CHAPTER 2.0
PATHOGENS OF CONCERN IN AQUATIC SYSTEMS AND DRINKING WATER SUPPLIES

Although the majority of pathogen-caused disease outbreaks in the United States occur as a result of ingestion during recreation or through consumption of contaminated food, the presence of pathogens in surface waters, albeit at low levels, is a subject of continuing interest for water suppliers because of the possibility of pathogens not being fully removed during treatment. In the widely studied Cryptosporidium contamination episode in Milwaukee in 1993 (MacKenzie et al., 1994; Eisenberg et al., 2005), the drinking water treatment plant in question had been meeting operational targets with respect to indicator coliforms, and yet managed to result in the infection of nearly 400,000 people, about 25% of the population supplied by the utility. Because of the potential failure of treatment and disinfection to remove and/or inactivate pathogens, there is a great deal of interest in minimizing, and at the very least, characterizing the abundance of pathogens in ambient waters used for municipal supply.

This chapter provides an overview of pathogens in aquatic systems, including a description of routes of transmission, a summary of known pathogens, and the role of indicators widely used in lieu of actual data on pathogens.

2.1 MECHANISMS OF WATERBORNE PATHOGEN TRANSMISSION

Many pathogens of concern in source waters for drinking water supply are spread by the fecal-to-oral route. In recent years there has been greater focus on pathogens that can infect both humans and animals, and for which the pathogens may originate from natural sources other than sewage. Figure 2-1 is a schematic of the processes by which pathogens may cause human infection. This schematic shows that pathogens may originate in six potential sources: domestic animals during grazing or in confined animal facilities, wild animals in natural lands, aquatic avian and mammalian species that inhabit surface waters, human water-contact recreational activity, urban stormwater runoff, and wastewater discharge. Pathogens that have animal hosts
(zoonoses) can be transported from the watershed to source waters from natural lands, from grazed lands, and from confined animal facilities. Some animal hosts can be aquatic species (such as geese) and contribute pathogens and other bacterial loads directly to water bodies. Stormwater runoff from urban/rural areas that contains organisms shed by domestic pets, birds, rodents and sewage spills that enter storm drain systems can also contaminate source waters. Municipal wastewater can also be a source because of the presence of pathogens in the feces of infected humans. The *Cryptosporidium* episode in Milwaukee cited above, the source of the pathogens was found to have been wastewater (Eisenberg et al., 2005). Once in the ambient environment, pathogens often die off at varying rates (depending on the organism), although in some instances they can survive and even reproduce in sediments. In most instances, the pathogens in source waters are removed by filtration or membranes or destroyed by disinfection techniques. Infections in humans may arise from pathogens that break through into treated drinking water or from external sources such as food ingestion and ingestion of untreated water during recreation.

Several pathogens have emerged as concerns to drinking water in recent years because of their newly recognized pathogenesis (e.g. *Helicobacter pylori*) or because of their involvement in outbreaks of waterborne diseases (Crittenden et al. 2005). Many of these organisms are opportunistic pathogens that can grow in natural water and soil environment and are transmitted through water and therefore are of concern to drinking water. Some of them are now considered by EPA as candidate contaminants for as part of the second update of the candidate contaminant list (CCL2) (US EPA, 2005). Table 2-1 lists these emerging microbial contaminants. Of
these candidate contaminants, cyanobacteria and the toxins they release have been discussed in the conceptual model for nutrients (Tetra Tech, 2006b).

Table 2-1. Emerging Microbial Contaminants Identified in USEPA’s Candidate Contaminant List 2 (Source: USEPA, 2005).

