Introduction to Waterborne Pathogens

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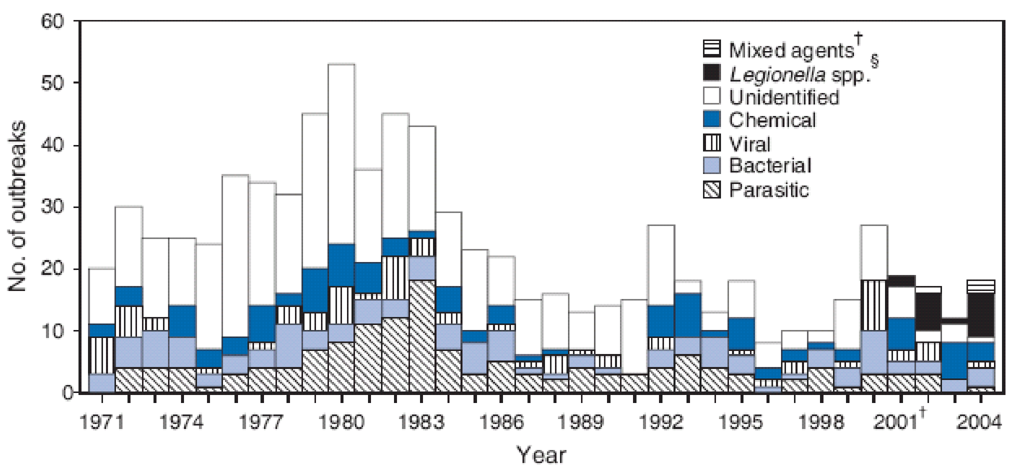
*Waterborne Pathogens*

Every year, dozens of waterborne disease outbreak occur in the United States. Statistics on the outbreaks associated with dringing water and recreational water are compiled and analyzed for the Centers for Disease Control and Prevention. This information is published biannually in the Morbidity and Mortality Weekly Reports Surveillance Summaries (http://www.cdc.gov/mmwr/). The latest report, published in 2006, includes information for 2003-2004 (CDC, 2006). Figure 1 shows a compilation of data on waterborne disease outbreaks associated with drinking water for the years 1971-2004. Causes of recreational outbreaks of illness are shown in Figure 2.

Figure 1. Number* of waterborne-disease outbreaks associated with drinking water, by year and etiologic agent — United States, 1971–2004 (CDC, 2006)

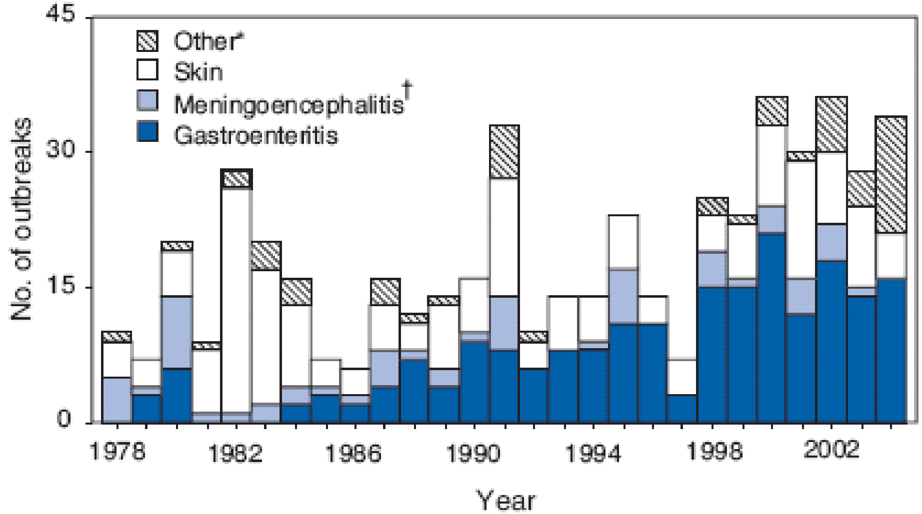



* n = 803.
† Beginning in 2003, mixed agents of more than one etiologic agent type were included in the surveillance system. However, the first observation is a previously unreported outbreak in 2002.
§Beginning in 2001, Legionnaires' disease was added to the surveillance system, and *Legionella* spp. were classified separately in this figure.

There are hundreds of different pathogens that can be transmitted by exposure to contaminated water. Many of these pathogens are enteric in nature, meaning that their primary site of infection is the intestines. Exposure to enteric pathogens is typically through consumption of food or water that contains the pathogens. In the body, the pathogen typically infects the gastrointestinal tract, the result of which is generally gastroenteritis, which has symptoms of nausea, vomiting, diarrhea, and/or fever. Some of the enteric pathogens infect other organs, such as the liver (*e.g.*, hepatitis A virus) or nervous system (*e.g.,* poliovirus), and thus cause more severe illnesses such as hepatitis and poliomyelitis. Infection by some enteric organisms has led to non-enteric sequelae. For example, *Campylobacter* infections may lead to development of Guillain-Barré syndrome, which is the most common cause of acute flaccid paralysis (a symptom of which is extreme weakness in the legs) in the United States

(Nachamkin, 2002). Another example is the production of reactive arthritis, in which the body's immune system reacts to certain bacterial infections by producing inflammation of the joints; this has been seen following infections by *Campylobacter*, Salmonellae, and Shigellae (Moe, 2002). Other pathogens, are transmitted through other routes. For example, *Pseudomonas* infections can be acquired through contact with the skin, leading to dermatitis. *Legionella* infections result from respiratory exposure. A list of some of the common waterborne enteric pathogens is shown in Table 1.

