

III.2 Host Animals Can Influence Pathogen Characteristics and Mechanisms of Rapid Evolution

There is evidence that zoonotic pathogens may change in infectivity, virulence, and the severity of disease caused in humans depending on their previous host environment. There is also evidence that some of these host-factor changes can influence subsequent infection cycles in exposed hosts. For example, in laboratory experiments using nutritionally deficient mice (selenium-deficient or vitamin E-deficient), Morse (1997) reported that a normally avirulent (mild) coxsackievirus B3 isolate gave rise to a virulent variant, albeit through an unknown mechanism. The virulent variant resembled other known virulent genotypes and was stable upon subsequent infection of other mice with adequate nutrition. In another demonstration of host influence on pathogen evolution, yeast with deletions in genes that normally suppress viral RNA recombination became “hotbeds” for viral recombination. The host (in this case yeast) genes could affect viral recombinant accumulation by up to 80-fold (Serviene et al., 2005). The key

mechanisms of phenotypic change in pathogens are genetic diversity (coinfection and quasispecies), cryptic genes, mutators, and epigenetic effects, and are summarized as follows.

Genetic diversity (co-infection and quasispecies): The pathogen population within a single host can be comprised of several or even numerous genetic variants of the same zoonotic pathogen. This variation can occur when the infectivity is such that several genotypes coinfect the host simultaneously, then "compete" or "cooperate" as infection progresses. The fluctuations in genotype prevalence depend on host-pathogen interactions as some genotypes gain dominance and others become rare. High genetic diversity also can occur when pathogens have high mutation rates. This latter phenomenon, referred to as "quasispecies," occurs mainly in RNA viruses because viral RNA replicase enzymes lack proofreading functions during replication, which leads to high mutation rates (Domingo et al., 1998; Morse, 1997). For example, caliciviruses may have as many as 1 to 10 mutations per template copy (Smith et al., 1998), and within-person HEV sequence diversity has been documented to range from 0.11 to 3.4 percent (Grandadam et al., 2004). For retroviruses, every virus particle may be genetically different from every other particle. In fact, the cooperativity[6] of many virus particles' genetic material may be necessary to complete the infection process (Lederberg, 1998).

Cryptic genes: Meiotropy is the ability of an organism to gain a phenotypic trait through mutation (Brubaker, 1991). One example of a cryptic gene is the yopA gene in *Yersinia pseudotuberculosis*. A stretch of deoxyadenosine nucleotides (DNA base A) can spontaneously change in length between 8 and 9 bases, which can result in the absence or presence of the gene product. In *Y. pseudotuberculosis*, the absence of the gene product results in increased virulence.[7] In *Y. pestis* (plague), the gene yopA is not normally expressed; however, molecular manipulation that causes expression of the yopA gene product reduces the virulence of *Y. pestis*. Thus, it is possible that endemic hosts of *Y. pestis* could harbor a strain of lower virulence, which by one mutation could become hypervirulent and potentially cause an outbreak of plague (Rosqvist et al., 1988). Another example is in *Salmonella*, in which flagellar antigens can be expressed or silenced in a reversible manner by inversion of a segment of DNA that moves the promoter from one locus to another (Lederberg, 1998). This type of genetic rearrangement may serve as a mechanism of phase variation for antigenic factors in the bacteria. More standard transcriptional control of the gene expression might allow for low levels of transcriptional leakage and small quantities of antigen to be produced. Prior trace levels of antigens could be sufficient to promote host immunity. Bacteria carry site-specific recombinases that are capable of scrambling bacterial genomes in order to suppress and unsuppress genes.

Mutators: Several genes have been identified that influence the mutation rate of bacteria. For example, bacterial cells that carry the MutD5 protein, which binds to DNA polymerase, accumulate a broad spectrum of base substitutions and frameshift mutations (the mutation

[6] The hypothesis that swarms of viral genotypes cooperate to comprise an "average phenotype" differs from quorum sensing, which is the coordination of bacterial cells via cell-to-cell communication with small signaling molecules to form biofilms and other group-related phenotypes.

[7] In this case, the presence of the gene leads to decreased virulence because the gene product facilitates chronic infection, which is less severe than acute infection.

frequency can be 20- to 4,000-fold) (Selifonova et al., 2001). Mutator strains can pass genetic material to other bacteria and thus transfer the mutator trait. Therefore, the ability of bacteria to evolve rapidly is heritable.

Epigenetic effects: Epigenetic effects are reversible, heritable changes in gene regulation that occur without a change in DNA sequence. Genomic imprinting through DNA methylation is one type of epigenetic effect that has been documented for pathogen regulation of virulence (Low et al., 2001). The zoonotic bacteria *E. coli, S. enterica* (serovar Typhimurium), *Vibrio cholerae, Y. pseudotuberculosis, Y. enterocolitica, Helicobacter pylori,* and *Campylobacter* are a few notable pathogens for which DNA methylases are known to regulate gene expression (Fälker et al., 2005; Low et al., 2001). Although DNA methylase promoters are known to be responsive to *in vivo* growth conditions, the degree to which host factors influence pathogen DNA methylation and whether those methylation patterns provide a memory system for subsequent generations of pathogens are not known.

All of these mechanisms can contribute to rapid evolution of zoonotic pathogens.[8] Rapid evolution is most often observed in new hosts, where new stresses are thought to lead to strong selection and rapid evolution (Ebert, 1998). Evolution in new host environments can lead to a reduction of virulence when the original host is encountered again. Serial passage experiments (SPEs) often result in attenuation of pathogenicity in one host along with an escalation of pathogenicity in the new host.[9] Attenuation can be so severe that it can even lead to an altered host range (complete loss of ability to infect the original host species).[10] In SPEs when only one or a few pathogens are transferred at each passage, however, genetic drift can result in decreased genetic variability and lead to a failure to adapt to new hosts and a decline in overall pathogen fitness. Text Box III.2-1 summarizes some feeding studies done in humans and pigs with zoonotic parasites. The examples help illustrate how changes in laboratory isolates due to passage through hosts should be considered during experimental design.

Although SPEs are useful for studying pathogen evolution, the trends observed in SPEs are not necessarily broadly applicable to pathogen evolution as a whole (Ebert, 1998). In fact, the observation that virulence increases in SPEs in new hosts seems contrary to the classic expectation that pathogens and hosts evolve over time in ways that render infections benign (Dieckmann, 2002; Wills, 1996). Currently, evolutionary biologists estimate the evolutionary success or failure (i.e., "fitness") of pathogens by their rate of spread through a given host population. Low virulence can lead to missed opportunities to spread due to low pathogen numbers. High virulence can lead to host death and a subsequent lack of spread. Those hypotheses are supported by the observation that intermediate levels of pathogen virulence can be stable. Thus, the dynamic between pathogen and host drives pathogen (and host) evolution

[8] For the purpose of this paper, rapid changes are phenotype (or genotype or epigenetic) changes that occur within one or a few passages of a zoonotic pathogen through a particular host species.

[9] The negative correlation in the fitness of a pathogen in different hosts is the antagonistic pleiotropy hypothesis—a gene that enhances fitness in one host decreases fitness in the other host (Ebert, 1998).

[10] Live vaccines such as Theiler's yellow fever vaccine and Sabin's polio vaccine are examples of attenuated pathogens that elicit an immune response without inducing disease. However, reemergence of pathogen virulence after vaccination remains a risk (Ebert,1998).

and is influenced by numerous factors including the infection status of the host population as a whole (Dieckmann, 2002).

Because pathogens can evolve quickly within one host, it is reasonable to suspect that zoonotic pathogen pools that were propagated in animals would be different from pathogen pools that came from human sources. However, the extent to which known waterborne zoonotic pathogens are attenuated or gain enhanced virulence in humans when passing through animal hosts remains unknown. Even if general trends could be characterized, it is unlikely that differences could be quantified adequately in the near-term to predict differences in human infectivity and virulence for pathogens passing through different host animals.

Another mechanism by which virulence and fecundity of a microorganism may be affected was recently reported by Jenkins et al. (2007) for *C. parvum.* These investigators infected dairy calves with oocysts from either *C. parvum* Beltsville (B) or *C. parvum* Iowa (I). Calves given the B isolate excreted 5-fold more oocysts than those receiving the I isolate. Quantitative reverse transcriptase-PCR indicated that the B isolate contained a 3-fold greater number of the symbiont *C. parvum* virus (CPV) than the I isolate. They concluded that CPV may have a role in fecundity and possibly virulence of *C. parvum.* The authors indicated that their results contrasted to those found by Miller et al. (1988) in which there was an inverse relationship between presence of a *Giardia lamblia* virus (GLV) and parasite growth. Miller et al. (1988) used virus-free isolate that they infected with various numbers of GLV, and Jenkins et al. (2007) noted that virus-free cysts may be more susceptible to the effects of virus infection. Jenkins et al. (2007) also indicated that *C. hominis* and *C. parvum*, the two species most often associated with human infections, are the only species reported thus far to be infected by CPV.

Text Box III.2-1. Examples of Feeding Studies with Zoonotic Parasites

For the widespread waterborne zoonotic protozoa *Cryptosporidium* and *Giardia*, human volunteers (immunocompetent adults) have been exposed to measured doses of various strains of *Cryptosporidium* and to a single *Giardia* isolate to evaluate their dose-response relationships. However, there are uncertainties associated with characterizing dose even in clinical settings. Microbes can exhibit variability in infectivity, virulence, or environmental survival within strains; even for "pure isolates," batches can differ. For microorganisms that cannot survive freezer storage or do not maintain their genetic integrity in freezer storage or tissue culture, *in vivo* passage in animals is required to maintain stocks. Even if the starting inoculum for a dose-response study is clonal, mutations will occur that may impact pathogen characteristics. When the starting inoculum is not clonal, which is most often the case, the subpopulation ratios within an individual host can differ from other hosts receiving the same inoculum. The subpopulation ratios also may vary as infection progresses, so collecting pathogens from a host on one day may not yield the same pool of pathogens as collecting on another day. In addition, some pathogens are not amenable to storage or maintenance in laboratory settings under any conditions and must be continuously collected from the field for each experiment. Although the challenges of properly characterizing and controlling pathogen variability in experimental research settings are considerable, the challenges are even greater for epidemiological studies.

Cryptosporidium in Humans

Data on the infectivity of *Cryptosporidium* in humans are available from studies conducted at the University of Texas, Houston, by DuPont, Chappell, Okhuysen, and colleagues. These studies all involved healthy adult volunteers ingesting different numbers of *Cryptosporidium* oocysts. Subjects were then evaluated for *Cryptosporidium* in stool samples and for diarrheal and other GI illness symptoms. Infectivity was estimated for five *C. parvum* isolates: TAMU (collected from a veterinary student); Iowa (derived from a calf); UCP (derived from a calf); Moredun, (collected from a red deer calf); 16W (from a calf); and TU502 (an isolate collected from an infected child and propagated in gnotobiotic piglets [Chappell et al., 2006]). *Cryptosporidium* from animal sources were found to be infectious in humans. No attempt was made to evaluate differences in potential infectivity due to repeated passage in animal hosts. However, the UCP isolate was less infectious in humans than the other isolates, and EPA's Science Policy Council speculated that this could be due to prolonged maintenance in calves. In the risk assessment that was conducted in support of EPA's Long Term 2 Enhanced Surface Water Treatment Rule (LT2) Economic Analysis, a dose-response relationship was chosen that weighs the UCP data less than the other isolates.

Cryptosporidium in Animals

Akiyoshi et al. (2003) investigated mixed infections of Type 1 (*C. hominis*) and Type 2 (*C. parvum*) in gnotobiotic piglets. In all the time intervals tested, Type 2 displaced Type 1, even if Type 1 was permitted to become established before inoculation with Type 2. This result raises significant questions regarding the relative perpetuation and survival of the two genotypes in mammalian hosts. The same researchers noted that field technicians very readily became infected with *C. hominis*, but that cross-contamination of *C. hominis* with *C. parvum* in the animals used to maintain stocks resulted in *C. parvum* overwhelming *C. hominis* (Tzipori, 2000). These observations suggest that discovering the mechanisms by which *C. hominis* is maintained in natural setting when *C. parvum* is also present should improve understanding of risks to humans.

Giardia in Humans

Rendtorff (1954) conducted a controlled, clinical study of male prison volunteers who were fed *Giardia* cysts obtained from a human source. *Giardia* cysts of known numbers varying from 1 to 10^6 were placed into gelatin capsules along with a small amount of saline. The capsules were given to the volunteers along with 4 to 6 ounces of water. Control subjects were given sterile saline in the same manner. A dose of 10 cysts was found to be sufficient to produce human infection, as determined by observing the presence of *Giardia* in fecal smears (Rendtorff, 1954). However, because cyst viability could not be determined prior to administration to volunteers, the failure to elicit infection in the five men treated with a dose that was calculated to contain only one cyst may have been due to dosing with inactive cysts. The *Giardia* Assemblages used in these studies are not known but presumably are A or B because these are the Assemblages known to infect humans.

IV. SUMMARY

Contamination of recreational waters with feces from warm-blooded animals poses a risk of zoonotic infection of humans with some of the pathogens in those waters. Although the risk and severity of human illness due to contamination with animal feces and zoonotic pathogens is most likely lower than the risk and severity of illness from treated or untreated human sewage, currently available data are insufficient to quantify the differences. At present, the six most important zoonotic waterborne pathogens are the following:

- Pathogenic *E. coli*;
- *Salmonella*;
- *Campylobacter*;
- *Leptospira*;
- *Cryptosporidium*; and
- *Giardia.*

All of these waterborne pathogens are likely to cause more severe symptoms in children and immunocompromised individuals and subpopulations than in the remainder of the population. Of these six, pathogenic *E. coli* has the most potential for severe adverse health effects that can even be fatal. Potential debilitating chronic sequelae such as Guillain-Barré Syndrome and reactive arthritis have been associated with *Campylobacter* infections. Although the most common recreational illnesses are probably due to human viruses causing short-term GI, the waterborne zoonotic pathogens discussed in this report have the potential to cause serious health effects. While serious health outcomes are likely to be rare in comparison with self-limiting illnesses as a result of ambient (recreational) water exposure, the adverse health impacts of the rare, but more serious illnesses remain an important public health challenge.

V. REFERENCES

Abbaszadegan, M. 2006. Rotoviruses, In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 46.

Ackman, D., Marks, S., Mack, P., Caldwell, M., Root, T., Birkhead, G. 1997. Swimming-associated haemorrhagic colitis due to *Escherichia coli* O157:H7 infection: evidence of prolonged contamination of a fresh water lake. Epidemiology and Infection 119(1): 1-8.