<table>
<thead>
<tr>
<th>Microbial Contaminants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoviruses</td>
</tr>
<tr>
<td>Microsporidia (Enterocytozoon &amp; Septata)</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
</tr>
<tr>
<td>Caliciviruses</td>
</tr>
<tr>
<td>Echoviruses</td>
</tr>
<tr>
<td>Coxsackieviruses</td>
</tr>
<tr>
<td>Mycobacterium avium intracellulare (MAC)</td>
</tr>
<tr>
<td>Cyanobacteria (blue-green algae), other freshwater algae, and their toxins</td>
</tr>
</tbody>
</table>

2.2 **FATE OF PATHOGENS IN THE AMBIENT ENVIRONMENT**

Organisms in general require energy and carbon sources for metabolism. Different organisms generally have their preferred temperature range, pH range and oxygen level for survival. For many pathogenic organisms that have reservoirs in human and warm blooded-animal intestines, warm temperatures are preferred. These parasites also rely on their hosts for energy and carbon sources and prefer anaerobic conditions. Although they may die off rapidly once excreted by their host, they may survive under warm, wet conditions. Many organisms of interest can also exist in an environmentally resistant stage. For example, viruses can exist as virons, bacteria can exist as spores, cystlike forms that are resistant to extreme environmental conditions, and protozoa can exist in the environmentally resistant stages as cysts and oocysts outside their hosts.

2.3 **BACTERIA THAT CAUSE DISEASE**

Bacteria are single celled organisms ranging in size from 0.1-1 µm. There are two groups of bacteria in surface waters: the autochthonous group and allochthonous group. Most bacteria of concern to public health are allochthonous. Allochthonous bacteria prefer the warm environment of intestines of warm-blood animals, and spend only a limited portion of their life span in natural waters. As shown schematically in Figure 2-1, they are carried to natural waters through contamination, rainfall and runoff. Outside the intestinal environment, these bacteria tend to die off. The presence of allochthonous bacteria is generally an indicator of stormwater, wastewater or fecal contamination.

There are a variety of bacterial pathogens that cause waterborne disease in humans. Some are classical pathogens that have been known to affect human health for a long time including *Vibrio cholerae*, *Salmonella spp.*, and *Shigella spp*. Some pathogens have become concerns to human health in recent years, including pathogenic *Escherichia coli* (*E. coli*), *Yersinia enterocolitica*, and *Campylobacter jejuni*. Many of these organisms live inside humans or animals and can be transmitted through human
or animal wastes. Most of these organisms have been related to recent waterborne disease outbreaks (summarized in Table 2-2). Key characteristics of these organisms are summarized below. Some emerging bacterial pathogens, including *Helicobacter pylori* and *Mycobacterium avium intracellulare* listed under US EPA CCL2, are gaining increasing interest from a human health standpoint and are also discussed below (Table 2-3, sections 2.3.4 - 2.3.8).

Table 2-2. Summary of bacteria recently associated with waterborne disease (After Crittenden et al. 2005)

<table>
<thead>
<tr>
<th>Organism or Bacteria</th>
<th>Size, µm</th>
<th>Motile</th>
<th>Health effects in healthy persons</th>
<th>Evidence of waterborne pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteropathogenic E. coli (EPEC)</td>
<td>0.3-0.5 × 1-2</td>
<td>No</td>
<td>Traveler’s diarrhea</td>
<td>Numerous waterborne outbreaks</td>
</tr>
<tr>
<td>Enteroaggregative E. coli. (EaggEC)</td>
<td>0.3-0.5 × 1-2</td>
<td>No</td>
<td>Childhood diarrhea and diarrhea among immunocompromised</td>
<td>Numerous waterborne outbreaks</td>
</tr>
<tr>
<td>Enteroinvasive E. coli (EIEC)</td>
<td>0.3-0.5 × 1-2</td>
<td>No</td>
<td>Childhood diarrhea</td>
<td>Numerous waterborne outbreaks</td>
</tr>
<tr>
<td>Enterohemorrhagic E. coli (EHEC)</td>
<td>0.3-0.5 × 1-2</td>
<td>No</td>
<td>Bloody diarrhea, occasionally hemolytic uremic syndrome</td>
<td>Six waterborne outbreaks, notably Cabool, MI and Walkerton, Ontario</td>
</tr>
<tr>
<td>Enterotoxigenic E. coli. (ETEC)</td>
<td>0.3-0.5 × 1-2</td>
<td>No</td>
<td>Traveler’s diarrhea</td>
<td>Numerous waterborne outbreaks</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>0.3-0.5 × 1-2</td>
<td>No</td>
<td>Fever, abdominal pain, gastroenteritis with diarrhea and vomiting</td>
<td>Outbreaks associated with contaminated spring water</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>0.3 × 1-5</td>
<td>Yes</td>
<td>Diarrhea, abdominal pain, nausea, fever, malaise</td>
<td>Numerous outbreaks</td>
</tr>
</tbody>
</table>