Figure 2. Number of recreational water-associated outbreaks (n = 508), by year and illness — United States, 1978–2004 (CDC, 2006)




* Includes keratitis, conjunctivitis, otitis, bronchitis, meningitis, hepatitis, leptospirosis, Pontiac fever, acute respiratory illness, and combined illnesses.
† Also includes data from report of ameba infections (**Source:** Visvesvara GS, Stehr-Green JK. Epidemiology of free-living ameba infections.J Protozool 1990;37:25S–33S).

Because the primary site of infection is the gastrointestinal tract, enteric microorganisms are shed from the body in the fecal material. The concentrations of these organisms can be very high - in the millions to billions of organisms per gram of feces (see Table 2). Pathogen excretion doesn't occur only while the infected individual is symptomatic. For many pathogens, the organisms continue to be shed in the feces for weeks to months after symptoms have disappeared. In addition, in the case of some pathogens, the microorganisms are shed in the feces prior to the appearance of symptoms. For example, the peak fecal concentrations of hepatitis A virus are found before the individual exhibits symptoms (CDC, 2007).

*Waterborne Pathogen Indicators*

Because there are hundreds of different pathogens that could be present in water, it is generally not economically, technologically, or practically possible to test water to determine whether it contains pathogens. Therefore the approach has been to use one microorganism (or group of microorganisms) to indicate whether or not a health risk exists. Different bacterial groups have been used as indicators of the microbiological quality of drinking water, and recreational waters for many years. For example, total coliform bacteria have been used for decades to assess the microbiological quality of drinking water.

Table 1. Common Waterborne Pathogens

| Organism | Source | Incubation Period | Symptoms | Duration of Illness |
|---|---|---|---|---|
| VIRUSES | | | | |
| Enteroviruses (polio, coxsackie, and echoviruses) | human feces | 1-14 days | respiratory illness, meningitis, diarrhea, encephalitis, paralytic disease | variable |
| Hepatitis A virus | human feces | 15-50 days | fever, malaise, jaundice, anorexia, nausea | 1-2 weeks – several months |
| Norovirus | human feces | 1-3 days | acute gastroenteritis | 1-3 days |
| Rotaviruses | human feces | 1-3 days | acute gastroenteritis | 3-7 days |
| BACTERIA | | | | |
| *Campylobacter jejuni* | human and animal feces | 3-5 days | acute gastroenteritis, possible Guillain-Barre syndrome, possible reactive arthritis | 1-4 days |
| *E. coli* | human feces | 0.5-6 days | watery diarrhea | 3 days-3 weeks |
| *E. coli* O157:H7 | human and animal feces | 3-8 days | watery, then bloody diarrhea; vomiting, possible hemolytic uremic syndrome | 1-12 days |
| Legionellae | freshwater, soil | Legionnaire's disease: 2-14 days Pontiac fever: 5-66 hours | Legionnaire's disease: pneumonia with anorexia, headache, chills, cough, chest pain, and diarrhea Pontiac fever: fever, chills, muscle pain | Legionnaire's disease: weeks - months Pontiac fever: 2-7 days |
| *Pseudomonas* | water | unknown | Dermatitis, ear infections, eye infections | unknown |
| Salmonellae | human and animal feces | 8-48 hours | loose, watery diarrhea; possible reactive arthritis | 3-5 days |
| Shigellae | human feces | 1-7 days | dysentery with fever; possible reactive arthritis | 4-7 days |
| *Vibrio cholerae* | human feces | 9-72 hours | profuse, watery diarrhea, vomiting, rapid dehydration | 3-4 days |
| PARASITES | | | | |
| *Cryptosporidium* | human and animal feces | 1-2 weeks | profuse, watery diarrhea | 4-21 days |
| *Entamoeba* | human feces | 2-4 weeks | abdominal pain, occasional mucoid or bloody diarrhea | weeks to months |
| *Giardia* | human and animal feces | 5-25 days | abdominal pain, bloating; loose, pale, greasy stools | 1-2 weeks - months |
| *Naegleria* | Freshwater in warm climates, soil, decaying vegetation | 3-7 days | meningoencephalitis, headache, fever, nausea, vomiting, usually fatal | 10 days |

Adapted from Moe, 2002

Table 2. Concentration and Duration of Excretion of Selected Enteric Pathogens in Feces

| Organism | Concentration in feces (#/g) | Duration of excretion (weeks) |
|---|---|---|
| *Giardia* | 1 million – 10 million | 24 |
| *Cryptosporidium* | 1 million – 10 million | 1 - 2 |
| Enterovirus | 1000 – 10 million | 2 - 16 |
| Hepatitis A virus | 100 million | 4 - 6 |
| Rotavirus | 10 billion | 1 - 3 |
| Salmonellae | 10,000 – 10 billion | 4 - 5 |
| Shigellae | 100,000 – 1 billion | 2 |