Adam, R.D. 2001. Biology of *Giardia lamblia*. Clinical Microbiology Reviews 14(3): 447-475.

Akiyoshi, D.E., Mor, S., Tzipori, S. 2003. Rapid displacement of *Cryptosporidium parvum* type 1 by type 2 in mixed infections in piglets. Infection and Immunity 71(10): 5765-5771.

Allen, L., Briggle, T., Pfaffenberger, C. 1982. Absorption and excretion of cyanuric acid in long-distance swimmers. Drug Metabolism 13: 499-516.

Allos, B.M. 1998. *Campylobacter jejuni* infection as a cause of the Guillain-Barre syndrome. Infectious Disease Clinics of North America 12(1): 173-184.

Allos, B.M. 2001. *Campylobacter jejuni* infections: update on emerging issues and trends. Clinical Infectious Diseases 32(8): 1201-1206.

Anderson, B.C. 1985. Moist heat inactivation of *Cryptosporidium* sp. American Journal of Public Health 75(12): 1433-1434.

Anderson, B.C. 1986. Effect of drying on the infectivity of *Cryptosporidia*-laden calf feces for 3- to 7-day-old mice. American Journal of Veterinary Research 47(10): 2272-2273.

APHA. 2004. Control of Communicable Diseases Manual, 18th Edition. American Public Health Association Heymann, D. (ed.) Washington, DC.

Appelbee, A.J., Thompson, R.C.A., Olson, M.E. 2005. *Giardia* and *Cryptosporidium* in mammalian wildlife - current status and future needs. Trends in Parasitology 21(8): 370-376.

Arrowood, M. 1997. Diagnosis. In: *Cryptosporidium* and Cryptosporidiosis; Fayer, R. (ed). CRC Press: New York, NY. Chapter 2.

Atwill, E.R., Sweitzer, R.A., Pereira, M.G., Gardner, I.A., Vuren, D V., Boyce, W.M. 1997. Prevalence of and associated risk factors for shedding *Cryptosporidium parvum* oocysts and *Giardia* cysts within feral pig populations in California. Applied and Environmental Microbiology 63(10): 3946-3949.

Atwill, E.R., Pereira, M.D.G.C., Alonso, L.H., Elmi, C., Epperson, W.B., Smith, R., Riggs, W., Carpenter, L.V., Dargatz, D.A., Hoar, B. 2006. Environmental load of *Cryptosporidium parvum* oocysts from cattle manure in feedlots from the central and western United States. Journal of Environmental Quality 35(1): 200-206.

Avery, L.M., Killham, K., Jones, D.L. 2005. Survival of *E. coli* O157:H7 in organic wastes destined for land application. Journal of Applied Microbiology 98(4): 814-822.

Axelsson-Olsson, D., Waldenstrom, J., Broman, T., Olsen, B., Holmberg, M. 2005. Protozoan *Acanthamoeba polyphaga* as a potential reservoir for *Campylobacter jejuni*. Applied and Environmental Microbiology 71(2): 1987-1992.

Baker, K., Degnan, A. 2006. *Helicobacter pylori*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 12.

Baldwin, A., Kingman, H., Darville, M., Foot, A.B., Grier, D., Cornish, J.M., Goulden, N., Oakhill, A., Pamphilon, D.H., Steward, C.G., Marks, D.I. 2000. Outcome and clinical course of 100 patients with adenovirus infection following bone marrow transplantation. Bone Marrow Transplant 26(12): 1333-1338.

Berk, S.G., Gunderson, J.H., Newsome, A.L., Farone, A.L., Hayes, B.J., Redding, K.S., Uddin, N., Williams, E.L., Johnson, R.A., Farsian, M., Reid, A., Skimmyhorn, J., Farone, M.B. 2006. Occurrence of infected amoebae in cooling towers compared with natural aquatic environments: implications for emerging pathogens. Environmental Science and Technology 40(23): 7440-7444.

Blankspoor, H. 2006. *Schistosomatidae.* In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 35.

Boczek, L.A., Rice, E.W., Johnston, B., Johnson, J.R. 2007. Occurrence of antibiotic-resistant uropathogenic *Escherichia coli* clonal group A in wastewater effluents. Applied and Environmental Microbiology 73(13): 4180-4184.

Bolin, C., Brown, C., Rose, J. 2004a. Emerging zoonotic diseases and water. In: Waterborne Zoonoses: Identification, Causes and Control; Cotruvo, J.A, Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R., Gannon, V.P.J. (eds), World Health Organization (WHO). IWA Publishing: London, UK. Chapter 2.

Bolin, C., Brown, C., Rose, J. 2004b. Leptosporidiosis and other potential zoonoses in water. In: Waterborne Zoonoses: Identification, Causes and Control; Cotruvo, J.A, Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R., Gannon, V.P.J. (eds), World Health Organization (WHO). IWA Publishing: London, UK. Chapter 20.

Booher, S.L., Cornick, N.A., Moon, H.W. 2002. Persistence of *Escherichia coli* O157:H7 in experimentally infected swine. Veterinary Microbiology 89(1): 69-81.

Borella, P., Montagna, M.T., Romano-Spica, V., Stampi, S., Stancanelli, G., Triassi, M., Neglia, R., Marchesi, I., Fantuzzi, G., Tato, D., Napoli, C., Quaranta, G., Laurenti, P., Leoni, E., Luca, G.D., Ossi, C., Moro, M., D'Alcala, G.R. 2004. *Legionella* infection risk from domestic hot water. Emerging Infectious Diseases 10(3): 457-464.

Brookes, J.D., Antenucci, J., Hipsey, M., Burch, M.D., Ashbolt, N.J., Ferguson, C. 2004. Fate and transport of pathogens in lakes and reservoirs. Environment International 30(5): 741-759.

Brooks, J.T., Sowers, E.G., Wells, J.G., Greene, K.D., Griffin, P.M., Hoekstra, R.M. Strockbine, N.A. 2005. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2002. Journal of Infectious Diseases 192(8): 1422-1429.

Brotman, M., Giannella, R., Alm, P., Bauman, M., AR, A.B., Black, R. 1995. Consensus conference statement: *Escherichia coli* 0157:H7 infections-an emerging national health crisis, July 11-13, 1994. American Gastroenterological Association 108: 1923-1934.

Brown, P., McShane, L.M., Zanusso, G., Detwile, L. 2006. On the question of sporadic or atypical bovine spongiform encephalopathy and Creutzfeldt-Jakob disease. Emerging Infectious Diseases 12(12): 1816-1821.

Brubaker, R.R. 1991. Factors promoting acute and chronic diseases caused by yersiniae. Clinical Microbiology Reviews 4(3): 309-324.

Buswell, C.M., Herlihy, Y.M., Lawrence, L.M., McGuiggan, J.T., Marsh, P.D., Keevil, C.W., Leach, S.A. 1998. Extended survival and persistence of *Campylobacter* spp. in water and aquatic biofilms and their detection by immunofluorescent-antibody and -rRNA staining. Applied and Environmental Microbiology 64(2): 733-741.

Caccio, M. 2005. Molecular epidemiology of human cryptosporidiosis. Parasitologia 47: 185-192.

Cali, A. 2006. Microsporidia. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 3.

Carey, C.M., Lee, H., Trevors, J.T. 2004. Biology, persistence and detection of *Cryptosporidium parvum* and *Cryptosporidium hominis* oocyst. Water Research 38(4): 818-862.

Carr, R., Bartram, J. 2004. The control envelope and risk management. In: Waterborne Zoonoses: Identification, Causes and Control; Cotruvo, J.A, Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R., Gannon, V.P.J. (eds), World Health Organization (WHO). IWA Publishing: London, UK. Chapter 5.

Casemore, D., Wright, S., Coop, R. 1997. Cryptosporidiosis - human and animal epidemiology. In: *Cryptosporidium* and Cryptosporidiosis; L.R. Fayer (ed.). CRC Press: New York, New York. Pp. 65-92.

U.S. Centers of Disease Control and Prevention (CDC). 1993a. Update: multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers - western United States, 1992-1993. Mortality and Morbidity Weekly Report 42(14): 258-263.

CDC. 1993b. Surveillance for waterborne disease outbreaks - United States, 1991-1992. Morbidity and Mortality Weekly Report 42 (SS-5): 1-22.

CDC. 1996. Surveillance for waterborne-disease outbreaks - United States, 1993-1994. Morbidity and Mortality Weekly Report 45 (SS-1): 1-33.

CDC 1998. Surveillance for waterborne-disease outbreaks - United States, 1995-1996. Morbidity and Mortality Weekly Report 47(SS-5): 1-33.

CDC. 2000. Surveillance for waterborne-disease outbreaks - United States, 1997-1998. Morbidity and Mortality Weekly Report 49(SS-4): 1-21.

CDC. 2002. Surveillance for waterborne-disease outbreaks - United States, 1999-2000. Morbidity and Mortality Weekly Report 51(SS-8): 1-47.

CDC. 2004. Surveillance for waterborne-disease outbreaks associated with recreational water - United States, 2001-2002, and surveillance for waterborne-disease outbreaks associated with drinking water: United States, 2001-2002. Morbidity and Mortality Weekly Report 53 (SS-8).

CDC. 2005. Cryptosporidiosis Surveillance - United States 1999-2002, and Giardiasis Surveillance - United States, 1998-2002. Morbidity and Mortality Weekly Report 54(SS-1).

CDC. 2007a. Summary of notifiable diseases - United States, 2005. Morbidity and Mortality Weekly Report 54(53).

CDC. 2007b. Cryptosporidiosis outbreaks associated with recreational water use - five states, 2006. Morbidity and Mortality Weekly Report (56)29: 729-732.

Chakrabarti, S., Mautner, V., Osman, H., Collingham, K.E., Fegan, C.D., Klapper, P.E., Moss, P.A.H., Milligan, D.W. 2002. Adenovirus infections following allogeneic stem cell transplantation: incidence and outcome in relation to graft manipulation, immunosuppression, and immune recovery. Blood 100(5): 1619-1627.

Chalker, R.B., Blaser, M.J. 1988. A review of human salmonellosis: III. Magnitude of *Salmonella* infection in the United States. Reviews of Infectious Diseases 10(1): 111-124.

Chalmers, R.M., Salmon, R.L., Willshaw, G.A., Cheasty, T., Looker, N., Davies, I., Wray, C. 1997. Vero-cytotoxin-producing *Escherichia coli* O157 in a farmer handling horses. Lancet 349(9068): 1816.

Chan, P.K.S. 2002. Outbreak of avian influenza A(H5N1) virus infection in Hong Kong in 1997. Clinical Infectious Diseases 34(Suppl 2): S58-S64.

Chapman, P.A., Siddons, C.A., Malo, A.T.G., Harkin, M.A. 1997. A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. Epidemiology and Infection 119(2): 245-250.

Chappell, C.L., Okhuysen, P.C., Sterling, C.R., Wang, C., Jakubowski, W., Dupont, H.L. 1999. Infectivity of *Cryptosporidium parvum* in healthy adults with pre-existing anti-C. parvum serum immunoglobulin G. American Journal of Tropical Medicine and Hygiene 60(1): 157-164.

Chappell, C.L., Okhuysen, P.C., Langer-Curry, R., Widmer, G., Akiyoshi, D.E., Tanriverdi, S., Tzipori, S. 2006. *Cryptosporidium hominis*: experimental challenge of healthy adults. American Journal of Tropical Medicine and Hygiene 75(5): 851-857.

Chart, H., Sussman, M., Stewart-Tull, D. 2000. *E. coli* – friend or foe? Supplement to Journal of Applied Microbiology Society for Applied Microbiology, Symposium Series No. 29.

Cieslak, P.R., Noble, S.J., Maxson, D.J., Empey, L.C., Ravenholt, O., Legarza, G., Tuttle, J., Doyle, M.P., Barrett, T.J., Wells, J.G., McNamara, A.M., Griffin, P.M. 1997. Hamburger-associated *Escherichia coli* O157:H7 infection in Las Vegas: a hidden epidemic. American Journal of Public Health 87(2): 176-180.

Clifford, C.P., Crook, D.W., Conlon, C.P., Fraise, A.P., Day, D.G., Peto, T.E. 1990. Impact of waterborne outbreak of cryptosporidiosis on AIDS and renal transplant patients. Lancet 335(8703): 1455-1456.

Cliver, D., Fayer, R. 2004. Categories of waterborne disease organisms. In: Waterborne Zoonoses: Identification, Causes and Control; Cotruvo, J.A, Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R., Gannon, V.P.J. (eds), World Health Organization (WHO). IWA Publishing, London, UK. Section V.

Cohen, M.L., Tauxe, R.V. 1986. Drug-resistant *Salmonella* in the United States: an epidemiologic perspective. Science 234(4779): 964-969.

Corsi, A., Nucci, C., Knafelz, D., Bulgarini, D., Iorio, L.D., Polito, A., Risi, F.D., Morini, F.A., Paone, F.M. 1998. Ocular changes associated with *Giardia lamblia* infection in children. British Journal of Ophthalmology 82(1): 59-62.

Couroucli, X.I., Welty, S.E., Ramsay, P.L., Wearden, M.E., Fuentes-Garcia, F.J., Ni, J., Jacobs, T.N., Towbin, J.A., Bowles, N.E. 2000. Detection of microorganisms in the tracheal aspirates of preterm infants by polymerase chain reaction: association of adenovirus infection with bronchopulmonary dysplasia. Pediatric Research 47(2): 225-232.

Covert, T., Meckes, M. 2006. *Salmonella*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 17.

Craun, G., Calderon, R., Craun, M. 2004a. Waterborne outbreaks caused by zoonotic pathogens in the USA. In: Waterborne Zoonoses: Identification, Causes and Control; Cotruvo, J.A, Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R., Gannon, V.P.J. (eds), World Health Organization (WHO*)*. IWA Publishing: London, UK. Chapter 8.

Craun, G., Till, D., McBride, G. 2004b. Epidemiological studies and surveillance. In: Waterborne Zoonoses: Identification, Causes and Control; Cotruvo, J.A, Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R., Gannon, V.P.J. (eds), World Health Organization (WHO). IWA Publishing, London, UK. Chapter 10.

Craun, G.F., Calderon, R.L., Craun, M.F. 2005. Outbreaks associated with recreational water in the United States. International Journal of Environmental Health Research 15(4): 243-262.

Craun, M.F., Craun, G.F., Calderon, R.L., Beach, M.J. 2006. Waterborne outbreaks reported in the United States. Journal of Water and Health 4(Suppl 2): 19-30.

Crawford-Miksza, L.K., Schnurr, D.P. 1996. Adenovirus serotype evolution is driven by illegitimate recombination in the hypervariable regions of the hexon protein. Virology 224(2): 357-367.