Table 2-3. Summary of bacteria of emerging concern in drinking water (After Crittenden et al. 2005)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Size, µm</th>
<th>Motile</th>
<th>Normal Habitat</th>
<th>Health effects in healthy persons</th>
<th>Modes of transmission</th>
<th>Evidence of waterborne pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Legionella pneumophila</td>
<td>0.3-0.9 × 2-20</td>
<td>Yes</td>
<td>Warm water</td>
<td>Legionnaire’s disease, Pontiac fever</td>
<td>Aerosols</td>
<td>Outbreaks associated with warm-water aerosols</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>0.3-1.0 × 1.0- 3.5</td>
<td>Yes</td>
<td>All fresh waters</td>
<td>Gastroenteritis (controversial)</td>
<td>Water exposure</td>
<td>Drinking contaminated water; water in open wounds</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>0.3-1.0 × 1.0- 3.5</td>
<td>Yes</td>
<td>Human stomach and upper intestinal tract</td>
<td>Dominant cause of peptic and duodenal ulcers; associated with gastric carcinoma</td>
<td>Fecal-oral</td>
<td>Some indirect evidence</td>
</tr>
<tr>
<td>Mycobacterium avium intercellulare</td>
<td>0.2-0.6 ×1-10</td>
<td>No</td>
<td>Soil, dust, water, and animals</td>
<td>Lung infection, fatigue</td>
<td>Inhalation or ingestion</td>
<td>Increasing incidence of gastrointestinal illness in AIDS patients</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>0.5-0.8 × 1.5-3</td>
<td>Polar flagellum</td>
<td>Inhabitant of soil and water; opportunistic human pathogen</td>
<td>Infections of urinary tract, respiratory system, and soft tissue; dermatitis, bacteremia, and systemic infections</td>
<td>Contact with compromised tissue</td>
<td>Only for immunocompromised patients</td>
</tr>
</tbody>
</table>
2.3.1 **Pathogenic *Escherichia coli***

*Escherichia coli* is a facultative, anaerobic, gram-negative rod shaped bacteria that lives in the gastrointestinal tract of warm blooded animals. The presence of *E. coli* normally is beneficial to the host through the suppression of harmful bacteria and synthesis of vitamins. However, some strains of *E. coli* are pathogenic. There are several groups of *E. coli* that have been identified to be pathogenic including: the enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), and enteroaggregative *E. coli* (EaggEC).

ETEC impacts human through generating toxins that result in diarrhea without invading the epithelial wall. EPEC also impacts human through the production of a toxin but by invading the epithelial wall, which can result in inflammation and fever. EIEC does not produce toxins, but can penetrate and grow in the epithelial cells of the intestine, which causes severe inflammation, fever and bacillary dysentery. EaggEC is less studied but is generally thought to adhere to intestine without inflammation or fever. EHEC, which includes the well known *E. coli* O157:H7 implicated in recent *E. coli* outbreaks from California produce (FDA, 2006), produces a Shiga-like toxin. *E. coli* O157:H7 is unique in that some patients, particularly children, can develop hemolytic uremic syndrome, leading to destruction of red blood cells and occasional kidney failure.

The presence of *E. coli* is generally an indicator of fecal contamination, either by human waste water or animal wastes. A recent case of waterborne outbreak occurred in Walkerton, Ontario during May to June 2000, was attributed to the contamination of Enterohemorrhagic *E. coli* in a drinking well by farm manure. Among the 1,346 cases reported, about half were infected with *E. coli* O157:H7.

2.3.2 **Campylobacter**

*Campylobacter* is a gram-negative, motile, rod shaped bacteria. *Campylobacter* can be found in natural waters throughout the year. The presence of *Campylobacter* is not directly related to indicators of fecal contamination. *Campylobacter jejuni* is commonly present in the gastrointestinal tract of healthy cattle, pig and poultry. The organism survives better in cold temperatures. When stressed, it can enter a state that can still be transmitted to animals. *Campylobacter* is a leading cause of bacterial gastroenteritis in U.S., followed by salmonellosis, shigellosis, and *E. coli* O157:H7 infection. Virtually all human illness associated with *Campylobacter* is caused by one species, *Campylobacter jejuni*, but 1% are caused by other species. *Campylobacter* infection in some rare cases may be followed by Guillain-Barre Syndrome (GBS), a form of neuromuscular paralysis. Strains of *Campylobacter* have developed resistance to antibiotics, resulting in clinical treatment difficulty.

2.3.3 **Yersinia enterocolitica**

*Yersinia enterocolitica* is a small rod-shaped, gram-negative bacterium and is not a normal flora in the human gastrointestinal tract. *Yersinia enterocolitica* is able to grow in cold temperature but dies off under normal room temperature. It also prefers neutral to alkaline pH. *Yersinia enterocolitica* is an invasive pathogen that penetrates
Chapter 2.0 Conceptual Model for Pathogens and Pathogen Indicators in the Central Valley

the intestinal lining and enters lymph nodes, causing systemic infection. *Yersinia enterocolitica* releases enterotoxins that cause yersiniosis, a disease exhibiting pain and intestine inflammation. *Yersinia enterocolitica* can live in different animals including pigs, rodents, rabbits, sheep, cattle, horses, dogs, and cats. *Yersinia enterocolitica* has been detected widely in environmental and food sources such as lakes, ponds, meats, and milk.

2.3.4 **LEGIONELLA PNEUMOPHILA**

*Legionella pneumophila* is a motile, rod-shaped, gram-negative, aerobic bacterium. *Legionella* grows in warm aquatic environments with rust, algae, and organic particles. The organism can survive in tap water at room temperature for over a year. Among the many species and serogroups, the strain responsible for Legionnaires’ disease is the *Legionella pneumophila* serogroup. *Legionella pneumophila* is transmitted to humans via inhalation of aerosols, particularly under high relative humidity since it allows the organism to survive longer. *Legionella pneumophila* can move into a host cell, reproduce and eventually lyse the cell. Keeping *Legionella pneumophila* out of the water distribution system is an effective way to prevent spread of the disease. Incidences of *Legionella pneumophila* infections are generally associated with air conditioning equipment and hot-water supplies.

2.3.5 **AEROMONAS HYDROPHILA**

*Aeromonas hydrophila* occur as gram-negative, motile, non-spore forming, facultatively anaerobic rods or coccobacilli. These organisms are heat sensitive, destroyed easily by pasteurization. *Aeromonas hydrophila* inhabits fresh or sea water and is found widely in different sources of water. *Aeromonas hydrophila* is of particular concern to immunocompromised hosts. *Aeromonas* causes diarrheal illness through production of heat-sensitive enterotoxins. *Aeromonas hydrophila* is opportunistic pathogen that is ubiquitous in the environment that can potentially be transmitted through contaminated drinking water but also can be transmitted through foods that come in contact with contaminated water.

2.3.6 **HELCOBACTER PYLORI**

*Helicobacter pylori* is a spiral-shaped, gram-negative rod that is motile. *Helicobacter pylori* lives in the stomach and duodenum of human and animals. It is thought to be the most common cause of gastritis in humans (Crittenden et al., 2005). *Helicobacter pylori* adheres to the plasma membranes of surface epithelial cells, which protects it from the immune system. It is found that *Helicobacter pylori* infection increases the risk of gastric cancer. *Helicobacter pylori* is transmitted through fecal-oral route and is considered a potential waterborne pathogen.