Cross, J., Sherchand, J. 2004. *Cyclosporiasis*. In: Waterborne Zoonoses: Identification, Causes and Control; Cotruvo, J.A, Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R., Gannon, V.P.J. (eds), World Health Organization (WHO). IWA Publishing: London, UK. Chapter 17.

Current, W.L., Garcia, L.S. 1991. Cryptosporidiosis. Clinical Microbiology Reviews 4(3): 325-358.

Czajkowska, D., Witkowska-Gwiazdowska, A., Sikorska, I., Boszczyk-Maleszakl, H., Horoch, M. 2005. Survival of *Escherichia coli* serotype 0157:H7 in water and in bottom-shore sediments. Polish Journal of Environmental Studies 14(4): 423-430.

Degremont, A., Sturchler, D., Wolfensberger, E., Osterwalder, B. 1981. Etude clinique et therapeutique d'un collectif de 217 patients atteints de giardiase et d'amibiase intestinales. Schweiz. Med. Wschr. 111: 2039-2046 [article in French, as cited in ICAIR 1984, Final draft for drinking water criteria document on *Giardia* prepared for Criteria and Standards Division, Office of Drinking Water, USEPA].

Degnan, A., Standridge, J. 2006. Enterohemorrhagic *E coli*. Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 9.

Dieckmann, U. 2002. Adaptive dynamics of pathogen-host interactions. In: Adaptive Dynamics of Infectious Diseases: In Pursuit of Virulence Management; Dieckmann, U., Metz, J.A.M., Sabelis, M.W., Sigmund, K. (eds), Cambridge University Press: Cambridge, UK. Chapter 4, pp. 39-59.

Domingo, E., Baranowski, E., Ruiz-Jarabo, C.M., Martin-Hernndez, A.M., Siz, J.C., Escarmis, C. 1998. Quasispecies structure and persistence of RNA viruses. Emerging Infectious *Diseases* 4(4): 521-527.

Donnison, A.M., Ross, C.M. 1999. Animal and human faecal pollution in New Zealand rivers. New Zealand Journal of Marine and Freshwater Research 33: 119-128.

Dubey, J. 2006. *Toxoplasma gondii*. In Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 36.

Dubey, J.P. 2004. Toxoplasmosis - a waterborne zoonosis. Veterinary Parasitology 126(1-2): 57-72.

Dufour, A.P., Evans, O., Behymer, T.D., Cantu, R. 2006. Water ingestion during swimming activities in a pool: a pilot study. Journal of Water and Health 4(4): 425-430.

DuPont, H.L., Chappell, C.L., Sterling, C.R., Okhuysen, P.C., Rose, J.B., Jakubowski, W. 1995. The infectivity of *Cryptosporidium parvum* in healthy volunteers. New England Journal of Medicine 332(13): 855-859.

DuPont, H.L., Formal, S.B., Hornick, R.B., Snyder, M.J., Libonati, J.P., Sheahan, D.G., LaBrec, E.H., Kalas, J.P. 1971. Pathogenesis of *Escherichia coli* diarrhea. New England Journal of Medicine 285(1): 1-9.

Dykes, A.C., Juranek, D.D., Lorenz, R.A., Sinclair, S., Jakubowski, W., Davies, R. 1980. Municipal waterborne giardiasis: an epidemiologic investigation, beavers implicated as a possible reservoir. Annals of Internal Medicine 92: 165-170.

Ebert, D. 1998. Experimental evolution of parasites. Science 282(5393): 1432-1435.

Eisenberg, J.N.S., Lei, X., Hubbard, A.H., Brookhart, M.A., Colford, J.M. 2005. The role of disease transmission and conferred immunity in outbreaks: analysis of the 1993 *Cryptosporidium* outbreak in Milwaukee, Wisconsin. American Journal of Epidemiology 161(1): 62-72.

El Mansoury, S.T.E., Naga, I.F.A.E., Negm, A.Y., Amer, E.E. 2004. Influence of temperature and salinity on the viability and infectivity of *Giardia lamblia* and *Cryptosporidia parvum*. Journal of the Egyptian Society of Parasitology 34(1): 161-172.

Endo, T., Morishima, Y. 2004. Major helminth zoonoses in water. Chapter 18 in waterborne zoonoses: identification, causes and control; Cotruvo, J.A, Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R., Gannon, V.P.J. (eds), World Health Organization (WHO). IWA Publishing: London, UK. Chapter 18.

Enriquez, C., Thurston-Enriquez, J. 2006. Adenoviruses. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 38.

Evans, O., Wymer, L., Behymer, T., Dufour, A. 2006. An observational study: determination of the volume of water ingested during recreational swimming activities. U.S. Environmental Protection Agency, Cincinnati, OH. Poster presentation at the National Beaches Conference, Niagara Falls, New York. October.

Falker, S., Schmidt, M.A., Heusipp, G. 2005. DNA methylation in *Yersinia enterocolitica*: role of the DNA adenine methyltransferase in mismatch repair and regulation of virulence factors. Microbiology 151(Pt 7): 2291-2299.

Farthing, M.J. 2000. Clinical aspects of human cryptosporidiosis. In: Cryptosporidiosis and Microsporidiosis; Petry, F. (ed). S. Karger AG: New York, NY. Pp. 50-74.

Farthing, M.J. 1996. Giardiasis. Gastroenterology Clinics of North America 25(3): 493-515.

Faubert, G. 2000. Immune response to *Giardia duodenalis*. Clinical Microbiology Reviews 13(1): 35-54.

Fayer, R., Xiau, L.X. (eds). 2007. *Cryptosporidium* and Cryptosporidiosis, Second Edition. CRC Press: Boca Raton, FL.

Fayer, R. 2004a. *Cryptosporidium*: a water-borne zoonotic parasite. Veterinary Parasitology 126(1-2): 37-56.

Fayer, R. 2004b. Waterborne zoonotic protozoa. In: Waterborne Zoonoses: Identification, Causes and Control; Cotruvo, J.A, Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R., Gannon, V.P.J. (eds), World Health Organization (WHO). IWA Publishing: London, UK. Chapter 16.

Fayer, R. 1994. Effect of high temperature on infectivity of *Cryptosporidium parvum* oocysts in water. Applied and Environmental Microbiology 60(8): 2732-2735.

Fayer, R., Morgan, U., Upton, S.J. 2000. Epidemiology of *Cryptosporidium*: transmission, detection and identification. International Journal for Parasitology 30(12-13): 1305-1322.

Fayer, R., Nerad, T. 1996. Effects of low temperatures on viability of *Cryptosporidium parvum* oocysts. Applied and Environmental Microbiology 62(4): 1431-1433.

Fayer, R., Nerad, T., Rall, W., Lindsay, D.S., Blagburn, B.L. 1991. Studies on cryopreservation of *Cryptosporidium parvum*. International Journal for Parasitology 77(3): 357-361.

Fayer, R., Speer, C., Dubey, J. 1997. The general biology of *Cryptosporidium*. In: *Cryptosporidium* and Cryptosporidiosis, Fayer, R. (ed). CRC Press: New York, New York.

Fayer, R., Trout, J.M., Jenkins, M.C. 1998. Infectivity of *Cryptosporidium parvum* oocysts stored in water at environmental temperatures. International Journal for Parasitology 84(6): 1165-1169.

Fayer, R. 2003. *Cryptosporidium*: from molecules to disease. In: *Cryptosporidium*: From Molecules to Disease, Thompson R.C.A., Armson A., Ryan U.M. (eds). Elsevier: Amsterdam, The Netherlands; pp. 11-18.

Fayer, R., Ungar, B.L. 1986. *Cryptosporidium* spp. and cryptosporidiosis. Microbiological Reviews 50(4): 458-483.

FDA. 2001. Draft risk assessment on the public health impact of *Vibrio parahaemolyticus* in raw molluscan shellfish. Technical Report. Center for Food Safety and Applied Nutrition, Food and Drug Administration, U.S. Department of Health and Human Services.

Feder, I., Wallace, F.M., Gray, J.T., Fratamico, P., Fedorka-Cray, P.J., Pearce, R.A., Call, J.E., Perrine, R., Luchansky, J.B. 2003. Isolation of *Escherichia coli* O157:H7 from intact colon fecal samples of swine. Emerging Infectious Diseases 9(3): 380-383.

Feltus, D.C., Giddings, C.W., Schneck, B.L., Monson, T., Warshauer, D., McEvoy, J.M. 2006. Evidence supporting zoonotic transmission of *Cryptosporidium* spp. in Wisconsin. Journal of Clinical Microbiology 44(12): 4303-4308.

Fredricksen, D., Geldreich, E., Karner, D.A. 2006. Cyanobacteria. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 8.

Fricker, C. 2006b. *Yersinia*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 22.

Fricker, C. 2006a. *Camplylobacter*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 7,

Frisby, H.R., Addiss, D.G., Reiser, W.J., Hancock, B., Vergeront, J.M., Hoxie, N.J., Davis, J.P. 1997. Clinical and epidemiologic features of a massive waterborne outbreak of cryptosporidiosis in persons with HIV infection. Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology 16(5): 367-373.

Frost, F., Craun, G. 1998. The importance of acquired immunity in the epidemiology of cryptosporidiosis and giardiasis. OECD (Organization for Economic Cooperation and Development) Workshop: Molecular Technologies for Safe Drinking Water. Interlaken, Switzerland, July 5-8.

Gagliardi, J.V., Karns, J.S. 2002. Persistence of *Escherichia coli* O157:H7 in soil and on plant roots. Environmental Microbiology 4(2): 89-96.

Ganoza, C.A., Matthias, M.A., Collins-Richards, D., Brouwer, K.C., Cunningham, C.B., Segura, E.R., Gilman, R.H., Gotuzzo, E., Vinetz, J.M. 2006. Determining risk for severe leptospirosis by molecular analysis of environmental surface waters for pathogenic *Leptospira*. Public Library of Science Medicine 3(8): 1329-1340.

Garcia, L. 2006c. *Isosprora belli*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 32.

Garcia, L. 2006b. *Blastocystis hominis*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 27.

Garcia, L. 2006a. *Balantidium coli*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 26.

Garg, A.X., Marshall, J., Salvadori, M., Thiessen-Philbrook, H.R., Macnab, J., Suri, R. S., Haynes, R.B., Pope, J., Clark, W., Investigators, W.H.S. 2006. A gradient of acute gastroenteritis was characterized, to assess risk of long-term health sequelae after drinking bacterial-contaminated water. Journal of Clinical Epidemiology 59(4): 421-428.

Garg, A.X., Moist, L., Matsell, D., Thiessen-Philbrook, H.R., Haynes, R.B., Suri, R.S., Salvadori, M., Ray, J., Clark, W.F. 2005. Risk of hypertension and reduced kidney function after acute gastroenteritis from bacteria-contaminated drinking water. Canadian medical Association Journal 173(3): 261-268.

Geldreich, E. 1996. Creating microbial quality in drinking water. In: Microbial Quality of Water Supply in Distribution Systems. Lewis Publishers. Chapter 2.

Geldreich, E., Degnan, A. 2006b. *Pseudomonas*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 16.

Geldreich, E., Degnan, A. 2006a. *Flavobacterium*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 11.

Geldreich, E., Standridge, J. 2006a. *Klebsiella*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 13.

Geldreich, E., Standridge, J. 2006b. *Serratia*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 18.

Geldreich, E., Standridge, J. 2006c. *Staphylococcus*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 20.

Gerba, C. 2006a. Enteroviruses and parechoviruses. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 41.

Gerba, C. 2006b. Hepatitis E virus. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 43.

Gillespie, I.A., O'Brien, S.J., Frost, J.A., Adak, G.K., Horby, P., Swan, A.V., Painter, M.J., Neal, K.R., *Campylobacter* Sentinel Surveillance Scheme Collaborators. 2002. A case-case comparison of *Campylobacter coli* and *Campylobacter jejuni* infection: a tool for generating hypotheses. Emerging Infectious Diseases 8(9): 937-942.

Glass, R. I. 2006. New hope for defeating rotavirus. Scientific American 294(4): 46-51, 54-5.

Graczyk, T.K., Kacprzak, M., Neczaj, E., Tamang, L., Graczyk, H., Lucy, F E., Girouard, A.S. 2007. Occurrence of *Cryptosporidium* and *Giardia* in sewage sludge and solid waste landfill leachate and quantitative comparative analysis of sanitization treatments on pathogen inactivation. Environmental Research 106(1): 27-33.

Grandadam, M., Tebbal, S., Caron, M., Siriwardana, M., Larouze, B., Koeck, J.L., Buisson, Y., Enouf, V., Nicand, E. 2004. Evidence for hepatitis E virus quasispecies. Journal of General Virology 85(Pt 11): 3189-3194.

Gray, G.C., Setterquist, S F., Jirsa, S.J., DesJardin, L.E., Erdman, D.D. 2005. Emergent strain of human adenovirus endemic in Iowa. Emerging Infectious Diseases 11(1): 127-128.

Griffin, D.W., Donaldson, K.A., Paul, J.H., Rose, J.B. 2003. Pathogenic human viruses in coastal waters. Clinical Microbiology Reviews 16(1): 129-143.

Griffin, P.M., Tauxe, R.V. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiologic Reviews 13: 60-98.

Guan, T.Y., Holley, R.A. 2003. Pathogen survival in swine manure environments and transmission of human enteric illness - a review. Journal of Environmental Quality 32(2): 383-392.

Haas, C.N., Thayyar-Madabusi, A., Rose, J.B., Gerba, C.P. 2000. Development of a dose-response relationship for *Escherichia coli* O157:H7. International Journal of Food Microbiology 56(2-3): 153-159.

Hall, N. 2006. *Legionella*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 14.

Hammermueller, J., Kruth, S., Prescott, J., Gyles, C. 1995. Detection of toxin genes in *Escherichia coli* isolated from normal dogs and dogs with diarrhea. Canadian Journal of Veterinary Research 59(4): 265-270.

Hammond, B.G., Barbee, S.J., Inoue, T., Ishida, N., Levinskas, G J., Stevens, M.W., Wheeler, A.G., Cascieri, T. 1986. A review of toxicology studies on cyanurate and its chlorinated derivatives. Environmental Health Perspectives 69: 287-292.

Hamnes, I.S., Gjerde, B.K., Forberg, T., Robertson, L.J. 2007. Occurrence of *Giardia* and *Cryptosporidium* in Norwegian red foxes (*Vulpes vulpes*). Veterinary Parasitology 143(3-4): 347-353.

Harcourt, B.H., Lowe, L., Tamin, A., Liu, X., Bankamp, B., Bowden, N., Rollin, P.E., Comer, J.A., Ksiazek, T.G., Hossain, M.J., Gurley, E.S., Breiman, R.F., Bellini, W.J., Rota, P.A. 2005. Genetic characterization of Nipah virus, Bangladesh, 2004. Emerging Infectious Diseases 11(10): 1594-1597.

Health Canada. 2006. Guidelines for Canadian Drinking Water Quality: Guideline Technical Document - Bacterial Waterborne Pathogens - Current and Emerging Organisms of Concern. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada: Ottawa, Ontario.

Heerden, J., Ehlers, M.M., Vivier, J.C., Grabow, W.O.K. 2005. Risk assessment of adenoviruses detected in treated drinking water and recreational water. Journal of Applied Microbiology 99(4): 926-933.

Hellard, M.E., Sinclair, M.I., Hogg, G.G., Fairley, C.K. 2000. Prevalence of enteric pathogens among community based asymptomatic individuals. Journal of Gastroenterology and Hepatology 15(3): 290-293.

Hogg, J.C. 2001. Role of latent viral infections in chronic obstructive pulmonary disease and asthma. American Journal of Respiratory and Critical Care Medicine 164(10 Pt 2): S71-S75.

Hopkins, R.S., Gaspard, G.B., Williams, F.P., Karlin, R.J., Cukor, G., Blacklow, N.R. 1984. A community waterborne gastroenteritis outbreak: evidence for rotavirus as the agent. American Journal of Public Health 74(3): 263-265.

Hopkins, R.S.,Juranek, D.D. 1991. Acute giardiasis: an improved clinical case definition for epidemiologic studies. American Journal of Epidemiology 133(4): 402-407.

Hrudey, S., Huck, P., Payment, P., Gillham, R., Hrudey, E. 2002. Walkerton: lessons learned in comparison with waterborne outbreaks in the developed world. Journal of Environmental Engineering and Science 1, 397-470.

Hrudey, S.E., Payment, P., Huck, P.M., Gillham, R.W., Hrudey, E.J. 2003. A fatal waterborne disease epidemic in Walkerton, Ontario: comparison with other waterborne outbreaks in the developed world. Water Science and Technology 47(3) 7-14.

Hunter, P.R. 2003. Drinking water and diarrhoeal disease due to *Escherichia coli*. Journal of Water and Health 1(2) 65-72.

Hunter, P.R., Nichols, G. 2002. Epidemiology and clinical features of *Cryptosporidium* infection in immunocompromised patients. Clinical Microbiology Reviews 15(1): 145-154.

Hunter, P.R., Thompson, R.C.A. 2005. The zoonotic transmission of *Giardia* and *Cryptosporidium*. International Journal for Parasitology 35(11-12): 1181-1190.

Hunter, P.R., Hughes, S., Woodhouse, S., Raj, N., Syed, Q., Chalmers, R.M., Verlander, N.Q., Goodacre, J. 2004. Health sequelae of human cryptosporidiosis in immunocompetent patients. Clinical Infectious Diseases 39(4): 504-510.

Inpankaew, T., Traub, R., Thompson, R.C.A., Sukthana, Y. 2007. Canine parasitic zoonoses in Bangkok temples. Southeast Asian Journal of Tropical Medicine and Public Health 38(2): 247-255.

Jenkins M.B., Bowman, D.D., Fogarty, E.A., Ghiorse, W.C. 2002. *Cryptosporidium parvum* oocysts inactivation in three soil types at various temperatures and water potentials. Soil Biology and Biochemistry 34: 1101-1109.

Jenkins, M., Higgins, J., Abrahante, J., Kniel, K., O'Brien, C., Trout, J., Lancto, C., Abrahamsen, M., Fayer, R. 2007. Fecundity of *Cryptosporidium parvum* is correlated with intracellular levels of the viral symbiont CPV. International Journal for Parasitology 11: 1-5.

Johnson, D., Enriquez, C., Pepper, I., Davis, T., Gerba, C.P., Rose, J. 1997. Survival of *Giardia*, *Cryptosporidium*, poliovirus and *Salmonella* in marine waters. Water Science and Technology 35(11/12): 261-268.

Jones, K. 2001. Campylobacters in water, sewage and the environment. Proceedings of the Society for Applied Microbiology Symposium (30): 68S-79S.

Kanarat, S. 2004. Symptoms, treatments, and health consequences of waterborne zoonotic diseases. In: Waterborne Zoonoses: Identification, Causes and Control; Cotruvo, J.A, Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R., Gannon, V.P.J. (eds), World Health Organization (WHO). IWA Publishing: London, UK. Chapter 9.

Kappus, K.D., Lundgren, R.G., Juranek, D.D., Roberts, J.M., Spencer, H.C. 1994. Intestinal parasitism in the United States: update on a continuing problem. American Journal of Tropical Medicine and Hygiene 50(6): 705-713.

Katz, A.R., Ansdell, V.E., Effler, P.V., Middleton, C. & Sasaki, D.M. 2001. Assessment of the clinical presentation and treatment of 353 cases of laboratory-confirmed leptospirosis in Hawaii, 1974–1998. Clinical Infectious Diseases 33: 1834-1841.

Kean, B.H., William, D.C., Luminais, S.K. 1979. Epidemic of amoebiasis and giardiasis in a biased population. Journal of Digestive Diseases 55(5): 375-378.

Keene, W. 2006. *Entamoeba histolytica*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 30.

Keene, W.E., McAnulty, J.M., Hoesly, F.C., Williams, L.P., Hedberg, K., Oxman, G.L., Barrett, T.J., Pfaller, M.A., Fleming, D.W. 1994. A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. New England Journal of Medicine 331(9): 579-584.

Keene, W.E., Sazie, E., Kok, J., Rice, D.H., Hancock, D.D., Balan, V.K., Zhao, T., Doyle, M.P. 1997. An outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. Journal of the American Medical Association 277(15): 1229-1231.

King, B.J., Keegan, A.R., Monis, P.T., Saint, C.P. 2005. Environmental temperature controls *Cryptosporidium* oocyst metabolic rate and associated retention of infectivity. Applied and Environmental Microbiology 71(7): 3848-3857.

Kudva, I.T., Hatfield, P.G., Hovde, C.J. 1996. *Escherichia coli* O157:H7 in microbial flora of sheep. Journal of Clinical Microbiology 34(2): 431-433.

LeChevallier, M. 2006. *Mycobacterium avium* complex. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 15.

LeChevallier, M.W., Giovanni, G.D.D., Clancy, J.L., Bukhari, Z., Bukhari, S., Rosen, J. S., Sobrinho, J., Frey, M.M. 2003. Comparison of method 1623 and cell culture-PCR for detection of *Cryptosporidium spp.* in source waters. Applied and Environmental Microbiology 69(2): 971-979.

Lederberg, J. 1998. Emerging infections: an evolutionary perspective. Emerging Infectious Diseases 4(3): 366-371.

Lengerich, E.J., Addiss, D.G., Juranek, D.D. 1994. Severe giardiasis in the United States. Clinical Infectious Diseases 18(5): 760-763.

Levett, P.N. 2001. Leptospirosis. Clinical Microbiology Reviews 14(2): 296-326.

Li, X., Atwill, E.R., Dunbar, L.A., Jones, T., Hook, J., Tate, K.W. 2005. Seasonal temperature fluctuations induces rapid inactivation of *Cryptosporidium parvum*. Environmental Science and Technology 39(12): 4484-4489.

Lightfoot, D. 2004. *Salmonella* and other enteric organisms. In: waterborne Zoonoses: Identification, Causes and Control; Cotruvo, J.A, Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R., Gannon, V.P.J. (eds), World Health Organization (WHO). IWA Publishing: London, UK. Chapter 14.

Low, D.A., Weyand, N.J., Mahan, M.J. 2001. Roles of DNA adenine methylation in regulating bacterial gene expression and virulence. Infection and Immunity 69(12): 7197-7204.

Mackenzie, J.S. 1999. Emerging viral diseases: an Australian perspective. Emerging Infectious Diseases 5(1): 1-8.

MacKenzie, W.R., Hoxie, N.J., Proctor, M.E., Gradus, M.S., Blair, K.A., Peterson, D.E., Kazmierczak, J.J., Addiss, D.G., Fox, K.R., Rose, J.B. 1994. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. New England Journal of Medicine 331(3): 161-167.

MacKenzie, W.R., Schell, W.L., Blair, K.A., Addiss, D.G., Peterson, D.E., Hoxie, N.J., Kazmierczak, J.J., Davis, J.P. 1995. Massive outbreak of waterborne *Cryptosporidium* infection in Milwaukee, Wisconsin: recurrence of illness and risk of secondary transmission. Clinical Infectious Diseases 21(1): 57-62.

Marrie, T.J., Raoult, D., Scola, B.L., Birtles, R.J., de Carolis, E., & The Canadian Community-Acquired Pneumonia Study Canadian Community-Acquired Pneumonia Study Group 2001. *Legionella*-like and other amoebal pathogens as agents of community-acquired pneumonia. Emerging Infectious Diseases 7(6): 1026-1029.

McBride, G. 1993. Faecal indicator density and illness risk to swimmers in coastal waters: a preliminary study for New Zealand. In: Proceedings of the Annual Conference of the New Zealand Water and Waste Association, Havelock North, 1-3 September 1993.

McBride, G., Till, D., Ryan, D.T., Ball, A., Lewis, D.G., Palmer, D.S., Weinstein, P. 2002. Freshwater microbiology research programme report, pathogen occurrence and human health risk assessment analysis. Technical Report. Ministry for the Environment/Ministry of Health. New Zealand.

McCarthy, T.A., Barrett, N.L., Hadler, J.L., Salsbury, B., Howard, R.T., Dingman, D. W., Brinkman, C.D., Bibb, W.F., Cartter, M.L. 2001. Hemolytic-uremic syndrome and *Escherichia coli* O121 at a Lake in Connecticut, 1999. Pediatrics 108(4): E59.

McCuin, R.M., Clancy, J.L. 2006. Occurrence of Cryptosporidium oocysts in US wastewaters. Journal of Water and Health 4(4): 437-452.

McGee, P., Bolton, D.J., Sheridan, J.J., Earley, B., Kelly, G., Leonard, N. 2002. Survival of *Escherichia coli* O157:H7 in farm water: its role as a vector in the transmission of the organism within herds. Journal of Applied Microbiology 93(4): 706-713.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., Tauxe, R.V. 1999. Food-related illness and death in the United States. Emerging Infectious Diseases 5(5): 607-625.

Meites, E., Jay, M.T., Deresinski, S., Shieh, W., Zaki, S.R., Tompkins, L., Smith, D.S. 2004. Reemerging leptospirosis, California. Emerging Infectious Diseases 10(3): 406-412.

Mendez-Hermida, F., Castro-Hermida, J.A., Ares-Mazas, E., Kehoe, S.C., McGuigan, K.G. 2005. Effect of batch-process solar disinfection on survival of *Cryptosporidium parvum* oocysts in drinking water. Applied and Environmental Microbiology 71(3): 1653-1654.

Michel, P., Wilson, J.B., Martin, S.W., Clarke, R.C., McEwen, S.A., Gyles, C.L. 1999. Temporal and geographical distributions of reported cases of *Escherichia coli* O157:H7 infection in Ontario. Epidemiology and Infection 122(2): 193-200.

Miller, R.L., Wang, A.L., Wang, C.C. 1988. Identification of *Giardia lamblia* isolates susceptible and resistant to infection by the double-stranded RNA virus. Experimental Parasitology 66(1): 118-123.

Mintz, E., Hudson-Wragg, M., Mshar, P., Cartter, M., Hadler, J. 1993. Foodborne giardiasis in a corporate office setting. Journal of Infectious Diseases 167: 250-253.

Moe, C. 2004. What are the criteria for determining whether a disease is zoonotic and water related? In: Waterborne Zoonoses: Identification, Causes and Control; Cotruvo, J.A, Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R., Gannon, V.P.J. (eds), World Health Organization (WHO). IWA Publishing: London, UK. Chapter 3.

Molbak, K., Scheutz, F. 2004. Verocytotoxin-producing *Escherichia coli* and other diarrhoeagenic *E. coli*. In: Waterborne Zoonoses: Identification, Causes and Control; Cotruvo, J.A, Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R., Gannon, V.P.J. (eds), World Health Organization (WHO). IWA Publishing: London, UK. Chapter 13.

Morgan-Ryan, U.M., Fall, A., Ward, L.A., Hijjawi, N., Sulaiman, I., Fayer, R., Thompson, R.C.A., Olson, M., Lal, A., Xiao, L. 2002. *Cryptosporidium hominis n. sp.* (Apicomplexa: Cryptosporidiidae) from *Homo sapiens*. Journal of Eukaryotic Microbiology 49(6): 433-440.

Morse, S.S. 1997. The public health threat of emerging viral disease. Journal of Nutrition 127(5 Suppl): 951S-957S.

Moyer, N., Degnan, A. 2006. *Shigella*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 19.

Moyer, N., Standridge, J. 2006. *Aeromonas*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 6.

Mwenda, J.M., Nyachieo, A., Langat, D.K., Steele, D.A. 2005. Serological detection of adenoviruses in non-human primates maintained in a colony in Kenya. East African Medical Journal 82(7): 371-375.

Nachamkin, I. 2002. Chronic effects of *Campylobacter* infection. Microbes and Infection 4(4): 399-403.

Nascimento, A.L.T.O., Ko, A.I., Martins, E.A.L., Monteiro-Vitorello, C.B., Ho, P.L., Haake, D.A., Verjovski-Almeida, S., Hartskeerl, R.A., Marques, M.V., Oliveira, M.C., Menck, C.F.M., Leite, L.C.C., Carrer, H., Coutinho, L.L., Degrave, W.M., Dellagostin, O.A., El-Dorry, H., Ferro, E.S., Ferro, M.I.T., Furlan, L.R., Gamberini, M., Giglioti, E.A., Goes-Neto, A., Goldman, G.H., Goldman, M.H.S., Harakava, R., Jeronimo, S.M.B., Junqueira-de-Azevedo, I.L.M., Kimura, E.T., Kuramae, E.E., Lemos, E.G.M., Lemos, M.V.F., Marino, C.L., Nunes, L.R., de Oliveira, R.C., Pereira, G.G., Reis, M.S., Schriefer, A., Siqueira, W.J., Sommer, P., Tsai, S.M., Simpson, A.J.G., Ferro, J.A., Camargo, L.E.A., Kitajima, J.P., Setubal, J.C., Sluys, M.A.V. 2004. Comparative genomics of two *Leptospira interrogans* serovars reveals novel insights into physiology and pathogenesis. Journal of Bacteriology 186(7): 2164-2172.