2.3.7 **MYCOBACTERIUM AVIUM COMPLEX**

*Mycobacterium avium* complex (*M. avium* and *M. intracellulare*) are an aerobic, non-spore-forming, non-motile family of bacilli. *Mycobacterium avium* complexes (MAC) are abundant in soil, food and water. Members of MAC are able to grow in water samples without any additional substrate and are resistant to chlorination. They can grow over a wide range of temperature and salinities. The cell walls of these organisms contain high level of lipid and can colonize the wet surfaces in water.
systems. MAC organisms attack healthy individuals and result in serious tuberculosis-like infections for immuno-compromised individuals. MAC organisms have been found in water, food and soil samples from patient care sites.

2.3.8 **Pseudomonas aeruginosa**

*Pseudomonas aeruginosa* is a gram-negative, aerobic motile rod of the Pseudomonadaceae family. *Pseudomonas aeruginosa* is one of the most vigorous, fast-swimming bacteria seen in pond water samples. *Pseudomonas aeruginosa* are usually found in soil and water. The organism has a wide array of virulence factors. Most *Pseudomonas* infections are both invasive and toxinogenic. *Pseudomonas* adhere to the epithelial cells of upper respiratory tract and other epithelial cells as well (e.g., the gastrointestinal tract). *Pseudomonas aeruginosa* is resistant to antibiotics and can be waterborne. However, it is uncertain whether drinking water is an important means for transmitting the organism.
2.4 VIRUSES OF CONCERN

Viruses have simpler structure than other organisms. A basic virus consists of a core of nucleic acid (either DNA or RNA) surrounded by a protein coat. Viruses are parasites that depend on the host for resources and reproduction. Viruses can survive outside the host for a longer time than bacteria since it has no metabolic needs. Viruses are small in size and are more host-specific. Enteric viruses shed by animals and humans are abundant in untreated surface waters. Water treatment plants are typically effective in removing viruses, however inadequate treatment can result in viruses passing through to drinking water. Some viruses that have been associated with waterborne disease are those that affect the gastrointestinal tract system such as polio, coxsackie, echo-virus, and more recently identified viruses such as rota-, calici-, and adeno-viruses (Crittenden et al. 2005). Most of these viruses are known to be present in wastewater effluent and are transmitted through the fecal-oral route. Among these, adenoviruses, caliciviruses, coxsackie viruses, and echoviruses are candidate contaminants in CCL2 identified in Table 2-1.

2.4.1 POLIOVIRUS

Poliovirus is the virus that causes poliomyelitis which was a common disease fifty years ago in U.S. Poliovirus is an enterovirus that is highly contagious. It is transmitted through the fecal-oral route. The virus has three serotypes. Type I is the most common cause of disease in human. Today the use of vaccines has greatly reduced the incidence of the disease.

2.4.2 HEPATITIS

Hepatitis is a virus that causes liver inflammation and sometimes leads to jaundice. The virus is classified into Types A, B, C, D, E and G. All these viruses can cause acute viral hepatitis. The hepatitis B, C, D, and G viruses can also cause chronic hepatitis. Type A and E are infectious hepatitis that are transmitted through the fecal-oral route. Type C, D, and G are serum hepatitis that are transmitted through direct exposure of the blood serum. Hepatitis A is a well documented waterborne disease and it is widespread worldwide. To date no outbreaks of Hepatitis E have been reported in the U.S. due to effective water treatment.