Nasser, A.M., Telser, L., Nitzan, Y. 2007. Effect of sunlight on the infectivity of *Cryptosporidium parvum* in seawater. Canadian Journal Microbiology 53: 1101-1105.

Nataro, J.P., Deng, Y., Cookson, S., Cravioto, A., Savarino, S.J., Guers, L.D., Levine, M.M.. Tacket, C.O. 1995. Heterogeneity of enteroaggregative *Escherichia coli* virulence demonstrated in volunteers. Journal of Infectious Diseases 171(2): 465-468.

Nataro, J.P., Kaper, J.B. 1998. Diarrheagenic *Escherichia coli*. Clinical Microbiology Reviews 11(1): 142-201.

Naumova, E.N., Christodouleas, J., Hunter, P.R., Syed, Q. 2005. Effect of precipitation on seasonal variability in cryptosporidiosis recorded by the North West England surveillance system in 1990-1999. Journal of Water and Health 3(2): 185-196.

National Research Council (NRC). 2004. Indicators for Waterborne Pathogens. National Academies Press: Washington, DC.

O'Donoghue, P. 1995. *Cryptosporidium* and cryptosporidiosis in man and animals. International Journal for Parasitology Research 25(2): 139-195.

Ohl, M.E., Miller, S.I. 2001. *Salmonella*: a model for bacterial pathogenesis. Annual Review of Medicine 52: 259-274.

Okhuysen, P.C., Chappell, C.L., Crabb, J.H., Sterling, C.R., DuPont, H.L. 1999. Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults. Journal of Infectious Diseases 180(4): 1275-1281.

Okhuysen, P.C., Rich, S.M., Chappell, C.L., Grimes, K.A., Widmer, G., Feng, X., Tzipori, S. 2002. Infectivity of a *Cryptosporidium parvum* isolate of cervine origin for healthy adults and interferon-gamma knockout mice. Journal of Infectious Diseases 185(9): 1320-1325.

Olsen, S.J., Miller, G., Breuer, T., Kennedy, M., Higgins, C., Walford, J., McKee, G., Fox, K., Bibb, W., Mead, P. 2002. A waterborne outbreak of *Escherichia coli* O157:H7 infections and hemolytic uremic syndrome: implications for rural water systems. Emerging Infectious Diseases 8(4): 370-375.

Olson, M., Ralston, B., O'Handley, R., Guselle, N., Appelbee, A. 2003. What is the clinical and zoonotic significance of cryptosporidiosis in domestic animals and wildlife? In: *Cryptosporidium*: From Molecules to Disease; Thompson, R., Armson, A., Ryan, U. (eds). Elsevier: Amsterdam, The Netherlands.

Olson, M.E., Goh, J., Phillips, M., Guselle, N., McAllister, T.A. 1999. *Giardia* Cyst and *Cryptosporidium* oocyst survival in water, soil, and cattle feces. Journal of Environmental Quality 28: 1991-1996.

Olson, M.E., O'Handley, R.M., Ralston, B.J., McAllister, T.A., Thompson, R.C.A. 2004. Update on *Cryptosporidium* and *Giardia* infections in cattle. Trends in Parasitology 20(4): 185-191.

Ortega, Y. 2006. *Cyclospora cayetanensis*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 29.

Ortega, Y.R., Adam, R.D. 1997. *Giardia*: overview and update. Clinical Infectious Diseases 25(3): 545-550.

Osewe, P., Addiss, D.G., Blair, K.A., Hightower, A., Kamb, M.L., Davis, J.P. 1996. Cryptosporidiosis in Wisconsin: a case-control study of post-outbreak transmission. Epidemiology and Infection 117(2): 297-304.

Ostroff, S.M., Kobayashi, J.M., Lewis, J.H. 1989. Infections with *Escherichia coli* O157:H7 in Washington State. The first year of statewide disease surveillance. Journal of the American Medical Association 262(3): 355-359.

Palmer, S., Biffin, A. & Group 1990. Cryptosporidiosis in England and Wales: prevalence and clinical and epidemiological features. British Medical Journal 30: 774-777.

Pardo, J., Carranza, C., Muro, A., Angel-Moreno, A., Martin, A., Martin, T., Hernandez-Cabrera, M., Perez-Arellano, J. 2006. Helminth-related Eosinophilia in African immigrants, Gran Canaria. Emerging Infectious Diseases 12(10): 1587-1589.

Parker, J., Smith, H.V. 1993. Destruction of oocysts of *Cryptosporidium parvum*. Water Research 27(4): 729-731.

Pesaro, F., Sorg, I., Metzler, A. 1995. In situ inactivation of animal viruses and a coliphage in nonaerated liquid and semiliquid animal wastes. Applied and Environmental Microbiology 61(1): 92-97.

Pokorny, N.J., Weir, S.C., Carreno, R.A., Trevors, J.T., Lee, H. 2002. Influence of temperature on *Cryptosporidium parvum* oocyst infectivity in river water samples as detected by tissue culture assay. International Journal for Parasitology 88(3): 641-643.

Poly, F., Guerry, P. 2008. Pathogenesis of *Campylobacter*. Current Opinion in Gastroenterology 24(1): 27-31.

Pond, K. 2005a. *E. coli* O157. In: Water Recreation and Disease: Plausibility of Associated Infections: Acute Effects, Sequelae and Mortality. World Health Organization (WHO). IWA Publishing: London, UK. Chapter 4.

Pond, K. 2005b. *Leptospira*. In: Water Recreation and Disease: Plausibility of Associated Infections: Acute Effects, Sequelae and Mortality. World Health Organization (WHO). IWA Publishing: London, UK. Chapter 4.

Rangel, J.M., Sparling, P.H., Crowe, C., Griffin, P.M., Swerdlow, D.L. 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. Emerging Infectious Diseases 11(4): 603-609.

Rendtorff, R.C. 1954. The experimental transmission of human intestinal protozoan parasites. II. *Giardia lamblia* cysts given in capsules. American Journal of Tropical Medicine and Hygiene 59(2): 209-220.

Reynolds, K.A. 2006. Identifying hazards of waterborne disease. Water Conditioning and Purification.

Rice, D.H., Hancock, D.D., Besser, T.E. 1995. Verotoxigenic *E. coli* O157 colonisation of wild deer and range cattle. The Veterinary Record, 524.

Roberts, J.D., Silbergeld, E.K., Graczyk, T. 2007. A probabilistic risk assessment of *Cryptosporidium* exposure among Baltimore urban anglers. Journal of Toxicology and Environmental Health Part A: Current Issues 70(18): 1568-1576.

Roberts, W.G., Green, P.H., Ma, J., Carr, M., Ginsberg, A.M. 1989. Prevalence of cryptosporidiosis in patients undergoing endoscopy: evidence for an asymptomatic carrier state. American Journal of Medical Sciences 87(5): 537-539.

Robertson, B., Sinclair, M.I., Forbes, A.B., Veitch, M., Kirk, M., Cunliffe, D., Willis, J., Fairley, C.K. 2002. Case-control studies of sporadic cryptosporidiosis in Melbourne and Adelaide, Australia. Epidemiology and Infection 128(3): 419-431.

Robertson, L.J. 1996. Severe giardiasis and cryptosporidiosis in Scotland, UK. Epidemiology and Infection 117(3): 551-561.

Robertson, L.J., Campbell, A.T., Smith, H.V. 1992. Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. Applied and Environmental Microbiology 58(11): 3494-3500.

Robertson, L.J., Gjerde, B.K. 2006. Fate of *Cryptosporidium* oocysts and *Giardia* cysts in the Norwegian aquatic environment over winter. Microbial Ecology 52(4): 597-602.

Roefer, P., Monscvitz, J., Rexing, D. 1996. The Las Vegas cryptosporidiosis outbreak. American Water Works Association September 1996. Pp. 95-106.

Rollins, D.M., Colwell, R.R. 1986. Viable but nonculturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. Applied and Environmental Microbiology 42(3): 531-538.

Rose, J.B., Haas, C.N., Regli, S. 1991. Risk assessment and control of waterborne giardiasis. American Journal of Public Health 81(6): 709-713.

Rosqvist, R., Skurnik, M., Wolf-Watz, H. 1988. Increased virulence of *Yersinia pseudotuberculosis* by two independent mutations. Nature 334(6182): 522-524.

Runde, V., Ross, S., Trenschel, R., Lagemann, E., Basu, O., Renzing-Kohler, K., Schaefer, U.W., Roggendorf, M., Holler, E. 2001. Adenoviral infection after allogeneic stem cell transplantation (SCT): report on 130 patients from a single SCT unit involved in a prospective multi center surveillance study. Bone Marrow Transplant 28(1): 51-57.

Ryan, U., Monis, P., Enemark, H., Sulaiman, I., Samarasnghe, B., Read, C., Buddle, R., Robertson, I., Zhou, L., Thompson, R., Xiao, L. 2003. *Cryptosporidium suis n. sp.* (Apicomplexa: Cryptosporidiidae) in pigs (*Sus scrofa*). International Journal for Parasitology 90: 769-773.

Samadpour, M., Stewart, J., Steingart, K., Addy, C., Louderback, J., McGinn, M., Ellington, J., Newman, T. 2002. Laboratory investigation of an *E. coli* O157:H7 outbreak associated with swimming in Battle Ground Lake, Vancouver, Washington. Journal of Environmental Health 64(10): 16-26.

Sargeant, J.M., Gillespie, J.R., Oberst, R.D., Flood, S.J.A. 1999. Prevalence of *Escherichia coli* 0157:H7 in white-tailed deer sharing rangeland with cattle. Veterinary Medicine Today: Public Veterinary Medicine 215(6): 792-794.

Sattar, S., Chauret, C., Springthorpe, V., Battigelli, D., Abbaszadegan, M., LeChevallier, M. 1999. *Giardia* cyst and *Cryptosporidium* oocyst survival in watersheds and factors affecting inactivation. American Water Works Association Research Foundation (AWWARF): Denver, CO.

Sattar, S., Springthorpe, V. 2006. Reoviruses. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 45.

Schaefer, F. 2006. *Giardia lamblia*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 31.

Schaub, S. 2004. A regulatory perspective on zoonotic pathogens in water. In: Waterborne Zoonoses: Identification, Causes and Control; Cotruvo, J.A, Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R., Gannon, V.P.J. (eds), World Health Organization (WHO). IWA Publishing: London, UK. Chapter 27.

Schoeni, J.L., Doyle, M.P. 1994. Variable colonization of chickens perorally inoculated with *Escherichia coli* O157:H7 and subsequent contamination of eggs. Applied and Environmental Microbiology 60(8): 2958-2962.

Schwab, K. 2006. Astroviruses. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 39.

Schwab, K., Hurst, C. 2006. Human caliciviruses. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 44.

Selifonova, O., Valle, F., Schellenberger, V. 2001. Rapid evolution of novel traits in microorganisms. Applied and Environmental Microbiology 67(8): 3645-3649.

Serviene, E., Shapka, N., Cheng, C., Panavas, T., Phuangrat, B., Baker, J., Nagy, P.D. 2005. Genome-wide screen identifies host genes affecting viral RNA recombination. Proceedings of the National Academy of Sciences 102(30): 10545-10550.

Shadduck, J.A., Greeley, E. 1989. Microsporidia and human infections. Clinical Microbiology Reviews 2(2): 158-165.

Sischo, W.M., Atwill, E.R., Lanyon, L.E., George, J. 2000. *Cryptosporidia* on dairy farms and the role these farms may have in contaminating surface water supplies in the northeastern United States. Preventative Veterinary Medicine 43(4): 253-267.

Smith, A.W., Skilling, D.E., Cherry, N., Mead, J.H., Matson, D.O. 1998. Calicivirus emergence from ocean reservoirs: zoonotic and interspecies movements. Emerging Infectious Diseases 4(1): 13-20.

Smith, H., Grimason, A., Holland, C. 2006a. *Ascaris lumbricoides*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 24.

Smith, H., Grimason, A., Holland, C. 2006b. *Trichuris trichiura*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 37.

Sobsey, M. 2006. Hepatitis A virus. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 42.

Sterling, C., Marshall, M. 2006. *Cryptosporidium parvum* and *Cryptosporidium hominis*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 28.

Stewart, M., Rochelle, P. 2006. *Acinetobacter*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 5.

Straub, T., Mena, H., Gerba, C. 1994. Viability of *Giardia muris* and *Cryptosporidium parvum* oocysts after aging, pressure, pH manipulations, and disinfection in mountain reservoir water. Proceedings of the 94th American Society Microbiology General Meeting, Las Vegas, NV.

Straub, T.M., zu Bentrup, K.H., Orosz-Coghlan, P., Dohnalkova, A., Mayer, B.K., Bartholomew, R.A., Valdez, C.O., Bruckner-Lea, C.J., Gerba, C.P., Abbaszadegan, M., Nickerson, C.A. 2007. *In vitro* cell culture infectivity assay for human noroviruses. Emerging Infectious Diseases 13(3): 396-403.

Sunderland, D., Graczyk, T.K., Tamang, L., Breysse, P.N. 2007. Impact of bathers on levels of *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts in recreational beach waters. Water Research 41(15): 3483-3489.

Suresh, K., Smith, H. 2004. Tropical organisms in Asia/Africa/South America. In: Waterborne Zoonoses: Identification, Causes and Control. Cotruvo, J., Dufour, A., G. Rees, J.B., Carr, R., Cliver, D., Craun, G., Fayer, R., Gannon, V. (eds), World Health Organization (WHO). IWA Publishing: London, UK. Chapter 6.

Swerdlow, D.L., Woodruff, B.A., Brady, R.C., Griffin, P.M., Tippen, S., Donnell, H.D., Geldreich, E., Payne, B.J., Meyer, A., Wells, J.G., Greene, K.D., Bright, M., Bean, N.H., Blake, P.A. 1992. A waterborne outbreak in missouri of *Escherichia coli* 0157:H7 associated with bloody diarrhea and death. Annals of Internal Medicine 117(10): 812-819.

Tarr, P.I. 1995. *Escherichia coli* O157:H7: clinical, diagnostic, and epidemiological aspects of human infection. Clinical Infectious Diseases 20(1): 1-10.

Tate, K.W., Atwill, E.R., George, M.R., McDougald, N.K., Larsen, R.E. 2000. *Cryptosporidium parvum* transport from cattle fecal deposits on California rangelands. Journal of Range Management 53(3): 295-299.

Tezcan-Merdol, D., Ljungstrom, M., Winiecka-Krusnell, J., Linder, E., Engstrand, L., Rhen, M. 2004. Uptake and replication of *Salmonella enterica* in *Acanthamoeba rhysodes*. Applied and Environmental Microbiology 70(6): 3706-3714.

Thomas, K.M., Charron, D.F., Waltner-Toews, D., Schuster, C., Maarouf, A.R., Holt, J.D. 2006. A role of high impact weather events in waterborne disease outbreaks in Canada, 1975 - 2001. International Journal of Environmental Health Research 16(3): 167-180.

Thompson R.C. 2000. Giardiasis as a re-emerging infectious disease and its zoonotic potential. International Journal for Parasitology 30: 1259-1267.

Thompson, R.C.A., Monis, P.T. 2004. Variation in *Giardia*: implications for taxonomy and epidemiology. Advances in Parasitology 58: 69-137.

Till, D., McBride, G. 2004. Potential public health risk of Campylobacter and other zoonotic waterborne infections in New Zealand. In: Waterborne Zoonoses: Identification, Causes and Control; Cotruvo, J.A, Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R., Gannon, V.P.J. (eds), World Health Organization (WHO). IWA Publishing: London, UK. Chapter 12.

Toranzos, G., Toro, A., Degnan, A. 2006. *Vibrio cholerae*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 21.

Tozzi, A.E., Niccolini, A., Caprioli, A., Luzzi, I., Montini, G., Zacchello, G., Gianviti, A., Principato, F., Rizzoni, G. 1994. A community outbreak of haemolytic-uraemic syndrome in children occurring in a large area of northern Italy over a period of several months. Epidemiology and Infection 113(2): 209-219.

Traub, R., Wade, S., Read, C., Thompson, A., Mohammed, H. 2005. Molecular characterization of potentially zoonotic isolates of *Giardia duodenalis* in horses. Veterinary Parasitology 130(3-4): 317-321.

Traub, R.J., Monis, P.T., Robertson, I., Irwin, P., Mencke, N., Thompson, R.C.A. 2004. Epidemiological and molecular evidence supports the zoonotic transmission of *Giardia* among humans and dogs living in the same community. Parasitology 128(Pt 3): 253-262.

Tupchong, M., Simor, A., Dewar, C. 1999. Beaver fever - a rare cause of reactive arthritis. The Journal of Rheumatology 26(12): 2701-2702.

Tzipori, S. 2000. Predicting human dose-response relationships from multiple biological models. Conference transcript from Conference on Predicting Human Dose-response Relationships from Multiple Biological Models: Issues with *Cryptosporidium parvum*, September 28, 2000, USDA Center at Riverside, Riverdale, MD.
http://www.foodrisk.org/IRAC/events/2000-09-28/speakers/tzipori.cfm.

Ungar, B.L. 1990. Enzyme-linked immunoassay for detection of *Cryptosporidium* antigens in fecal specimens. Journal of Clinical Microbiology 28(11): 2491-2495.

U.S. Department of Agriculture (USDA). 2001. Risk draft assessment of the public health impact of *Escherichia coli* O157:H7 from ground beef'. U.S. Department of Agriculture. September.
http://www.fsis.usda.gov/Science/Risk_Assessments/index.asp.

U.S. Department of Agriculture (USDA). 2005. Risk Assessment of the Impact of Lethality Standards on Salmonellosis from Ready-to-Eat Meat and Poultry Products. Final Report. U.S. Department of Agriculture, The Food Safety and Inspection Service, Office of Public Health Science, Risk Assessment Division.
http://www.fsis.usda.gov/Science/Risk_Assessments/index.asp.

U.S. Environmental Protection Agency (USEPA). 1986. Bacteriological Ambient Water Quality Criteria for Bacteria. U.S. Environmental Protection Agency, Office of Emergency and Remedial Response: Washington, DC.

USEPA. 1989. Risk Assessment Guidance for Superfund Volume I. Human Health Evaluation Manual. U.S. Environmental Protection Agency, Office of Emergency and Remedial Response: Washington, DC. EPA/540/1-89/002.

USEPA. 1997. Exposure Factors Handbook. U.S. Environmental Protection Agency, Office of Research and Development National Center for Environmental Assessment. EPA/600/P-95/002Fa.

USEPA. 1998. *Giardia*: Human Health Criteria Document. U.S. Environmental Protection Agency, Office of Water: Washington, DC. EPA-823-R-002.

USEPA. 1999. Drinking Water Criteria Document for Viruses: An Addendum. U.S. Environmental Protection Agency, Office of Water: Washington, DC.

USEPA. 2001a. *Cryptosporidium*: Human Health Criteria Document. U.S. Environmental Protection Agency, Office of Water: Washington, DC.

USEPA. 2001b. *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA. U.S. Environmental Protection Agency, Office of Water: Washington, DC.

USEPA. 2002. Implementation Guidance for Ambient Water Quality Criteria for Bacteria. U.S. Environmental Protection Agency, Office of Water: Washington, DC.

USEPA. 2005a. Occurrence and Exposure Assessment for the Final Long Term 2 Enhanced Surface Water Treatment Rule. U.S. Environmental Protection Agency, Office of Water: Washington, DC.

USEPA. 2005b. Appendices to the Occurrence and Exposure Assessment for the Final Long Term 2 Enhanced Surface Water Treatment Rule. U.S. Environmental Protection Agency, Office of Water: Washington, DC.

Visvesvara, G., Moura, H. 2006a. *Acanthamoeba* spp. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 23.

Visvesvara, G., Moura, H. 2006b. *Balamuthia mandrillaris*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 25.

Visvesvara, G., Moura, H. 2006c. *Naegleria fowleri*. In: Waterborne Pathogens, AWWA Manual M48, Second Edition. American Water Works Association: Denver, CO. Chapter 34.

Walker, M., Leddy, K., Hagar, E. 2001. Effects of combined water potential and temperature stresses on *Cryptosporidium parvum* oocysts. Applied and Environmental Microbiology 76(12): 5526-5529.

Wang, G., Doyle, M.P. 1998. Heat shock response enhances acid tolerance of *Escherichia coli* O157:H7. Letters in Applied Microbiology 26(1): 31-34.

Whitman, R.L., Nevers, M.B, Byappanahalli, M.N. 2006. Examination of the watershed-wide distribution of *Escherichia* along southern Lake Michigan: an integrated approach. Applied and Environmental Microbiology 72(11): 7301-7310.

WHO. 2003. Human Leptospirosis: Guidance for Diagnosis, Surveillance and Control. Technical Report. World Health Organization (WHO) and the International Leptospirosis Society. http://whqlibdoc.who.int/hq/2003/WHO_CDS_CSR_EPH_2002.23.pdf.

WHO. 2004. Waterborne Zoonoses: Identification, Causes and Control. World Health Organization (WHO); Cotruvo, J.A., Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R., Gannon, V.P.J (eds). IWA Publishing: London, UK. ISBN: 1 84339 058 2. http://www.who.int/water_sanitation_health/diseases/zoonoses/en/.

WHO. 2006. Guidelines for Drinking Water Quality: *Cryptosporidium.* Technical report, World Health Organization.

Widmer, G., Tchack, L., Chappell, C.L., Tzipori, S. 1998. Sequence polymorphism in the beta-tubulin gene reveals heterogeneous and variable population structures in *Cryptosporidium parvum*. Applied and Environmental Microbiology 64(11): 4477-4481.

Wills, C. 1996. Fever Black Goddess: The Co-Evolution of People and Plagues. Addison-Wesley Publishing Company, Inc.: New York, NY. P.44.

Wolfe, M., Jakubowski, W., Hoff, J. (editors) 1979. Managing the patient with giardiasis: clinical, diagnostic and therapeutic aspects. In: Waterborne Transmission of Giardiasis. U.S. Environmental Protection Agency (USEPA): Cincinnati, OH.

Wolfe, M.S. 1992. Giardiasis. Clinical Microbiology Reviews 5(1): 93-100.

Xiao, L., Ryan, U.M. 2004. Cryptosporidiosis: an update in molecular epidemiology. Current Opinion in Infectious Diseases 17(5): 483-490.

Yamahara, K.M., Layton, B.A., Santoro, A.E., Boehm, A.B. 2007. Beach sands along the California Coast are diffuse sources of fecal bacteria to coastal waters. Environmental Science and Technology 41: 4515-4521. Supporting information for beach sands along the California coast are diffuse sources of fecal bacteria to coastal waters. Environmental Science and Technology Supplemental to 41: 4515-4521, 17 pp.

Yang, S., Benson, S.K., Du, C., Healey, M.C. 2000. Infection of immunosuppressed C57BL/6N adult mice with a single oocyst of *Cryptosporidium parvum*. International Journal for Parasitology 86(4): 884-887.

Yarze, J.C., Chase, M.P. 2000. *E. coli* O157:H7 - another waterborne outbreak! American Journal of Gastroenterology 95(4): 1096.

Yoder, J.S., Blackburn, B.G., Craun, G.F., Hill, V., Levy, D.A., Chen, N., Lee, S.H., Calderon, R.L., Beach, M.J. 2004. Surveillance for waterborne-disease outbreaks associated with recreational water - United States, 2001-2002. Mortality and Morbidity Weekly Report CDC Surveillance Summaries 53(8): 1-22.

Zhou, L., Singh, A., Jiang, J., Xiao, L. 2003. Molecular surveillance of *Cryptosporidium* spp. in raw wastewater in Milwaukee: implications for understanding outbreak occurrence and transmission dynamics. Journal of Clinical Microbiology 41(11): 5254-5257.

APPENDIX A

WATERBORNE PATHOGENS

As described in Section I.2 of this paper, four attributes were used to select the waterborne zoonotic pathogens of concern for recreational uses of ambient waters (partially adapted from Bolin et al., 2004a). Table A-1 lists all the pathogens that were evaluated for potential inclusion in this paper. Information provided in Table A-1 includes whether the pathogen is considered waterborne, the species that are zoonotic hosts, whether the zoonotic hosts are warm-blooded, what illnesses the pathogen causes in humans, and the importance of considering the pathogen as EPA decides whether animal sources of fecal material should be considered differently from human sources for CWA §304(a) AWQC.

Table A-1. Known and Potential Zoonotic Waterborne Pathogens

Type	Pathogen	Waterborne	Zoonotic Host	Zoonotic Host is Warm-blooded	Illnesses and Symptoms in Humans (less common symptoms)	U.S. Outbreaks	Importance to consider if animal fecal sources are discounted in AWQC	Reference(s)
Bacteria	*Acinetobacter*	Yes (generally in environment)	None	NA	Septicemia, meningitis, endocarditis, brain abscesses, pneumonia, empyema, urinary tract infections, eye infections, and skin and wound infections	Hospital settings	No	Stewart and Rochelle, 2006
Bacteria	*Aeromonas*	Yes (generally in environment)	None	NA	Gastroenteritis (septicemia)	None reported	No	Lightfoot, 2004; Moyer and Standridge, 2006
Bacteria	*Campylobacter*	Yes	Poultry, cattle, sheep, and wild birds	Yes	Diarrhea, abdominal pain, malaise, fever, nausea, and vomiting (typhoid-like syndrome, febrile convulsions, meningeal arthritis, reactive arthritis, and GBS)	Mainly foodborne and drinking water	Important	Allos, 1998; 2001; APHA, 2006; Fricker, 2006a
Bacteria	*Cyanobacteria*	Yes (generally in environment)	None	NA	Rash and gastroenteritis	Drinking water and recreational water	No	Fredericksen et al., 2006

Type	Pathogen	Waterborne	Zoonotic Host	Zoonotic Host is Warm-blooded	Illnesses and Symptoms in Humans (less common symptoms)	U.S. Outbreaks	Importance to consider if animal fecal sources are discounted in AWQC	Reference(s)
Bacteria	Verotoxin-producing *E.coli* (VTEC) – includes enterohemorrhagic *E. coli* (EHEC), including O157:H7	Yes	Cattle, chicken, sheep, pigs, horses, dogs, and deer	Yes	Diarrhea (bloody), severe abdominal cramping, headache, hemorrhagic colitis, and hemolytic uremic syndrome	Foodborne and waterborne (drinking water and recreational water)	Important	APHA, 2004; Degnan and Standridge, 2006; Mølbak and Scheutz, 2004
Bacteria	Enterotoxigenic *E. coli* (ETEC)	Yes	Same as VTEC	Yes	Acute, watery diarrhea	Waterborne	Possibly important	Hunter, 2003; Mølbak and Scheutz, 2004
Bacteria	Attaching and effacing *E. coli* (A/EEC)	Yes	Same as VTEC	Yes	Acute or persistent diarrhea	No waterborne reported	Possibly important	Mølbak and Scheutz, 2004
Bacteria	Enteropathogenic *E. coli* (EPEC)	Yes	Same as VTEC	Yes	Acute or persistent diarrhea	Waterborne	Possibly important	Lee et al, 2002; Mølbak and Scheutz, 2004
Bacteria	Enteroaggregative *E. coli* (EAggEC)	Yes	Same as VTEC	Yes	Acute, watery, and often protracted diarrhea	No waterborne reported	Possibly important	Mølbak and Scheutz, 2004
Bacteria	Diffuse adherent *E. coli* (DAEC)	Yes	Same as VTEC	Yes	Acute or persistent diarrhea	No waterborne reported	Possibly important	Mølbak and Scheutz, 2004
Bacteria	Enteroinvasive *E. coli* (EIEC)	Yes	None	NA	Acute, often inflammatory diarrhea; dysentery	No waterborne reported	No	Mølbak and Scheutz, 2004

Type	Pathogen	Waterborne	Zoonotic Host	Zoonotic Host is Warm-blooded	Illnesses and Symptoms in Humans (less common symptoms)	U.S. Outbreaks	Importance to consider if animal fecal sources are discounted in AWQC	Reference(s)
Bacteria	*Flavobacterium*	Yes	None	NA	Gastroenteritis, meningitis, pneumonia, endocarditis, and septicemia	Rare waterborne (stagnation in drinking water), hospital settings more common	No	Geldreich and Degnan, 2006a
Bacteria	*Helicobacter pylori*	Possibly	Weak evidence for ferrets, racoons, swine, sheep, rodents, and primates	Yes	Gastric disorders, pepticand duodenal ulcer disease, lymphoma of the digestive tract, and ademenocarcinoma of the stomach	No waterborne reported	No	Baker and Degnan, 2006; Health Canada, 2006
Bacteria	*Klebsiella*	Yes (generally in environment)	Warm-blooded animals	Yes	Infections in respiratory system, genitourinary tract, nose, and throat, (meningitis and septicemia)	Hospital settings	No	Geldreich and Standridge, 2006
Bacteria	*Legionella*	Yes	None	NA	Legionellosis, pneumonia, Legionnaire's disease, and Pontiac fever	Hospitals, pools, and spas	No	Hall, 2006
Bacteria	*Leptospira*	Yes	Rats, dogs, raccoons, swine, and cattle	Yes	Leptospirosis (Weil's disease)	Recreational waterborne over 50% of cases in Hawaii	Important	Levett, 2001