2.4.3 ROTAVIRUS

Rotaviruses are the most important viruses causing diarrhea worldwide. Rotavirus was estimated to contribute to 30 to 50 percent of severe diarrhea disease in humans. Rotavirus group A, B, and C are particularly of interest for human gastroenteritis. Among these, the group A subtypes 1, 2, 3 and 4 are the leading cause of severe diarrhea in infants and children. The rotaviruses are round particles about 80nm in diameter. Persons with gastroenteritis caused by rotavirus can excrete large numbers of viruses ($10^5$ to $10^{10}$ infective units per ml of stool) and the infective dose is on the order of 10 to 100 infectious units. The virus can be transmitted through fecal-oral route and via contaminated food and water.
2.4.4 **Adenoviruses**

Adenoviruses are large, non-enveloped viral particles. Adenoviruses are the only human enteric viruses with double-stranded DNA. Adenoviruses 40 and 41 are thought to infect human intestines. Adenoviruses are more commonly found in patients with gastroenteritis. Person to person transmission is presumably the principal mechanism for infection. Neither food nor water has been demonstrated as a means of transmission, although adenoviruses have been reported as possible agents in waterborne outbreaks and have been found in raw and finished drinking water.

2.4.5 **Human Caliciviruses**

The Norwalk and Norwalk-like viruses (Norovirus) are groups of single stranded RNA nonenveloped viruses in *Caliciviridae* family that cause acute gastroenteritis. Noroviruses have been associated with many waterborne and foodborne outbreaks. The infectious doses are low, with perhaps 10 to 100 particles constituting a 50% infectious dose. Some instances associated with contaminated food have also been shown to be related to contaminate drinking water as well.

2.5 **Protozoans of Concern**

The protozoans are a group of unicellular, non-photosynthetic organisms. Protozoans are usually motile. Several protozoans are transmitted by the fecal-oral route. Protozoans associated with waterborne disease mainly include *Entamoeba histolytica*, *Entamoeba dispar*, *Giardia lamblia*, and *Cryptosporidium parvum* (Crittenden et al. 2005). Parasitic protozoa can live in resting and living stages. In resting stages, cysts, oocysts and spores can survive outside the host. Inside the host, in living stages, trophozoites (*Giardia*) and sporozoites (*Cryptosporidium*) are released and grow.

2.5.1 **Entamoeba**

*Entamoebas* are single-celled parasitic amoeboid protozoa. *Entamoebas* can exist in two forms: trophozoites measuring 20 to 40 µm in diameter and sporozoites measuring 10 to 16 µm in diameter. In recent years, it was found that *Entamoebas histolytica* and *Entamoebas dispar* are the two species that cause invasive and noninvasive infections in humans. Infection mostly occurs in the digestive tract. *Entamoebas* infect mostly human and other animals such cats and dogs. The *Entamoebas* are transferred through fecal contamination of drinking water, but also through direct contact with contaminated hands or objects.

2.5.2 **Giardia**

*Giardia* is a single-celled, microscopic parasite that can be found in intestinal linings of a wide range of animals (in trophozoite form) and in feces of infected individuals and in contaminated water (in cyst form). *Giardia* can exist in the environment in sporozoite form (a cyst) or a trophozoite. As cysts, *Giardia* is about 11 to 14 µm long and 7 to 10 µm wide. *Giardia* can survive a wide range of temperature from ambient temperature of fresh water to internal temperature of animals. Among the many species of *Giardia*, *Giardia lamblia* (also known as *Giardia intestinalis*) infects humans. Infection by *Giardia lamblia* causes diarrhea and abdominal pain. *Giardia lamblia* has been found in wastewater and have also been related to several outbreaks.
of waterborne disease. *Giardia* infection is transmitted by the fecal-oral route. The infectious dose is low: ingestion of 10 cysts has been reported to cause infection. In the U.S., nearly 20,000 or more cases of giardiasis were reported each year during 1998 to 2002 (Hlavsa et al. 2005) as shown in Figure 2-3. The number of reported cases peaked in June to October, suggesting a relationship to recreational activities.

![Figure 2-3 Incidence of Giardiasis (upper panel) and Cryptosporidiosis (lower panel) in the U.S. by month between 1998 and 2002 (Source Hlavasa et al., 2005). Giardiasis numbers are reported in thousands.](image)

### 2.5.3 *Cryptosporidium*

*Cryptosporidium* spp. are single-celled, intestinal parasites that infect human and a variety of animals. *Cryptosporidium* can infect epithelial cells of the intestine wall and be excreted in feces as oocysts. An oocyst is approximately 3 to 7 μm in diameter.