Type	Pathogen	Waterborne	Zoonotic Host	Zoonotic Host is Warm-blooded	Illnesses and Symptoms in Humans (less common symptoms)	U.S. Outbreaks	Importance to consider if animal fecal sources are discounted in AWQC	Reference(s)
Bacteria	*Listeria monocytogenes*	No	Domestic and wild animals	Yes	Listeriosis, meningoencephalitis, fever, and abortion	Foodborne	No	APHA, 2004
Bacteria	*Mycobacterium avium* complex (MAC) and ssp. Paratuberculosis (MAP)	Yes (generally in environment)	Possibly sheep, cattle, goats, and birds	Yes	Respiratory infection, fever, and Crohn's disease	No recreational waterborne reported	Possibly important	Bolin et al., 2004b; Carr and Bartram, 2004; LeChevallier, 2006
Bacteria	*Pseudomonas*	Yes (generally in environment)	None	NA	Dermatitis	Recreational waterborne	No	Geldreich and Degnan, 2006b
Bacteria	*Salmonella*	Yes	Poultry, swine, cattle, rodents, wild birds, turtles, dogs, and cats	Yes	Gastroenteritis (enteric fever and septicemia)	Mainly foodborne and drinking water	Important	APHA, 2004; Covert and Meckes, 2006
Bacteria	*Serratia*	Yes (generally in environment)	None	NA	Opportunisitic infection (cystitis)	Hospital settings	No	Geldreich and Standridge, 2006a
Bacteria	*Shigella*	Yes	None (except primate colonies)	NA	Shigellosis, acute gastroenteritis, dysentary, fever, nausea, vomiting, and cramps	Recreational and drinking water	No	APHA, 2004; Cliver and Fayer, 2004; Moyer and Degnan, 2006
Bacteria	*Staphylococcus*	Yes	Skin of warm-blooded hosts	Yes	Cellulitis, pustules, boil, carbuncles, and impetigo (diarrhea and vomiting)	Hospital settings, pools, and spas	No	Geldreich and Standridge, 2006b

Type	Pathogen	Waterborne	Zoonotic Host	Zoonotic Host is Warm-blooded	Illnesses and Symptoms in Humans (less common symptoms)	U.S. Outbreaks	Importance to consider if animal fecal sources are discounted in AWQC	Reference(s)
Bacteria	*Vibrio cholerae*	Yes	Copepods, zooplankton	No	Profuse, watery diarrhea; vomiting	None recently	No	APHA, 2004; Toranzos et al., 2006
Bacteria	*Vibrio parahaemolyticus*	Yes (generally in environment)	Molluscan shellfish	No	Acute gastroenteritis (septicemia)	Foodborne	No	FDA, 2001
Bacteria	*Yersinia*	Yes	Pigs	Yes	Yersiniosis; acute, febrile diarrhea	Mainly foodborne	Possibly important	APHA, 2004; Fricker, 2006b
Protozoa	*Acanthamoeba*	Yes (free-living)	None	NA	Granulomatus ameobic encephalitis	None reported	No	Fayer, 2004b; Visvesvara and Moura, 2006a
Protozoa	*Ascaris lumbricoides*	Yes	None	NA	Ascariasis and roundworm infection	Foodborne	No	APHA, 2004; Smith et al., 2006a
Protozoa	*Balamuthia mandrillaris*	Yes (free-living)	Primates, sheep, dogs, and horses	Yes	Granulomatus ameobic encephalitis	None reported	No	Visvesvara and Moura, 2006b
Protozoa	*Balantidium coli*	Yes	Primates and pigs	Yes	Severe dysentery	None reported	No	Garcia, 2006a
Protozoa	*Blastocystis hominis*	Yes	Primates, cattle, sheep, pigs, horses, dogs, chickens, wild birds, alpacas, llamas, koalas, and wombats	Yes	Diarrhea, cramps, nausea, fever, vomiting, and abdominal pain	None reported	No	Garcia, 2006b

Type	Pathogen	Waterborne	Zoonotic Host	Zoonotic Host is Warm-blooded	Illnesses and Symptoms in Humans (less common symptoms)	U.S. Outbreaks	Importance to consider if animal fecal sources are discounted in AWQC	Reference(s)
Protozoa	*Cryptosporidium parvum* and *Cryptosporidium hominis*	Yes	Cattle, sheep, goats, pigs, horses, cats, dogs, wild animals, birds, reptiles, and fish	Yes	Cryptosporidiosis, profuse watery diarrhea, malaise, fever, anorexia, nausea, and vomiting	Recreational and drinking water	Important	APHA, 2004; CDC, 2007b; Olson, et al., 2003; Sterling and Marshall, 2006
Protozoa	*Cyclospora cayetanensis*	Yes	None	NA	Watery diarrhea, malaise, fever, anorexia, nausea, and vomiting	Foodborne and waterborne (drinking water and recreational water)	No	APHA, 2004; Cliver and Fayer, 2004; Cross and Sherchand, 2004; Ortega, 2006
Protozoa	*Entamoeba histolytica*	Yes	Potentially primates, dogs, cats, pigs, rats, and possibly cattle	Yes	Amoebiasis, dysentery, and diarrhea	None recently	No	APHA, 2004; Fayer, 2004b; Keene, 2006
Protozoa	*Giardia intestinalis* (also known as *G. duodenalis* and *G. lamblia*)	Yes	Beavers, cats, lemurs, sheep, calves, dogs, foxes, chinchillas, alpacas, horses, pigs, cows, and muskrats	Yes	Giardiasis; diarrhea (chronic); steatorrhea; abdominal cramps; bloating; frequent loose, pale, greasy stools; fatigue; and malabsorption	Recreational and drinking water	Important	APHA, 2004; Appelbee et al., 2005; Schaefer, 2006

Type	Pathogen	Waterborne	Zoonotic Host	Zoonotic Host is Warm-blooded	Illnesses and Symptoms in Humans (less common symptoms)	U.S. Outbreaks	Importance to consider if animal fecal sources are discounted in AWQC	Reference(s)
Protozoa	*Isosprora belli*	Yes	None	NA	Foul smelling, foaming diarrhea (months to years), abdominal colic, and fever	None reported	No	Garcia, 2006c
Protozoa	*Microsporidia (Enterocytozoon bieneusi, Encephalitozoon cuniculi, E. intestinalis)*	Yes	Cattle, pigs, cats, rabbits, and sheep	Yes	Diarrhea	None reported	Possibly important	Bolin et al., 2004b; Cali, 2006; Shadduck and Greely, 1989
Protozoa	*Naegleria fowleri*	Yes (free-living)	None	NA	Primary amebic meningoencephalitis	Mainly ambient recreational waters	No	Fayer, 2004b; Visvesvara and Moura, 2006c
Virus	Adenoviruses	Yes	None	NA	Acute gastroenteritis and respiratory disease	Waterborne	No	Enriquez and Thurston-Enriquez, 2006; Griffin et al., 2003; Reynolds, 2006
Virus	Astroviruses	Yes	None	NA	Gastroenteritis	Waterborne	No	Reynolds, 2006; Schwab, 2006
Virus	Avian influenza (H5N1)	Unknown	Birds	Yes	Mild upper respiratory illness to severe pneumonia and multiple organ failure	None reported	No	Chan, 2002; Suresh and Smith, 2004

Type	Pathogen	Waterborne	Zoonotic Host	Zoonotic Host is Warm-blooded	Illnesses and Symptoms in Humans (less common symptoms)	U.S. Outbreaks	Importance to consider if animal fecal sources are discounted in AWQC	Reference(s)
Virus	Coronaviruses (e.g., severe acute respiratory syndrome (SARS)-CoV)	Potentially (aerosolized wastewater)	Possibly civets and other wild animals	NA	Fever, dry cough, dyspnoea, and myalgia (diarrhea)	None reported	No	Moe, 2004; Reynolds, 2006; Suresh and Smith, 2004
Virus	Enteroviruses (e.g., coxsackie)	Yes	None	NA	Gastroenteritis, exanthema, diarrhea, fever, pharyngeal lesions, myocarditis, respiratory disease, and pneumonia	Recreational and drinking water	No	APHA, 2004; Gerba, 2006a; Griffin, 2003; Reynolds, 2006
Virus	Hendra virus	Unknown	Horses	Yes	Severe respiratory disease and meningoencephalitis	None reported	No	MacKenzie, 1999; Suresh and Smith, 2004
Virus	Hepatitis A virus	Yes	None	NA	Hepatitis - acute inflammation of the liver	None recently	No	Sobsey, 2006
Virus	Hepatitis E virus	Yes	Possibly pigs, chickens, and rats (close viral relatives to human form)	NA	Hepatitis - acute inflammation of the liver (fatality in pregnant women)	Rare in United States (common in other countries)	No	Cliver and Moe, 2004; Craun, 2004a; Gerba, 2006b
Virus	Human caliciviruses (norovirus, sapovirus)	Yes	None	NA	Diarrhea and vomiting	Very common (waterborne and foodborne)	No	Reynolds, 2006; Schwab and Hurst, 2006
Virus	Nipah virus	Unknown	Pigs, bats, and flying foxes	Yes	Acute and febrile encephalitis	None reported	No	Harcourt, 2005; Suresh and Smith, 2004

Type	Pathogen	Waterborne	Zoonotic Host	Zoonotic Host is Warm-blooded	Illnesses and Symptoms in Humans (less common symptoms)	U.S. Outbreaks	Importance to consider if animal fecal sources are discounted in AWQC	Reference(s)
Virus	Parechoviruses	Yes	None	NA	Pericarditis, herpangina, and respiratory disease	None reported (probably occurs, but unidentified)	No	Gerba, 2006a
Virus	Reoviruses	Yes	None	NA	Mostly mild or subclinical (rarely biliary atresia, juvenile onset diabetes, fever, rash, respiratory disease, and diarrhea	None reported (probably occurs, but unidentified)	No	Sattar and Springthrope, 2006
Virus	Rotaviruses	Yes	None	NA	Diarrhea	Very common waterborne	No	Abbaszadegan, 2006; Reynolds, 2006
Helminths	*Ancylostoma braziliense*	Mainly soil	Dogs or cats	Yes	Larval migration leads to creeping eruption	None reported (most important hookworm in humans - soil route)	No	Endo and Morishima, 2004
Helminths	*Angiostrongylus*	Yes	Mollusks	No	Meningitis	None reported	No	Endo and Morishima, 2004
Helminths	*Ascaris lumbricoides*	Mainly soil (drinking water possible)	None	NA	Ascaris pneumonia (lung hemorrhage)	None reported	No	Endo and Morishima, 2004; Smith et al., 2006

Type	Pathogen	Waterborne	Zoonotic Host	Zoonotic Host is Warm-blooded	Illnesses and Symptoms in Humans (less common symptoms)	U.S. Outbreaks	Importance to consider if animal fecal sources are discounted in AWQC	Reference(s)
Helminths	*Ascaris suum*	Mainly soil	Pigs	Yes	Ascaris pneumonia (lung hemorrhage)	None reported	No	Endo and Morishima, 2004
Helminths	*avian schistosomes (includes S. mansonni)*	Yes	Snails (birds are infected but not sources)	Yes	Cercarial dermatitis (swimmer's itch)	None reported	Low importance	Endo and Morishima, 2004
Helminths	*Baylisascaris procyonis*	Mainly soil	Raccoons	Yes	Larval migration may damage to the visceral and ocular systems (migration to brain)	None reported	No	Endo and Morishima, 2004
Helminths	*Dracunculus medinensis*	Yes	Crustaceans	No	Connective tissue migration, emerging in lower limb blister	None reported	No	Endo and Morishima, 2004
Helminths	*Echinococcus*	Yes	Foxes, dogs	Yes	Cystic hydatid disease, alveolar hydatid disease, polycystic hydatid disease, and polycystic hydatid disease	None reported	Low importance	Endo and Morishima, 2004
Helminths	*Fasciola hepatica*	Yes	Snails (herbivores and humans infected)	Yes	Fascioliasis	None reported	Low importance	Endo and Morishima, 2004
Helminths	*Pseudophyllid cestodes*	Yes	Copepods, fish	No	Cutaneous or mucocutaneous invasion	None reported	No	Endo and Morishima, 2004

Type	Pathogen	Waterborne	Zoonotic Host	Zoonotic Host is Warm-blooded	Illnesses and Symptoms in Humans (less common symptoms)	U.S. Outbreaks	Importance to consider if animal fecal sources are discounted in AWQC	Reference(s)
Helminths	*Schistosomes*	Yes	Snails (many animals can be infected, but are not sources)	No	Schistosomiasis (chronic infection - liver fibrosis, portal hypertension)	None reported	No	APHA, 2004; Blankespoor, 2006; Endo and Morishima, 2004
Helminths	*Strongyloides*	Mainly soil	Dogs	Yes	Eosinophilia	None reported	No	Endo and Morishima, 2004; Pardo, 2006
Helminths	*Taenia solium* (pork tapeworm)	Yes	Pigs	Yes	Cysticercosis and myositis	None reported	Low importance	Endo and Morishima, 2004
Helminths	*Toxocara canis*	Yes	Dogs	Yes	Toxocariasis (larval migration leads to hemorrhage and granulomatous lesions in the central nervous system)	None reported	Low importance	Endo and Morishima, 2004
Helminths	*Toxocara cati*	Yes	Cats	Yes	Toxocariasis (larval migration leads to hemorrhage and granulomatous lesions in the central nervous system)	None reported	Low importance	Endo and Morishima, 2004

Type	Pathogen	Waterborne	Zoonotic Host	Zoonotic Host is Warm-blooded	Illnesses and Symptoms in Humans (less common symptoms)	U.S. Outbreaks	Importance to consider if animal fecal sources are discounted in AWQC	Reference(s)
Helminths	*Toxoplasma gondii*	Yes	Cats	Yes	Toxoplasmosis, (in fetally exposed children - mental retardation, loss of vision, hearing impairment, and mortality)	None reported in recreational water	Potentially important	APHA, 2004; Dubey, 2004, 2006
Helminths	*Trichinella spiralis*	Mainly soil	Variety of animals	Yes	Trichinosis	Foodborne (mainly consumption of undercooked pork)	No	Endo and Morishima, 2004
Helminths	*Trichuris trichiuria*	Mainly soil	Monkeys, pigs, dogs, cats, and chicken	Yes	Trichuris dysentary syndrome, chronic diarrhea, anemia, and growth retardation	Foodborne	No	Endo and Morishima, 2004; Smith et al., 2006b
Prion	Bovine Spongiform Encephalopathy (BSE)	Unknown	Cattle	Yes	Variant Creutzfeldt-Jakob disease	Foodborne	No	Brown et al., 2006

NA = Not applicable

APPENDIX B

LITERATURE SEARCH STRATEGY AND RESULTS

The literature search strategy consisted of a number of combined approaches. Search terms and a synopsis of information needed were given to a professional librarian to search the online DIALOG databases. To supplement the DIALOG searches, individual authors used free search engines on the internet to find articles pertaining to specific information needed. Experts that participated in EPA's Experts Scientific Workshop on Critical Research Needs for the Development of New or Revised Recreational Water Quality Criteria[11] were contacted by email and requested to contribute literature they felt was important. The titles of literature cited in specific reports, books, review articles, and conference proceedings were evaluated for relevance.