*Cryptosporidium* is primarily transmitted through fecal-oral route with infection occurring as a result of the ingestion of oocysts in contaminated food or water. Two species of *Cryptosporidium*, *Cryptosporidium hominis* and *Cryptosporidium parvum*, are most relevant to humans. *Cryptosporidium hominis* only infects humans while *Cryptosporidium parvum* can infect a wide range of animals including humans. Symptoms of cryptosporidiosis, a disease caused by ingestion of *Cryptosporidium*, include diarrhea, stomach cramps, upset stomach, and slight fever. The infectious dose of *Cryptosporidium* is relatively low. The range of the dose-to-cause infection in 50 percent of the subjects (ID50) is reported to range from 9 to 1,042 oocysts (Okhuysen et al., 1999). *Cryptosporidiosis* is a major cause of gastrointestinal illness.
around the world. Outbreaks of cryptosporidiosis have been reported in several countries, most notably the waterborne outbreak in Milwaukee in 1993 with more than 400,000 people infected (MacKenzie et al., 1994; Edwards, 1993). In the U.S., there were usually two-to-three thousand cases reported annually between 1998 and 2002 (Figure 2-3 from Hlavsa et al., 2005). For 2002, cryptosporidiosis incidence ranged from 0.2 cases to 9.5 cases per 100,000 people. Numbers of cryptosporidiosis cases reported by illness onset are five-fold to six-fold higher during June-October than other months (Hlavsa et al., 2005).

*Cryptosporidium* has a wide range of hosts including cows, goats, sheep, pigs, horses, dogs, cats and wild animals. When infected, these animals can shed large numbers of oocysts in the environment. In watersheds with grazed lands in proximity to water bodies, infected cattle may be a source of oocysts in surface waters.

*Cryptosporidium* oocysts once shed by the animals can be retained by soil particles and vegetation or retained in soil matrix during infiltration, with only a portion of the oocysts being transported to surface waters through runoff. In the aquatic environment, *Cryptosporidium* oocysts can be aggregated to larger particles and are subject to settling, grazing, and inactivation due to temperature and solar radiation (Brooks et al. 2004). Oocysts are subject to grazing by rotifers, ciliates and other predators that can ingest these organisms. Temperature is one of the most important factors regulating the fate of *Cryptosporidium* oocysts in the environment (King and Monis, 2006). *Cryptosporidium* oocysts can persist in the environment for longer than a year at temperatures below 15°C (Jenkins et al. 2002). However, a slight increase of temperature to 20 or 25 °C significantly increases oocysts inactivation rates. Very low temperature (below freezing) also affects oocyst survival. Oocysts are also susceptible to inactivation via solar radiation, and when present on soil surfaces or near the surface of waters, can be inactivated quickly due to solar exposure. Moisture is another factor that influences the survival of *Cryptosporidium* oocysts in the environment. Desiccation of soil in arid environments increases rates of oocysts inactivation.

### 2.6 Median Dose to Cause Infection

Median infectious dose, N50, is usually used as a measure of the “typical” dose required to infect humans. N50 varies significantly among different microorganisms. The infection dose for some pathogenic *E. coli* is relatively high (> 10^6), however low infection dose of generally ranging from 10-100 has been observed for *E. coli* O157:H7 in various studies (Mead and Griffin, 1998; Hancock et al. 1997; U.S.FDA 2002). Some other microorganisms also require substantially low doses to cause infection. For example, the median dose of *Campylobacter jejuni* is around 1,000. *Giardia lamblia* only requires a median dose of 10 and median dose of *Cryptosporidium parvum* range from 10 to 100. Adenovirus 4 has the lowest dose of less than 10. *Rotavirus* also has a median dose less than 10. Figure 2-4 shows the median dose of organisms required in drinking water to cause infection. The median doses highlight the highly infectious nature of some organisms, the detection of which is very challenging at these low levels.