B.1 Initial Literature Search Strategy Conducted by Professional Librarian

Selection of DIALOG data base files used for this search:

```
  File 155:MEDLINE(R) 1950-2007/Nov 30
         (c) format only 2007 Dialog
  File 266:FEDRIP 2007/Sep
         Comp & dist by NTIS, Intl Copyright All Rights Res
  File 144:Pascal 1973-2007/Nov W3
         (c) 2007 INIST/CNRS
  File 110:WasteInfo 1974-2002/Jul
         (c)  2002 AEA Techn Env.
  File 245:WATERNET(TM) 1971-2007Jul
         (c) 2007 American Water Works Association
  File 117:Water Resources Abstracts 1966-2007/Aug
         (c) 2007 CSA.
  File   5:Biosis Previews(R) 1926-2007/Nov W4
         (c) 2007 The Thomson Corporation
  File  40:Enviroline(R) 1975-2007/Oct
         (c) 2007 Congressional Information Service
  File 143:Biol. & Agric. Index 1983-2007/Oct
         (c) 2007 The HW Wilson Co
  File   6:NTIS 1964-2007/Dec W3
         (c) 2007 NTIS, Intl Cpyrght All Rights Res
  File  72:EMBASE 1993-2007/Dec 04
         (c) 2007 Elsevier B.V.
```

Main search strategy used for this search:
Fecal set AND Composition set AND (Pathogen or Waterborne sets): 1985-present

```
S1   240528  FECAL OR FECES OR FAECAL OR FAECES OR DUNG OR SCAT OR EXCREMENT
             OR MANURE OR STOOLS)
S2  1686966  COMPOSITION OR COMPOSED OR ANIMAL(1N)HUMAN()(TRANSMISSION OR
             CONTAMINATION)
```

[11] Report from this workshop: http://www.epa.gov/waterscience/criteria/recreation/.

```
S3 3842348  PATHOGEN? ? OR INDICATOR? ? OR RISK OR RISKS
S4     983  WATERBORNE()(PATHOGEN? ? OR ZOONO? OR ILLNESS?)
S6    1140  S1 AND S2 AND (S3 OR S4)
S719961011  PY=1920:1984
S8    1037  S6 NOT S7 (limited to 1985-present; foreign language OK)
S9     704  RD S8  (unique items, deduped)
```

Dates: 1985-present
Language: No restrictions
Retrieve: Titles and year
Format: MS Word
Interested in international and domestic journals and government reports.

Descriptions of these files are available at http://library.dialog.com/bluesheets/.

Search terms:
Waterborne pathogen*
Waterborne illness*
Waterborne zoon*
Emerging waterborne zoonotic pathogen*
Antibiotic resistan* AND zoonotic pathogen*
Transmission rate waterborne pathogen*
Evolution zoon* pathogen*
Human fecal composition micro*

Enterohemorrhagic *E. coli* symptoms
Salmonella symptoms
Shigella symptoms
Campylobacter symptoms
Listeria symptoms
Cryptosporidium symptoms
Giardia symptoms
norovirus symptoms
rotavirus symptoms
hepatitis E symptoms

waterborne dermal infection*
waterborne eye infection*
waterborne ear infection*
waterborne respiratory infection*

What we want from the literature search:

- Fecal composition of species that harbor zoonotic pathogens (species of livestock and wild animals
- Fecal composition of humans – pathogens and indicators
- The extent to which strains found in animals can be transmitted to humans

- An evaluation of the extent to which the information identified can be used to support the differentiation of risk from animal and human sources of fecal contamination
- Which organisms are of substantial public health concern that occur in ambient waters and are pathogenic to humans
- Which of these organisms are also present in animal populations
- The extent to which these organisms found in animals can be transmitted to humans
- The potential outcomes of human infection and disease from animal sources
- Specific pathogens: *E. coli* O157-H7, *Salmonella*, *Shigella*, *Campylobacter*, *Listeria*, *Cryptosporidium*, *Giardia*, norovirus, rotavirus, Hepatitis E, emerging pathogens. For each:
    - Describe illness symptoms (range asymptomatic to severe)
    - Describe route of exposure from recreational immersion in water including, inhalation, skin and mucus, eyes, ears
    - Incidence (morbidity and mortality data, through recent time)
    - Zoonotic potential – which animal species
- Variations in strains that effect infectivity, severity of symptoms, environmental survival and treatability
- Short summary review of emerging pathogen mechanisms (not too deep into molecular mechanisms of evolution)
- Anything in the water matrix that affects survivability and infectivity, and virulence
- Pathogen:indicator ratios

B.2 Summary of Literature Search Results

This process resulted in a total order of 535 documents (primarily peer reviewed scientific articles), of which a total of 332 (62 percent) were received during the expedited writing process, not all of which could be reviewed. There are many more papers in the peer-reviewed literature, and this by no means represents all of them. 319 citations were included in the white paper.

B.3 Supplemental Free Online Search Engines

The following terms were searched on Google (http://www.google.com/):

Search Topic	Approximate # Titles Reviewed	# Titles of Interest
E. coli + deer	10	1
Adenoviruses + zoonotic	30	3
Hepatitis E+ condition + symptoms	30	2
Rotavirus + illness + symptoms	20	1

The following terms were searched on Google Scholar (http://scholar.google.com/):

Search Topic	Approximate # Titles Reviewed	# Titles of Interest
Adenoviruses + zoonotic	10	1
Rotavirus + illness + symptoms	30	3
Giardiasis + swimming	200	5
Giardiasis + surveillance	200	3
Giardia + symptoms	100	5
Giardia + cyst	100	2
Giardia + beach	200	1
Cryptosporidium + *hominis*	100	1
Cryptosporidium + beach	150	1
Cryptosporidium + incidental ingestion	250	4
Cryptosporidium + review	100	2
Cryptosporidium + exposure factors	150	3
Cryptosporidium + exposure	50	2
Cryptosporidium + symptoms	50	3
Cryptosporidium + risk	50	0
Campylobacter + chronic	50	3
Campylobacter + illness	50	3
Campylobacter + infection	50	4
Campylobacter + symptoms	50	1
Campylobacter + water	15	2
Campylobacter + pathogenesis	5	1
Salmonella + transmission	20	8
Salmonella + transmission in water	11	2
Salmonella + antibiotic resistance	20	3
Salmonella + pathogenesis	3	1
Shigella + outbreak	23	4
Shigellosis	35	4

The following terms were searched on PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez):

Search Topic	# Titles Reviewed	# Titles of Interest
E. coli O157:H7 + survival + environment	204	23
Salmonella + survival + environment	376	4
Shigella + survival + environment	65	10
Campylobacter + survival + environment	57	17
Norwalk Virus + survival + environment	2	Yes
Rotavirus + survival + environment	54	17
Hepatitis E + survival + environment	3	Yes

The following terms were searched on Scirus (http://www.scirus.com/):

Search Topic	Approximate # Titles Reviewed	# Titles of Interest
Helicobacter + environmental survival	30	1
Leptospira + CID	10	1
Leptospira + environmental survival	30	2
Leptosporidiosis + symptoms	30	0

The following terms were searched on the CDC website (http://www.cdc.gov/):

Search Topic	Approximate # Titles Reviewed	# Titles of Interest
Legionella + EID	10	1
Leptospira + EID	10	1
Avian influenza + EID	10	1
H5N1 + EID	20	0
SARS + EID	30	2
Hendra virus + EID	20	0
Nipah virus + EID	20	2
Strongyloides + EID	10	1
BSE + EID	10	1

B.4 Experts Contacted

The flowing experts in the field were contacted directly by email and asked to suggest references:

Nicholas Ashbolt, USEPA
Thomas Atherholt, New Jersey Department of Environmental Protection
Michael Beach, Centers for Disease Control and Prevention
Bart Bibler, Florida Department of Health
Alexandria Boehm, Stanford University, California
Rebecca Calderon, USEPA
Jennifer Clancy, Clancy Environmental Consultants
Jack Colford, University of California, Berkeley
Elizabeth Doyle, USEPA
Alfred Dufour, USEPA
Lee Dunbar, Connecticut Department of Environmental Protection
Lora Fleming, University of Miami School of Medicine and Rosenstiel School of Marine and Atmospheric Sciences, Florida
Charles Hagedorn, Virginia Tech
Joel Hansel, USEPA

Lawrence Honeybourne, Orange County Health Care Agency, Santa Ana, California
Donna Francy, U.S. Geological Survey
Roger Fujioka, University of Hawaii, Manoa
Toni Glymph, Wisconsin Department of Natural Resources
Mark Gold, Heal the Bay, California
Paul Hunter, University of East Anglia, U.K.
Dennis Juranek, Centers for Disease Control and Prevention (retired)
David Kay, University of Wales, U.K.
Sharon Kluender, Wisconsin State Laboratory of Hygiene
Erin Lipp, University of Georgia
Graham McBride, National Institute of Water and Atmospheric Research, New Zealand
Charles McGee, Orange County Sanitation District, California
Samuel Myoda, Delaware Department of Natural Resources
Charles Noss, USEPA
Robin Oshiro, USEPA
James Pendergast, USEPA
Mark Pfister, Lake County Health Department, Illinois
John Ravenscroft, USEPA
Stephen Schaub, USEPA
Mark Sobsey, University of North Carolina, Chapel Hill
Jeffrey Soller, Soller Environmental, California
Michael Tate, Kansas Department of Health and Environment
Peter Teunis, RIVM (National Institute of Public Health and the Environment), Netherlands
Gary Toranzos, University of Puerto Rico, Rio Piedras
Timothy Wade, USEPA
John Wathen, USEPA
Stephen Weisberg, Southern California Coastal Water Research Project
David Whiting, Florida Department of Environmental Protection
Richard Zepp, USEPA

B.4 Previously Cited References

The following specific reports were obtained and the titles of the references cited in the reports were reviewed for relevance:

- NRC. (2004) Indicators for Waterborne Pathogens. The National Academies Press
- USEPA. (2007) Report of the Experts Scientific Workshop on Critical Research Needs for the Development of New or Revised Recreational Water Quality Criteria http://www.epa.gov/waterscience/criteria/recreation
- WHO. (2004) Waterborne Zoonoses. http://www.who.int/water_sanitation_health/diseases/zoonoses.pdf
- References cited by the Natural Resources Defense Council reviewers of the EPA Critical Path Science Plan
- USEPA Internal draft Adenovirus criteria document
- USEPA Internal draft pathogenic *E. coli* criteria document

- Boehm et al. (2008) A sea change ahead for recreational water quality criteria. (peer review in progress)

In addition, Clancy Environmental Consultants, Inc., ICF International, Soller Environmental, WaltJay Consulting, and EPA's Health and Ecological Criteria Division all maintain extensive literature databases and reference lists from previously completed projects. All of those in house resources were also sources of literature.

APPENDIX C

INCIDENTAL INGESTION OF AMBIENT WATER DURING RECREATIONAL ACTIVITIES

There is a paucity of data concerning rates of incidental ingestion of surface water during recreational activities. Most of the available estimates address exposures during swimming in swimming pools, which may not necessarily be representative of typical "incidental" exposures in ambient waters. Dufour et al. (2006) reviewed early estimates of swimming-related water ingestion and concluded that incidental ingestion ranged from 10 to 50 mL per hour. None of the early estimates, however, were based on actual studies of water ingestion. EPA's *Risk Assessment Guidance for Superfund* (USEPA, 1989) recommended a value of 50 mL per hour for ingestion during water recreation, citing an early version of EPA's *Exposure Factors Handbook* (EFH). The latest version of the EFH (USEPA, 1997) contains no recommendation concerning recreational water intake. Hammond et al. (1986), in their assessment of the potential toxicity of swimming pool disinfectants, estimated that a 70-kg adult might ingest "1 to 2 cups" of water, meaning approximately 500 mL. However, this is a "ballpark" estimate and is not supported by observational data.

Allen et al. (1982) estimated water ingestion by competitive swimmers by measuring urinary excretion of isocyanuric acid, an unmetabolized compound used to stabilize chlorine levels in swimming pools. The average estimated water intake among the five swimmers that were studied was 161 mL per hour. The investigators also determined that (1) essentially all of the ingested isocyanuric acid appeared in urine within 24 hours, thus negating concern for elimination by other pathways; and (2) dermal absorption of the tracer compound was insignificant compared to the ingestion intake.

Using methods similar to those used by Allen et al. (1982), Dufour et al. (2006) estimated water intake in 12 adults and 41 "nonadults" engaged in less vigorous water recreation at a community swimming pool. Based on the amounts of isocyanurate excreted in urine, they estimated that 45 minutes of water recreation resulted in average water intakes of 37 mL (49 mL/hr) for nonadults and 16 mL (21 mL/hr) for adults. The exposures measured by Dufour et al. (2006) are perhaps more likely to be representative of typical "incidental" exposures than those of the competitive swimmers measured by Allen et al. (1982), suggesting the lower values may provide a better basis for estimating incidental exposures.

A larger follow-up study of 549 participants was subsequently conducted at several public and private outdoor swimming pools (Evans et al., 2006). Participants were requested to engage in active swimming for between 45 and 60 minutes. The overall average incidental ingestion rate was 32 mL/hr, with a range of 1 to 280 mL/hr. Adults averaged 24 mL/hr, and children averaged 47 mL/hr. Children (ages not specified) swallowed approximately twice as much water as adults. The follow-up study also showed that males ingested more than females and that adult men ingested more than adult women.

The small number of studies that are available measured water intake in only a few subjects and characterized water ingestion during either very active swimming or poorly defined water recreational activities. In addition, the incidental ingestion data that are available are for "clean" pool water and may not represent incidental ingestion for surface waters, which may provoke stronger avoidance behaviors due to the perception that surface waters are nonpotable.