Adapted from CDC, 2007; Rusin et al., 2000

The term “indicator” or “index organism” is typically used for a surrogate that is measured instead of analyzing a sample for pathogenic microorganisms. The first set of criteria for an ideal indicator was developed in 1966 by Bonde (Bonde,1966). Bonde’s criteria are that an ideal indicator should:

- Be present whenever the pathogens are present;
- Be present only when the presence of pathogens is an imminent danger, *i.e.* they must not proliferate to any greater extent in the aqueous environment;
- Occur in much greater numbers than the pathogens;
- Be more resistant to disinfectants and to the aqueous environment than the pathogens;
- Grow readily on simple media;
- Yield characteristic and simple reactions enabling as far as possible an unambiguous identification of the group;
- Be randomly distributed in the sample to be examined, or it should be possible to obtain a uniform distribution by simple homogenization procedures; and
- Grow widely independent of other organisms present, when inoculated in artificial media, *i.e.*, the indicator bacteria should not be seriously inhibited in their growth by the presence of other bacteria.

Payment *et al.* (2003) have modified Bonde’s original criteria in response to newly-identified uses for indicators and new analytical methods that have been developed. These criteria include:

- Association with fecal material (*i.e.,* pathogens)
- Risk to analyst
- Speed of measurement
- Cost
- Technical difficulty
- Survival in the environment
- Resistance to treatment

In a recent report by a committee of the National Research Council’s Water Science and Technology Board, the characteristics of the ideal indicator were divided into two categories by separating the desirable attributes of the methods from the desirable attributes of the indicators themselves (NRC, 2004). Desirable attributes of the methods to detect the indicators are (NRC, 2004):

- Specificity (independent of matrix effects)
- Broad applicability
- Precision
- Adequate sensitivity
- Rapidity of results
- Quantifiable
- Measures viability or infectivity
- Logistical feasibility

The desirable biological attributes of indicators are (NRC, 2004):

- Correlated to health risk
- Similar (or greater) survival to pathogens under environmental conditions
- Similar (or greater) transport to pathogens
- Present in greater numbers than pathogens
- Specific to a fecal source or identifiable as to source of origin

It is critical that the choice of an indicator be based on the particular application of interest. Potential applications of an indicator include being an indicator of (Yates, 2007):

- fecal contamination;
- the presence of domestic sewage;
- the presence of pathogens;
- the efficiency of a particular water or waste treatment process;
- the environmental fate of a pathogen of interest;
- the movement of particles suspended in water during subsurface transport

Using their modified criteria, Payment *et al.* (2003) have assessed the applicability of many of current and proposed indicators and pathogens for a variety of uses, as shown in Table 3. This systematic evaluation clearly shows that different indicators are better suited for some purposes than others, and that some indicators should not be used in certain situations.

*Monitoring for Waterborne Pathogens*

The direct detection of pathogens, if feasible, would seem to be the best option for obtaining data to allow a thorough assessment of the public health impact for a waterborne pathogen. With the relatively recent application of a number of molecular methods to the detection of microorganisms in environmental samples, direct pathogen monitoring is a more feasible (from a technological viewpoint) approach for assessing the microbiological quality of water than it has been in the past.

However, direct pathogen detection is not without its limitations. For example, as stated by Payment *et al.* (2003), because this approach depends on detecting an *actual* risk (i.e., the pathogen itself), rather than a *potential* risk (which detection of the indicator suggests), it does not provide the same level of public health protection as that obtained by indicator organisms. In addition, even with the advancements in molecular biology, such as microarrays, it is not possible to detect all known pathogens in a water sample. Reliance on molecular methods requires knowledge of the pathogens for which one is searching, and thus the risk from currently unknown pathogens cannot be assessed. Another drawback of most, if not all, of the molecular methods is that they can only analyze a very small volume of water; *i.e.,* a few microliters or less.

From a public health risk standpoint, the microbiological quality of hundreds of liters of water is of interest.

Table 3. Microbial parameter applicability and suitability (from Payment *et al.*, 2003)

| Parameter | Sanitary survey (catchment) | Source water characterization | Groundwater characterization | Treatment efficacy (removal) | Treatment efficiency (disinfection) | Treated water | Distribution system (ingress) | Distribution system (regrowth) | Outbreak investigation |
|---|---|---|---|---|---|---|---|---|---|
| Total coliforms | NR | NR | NR | NR | SA | S | SA* | S | S |
| *Escherichia coli* | S | S | S | S | S | SA | S* | NA | S |
| Fecal streptococci (enterococci) | SA | SA | NA | NA | NA | NA | NA | NA | S |
| Heterotrophic bacteria | NA | NA | NA | S | S | NR | S | S | S |
| Somatic coliphages | SA | SA | SA | NA | SA | NA | NA | NA | S |
| F-specific RNA phages | SA | SA | SA | NA | SA | NA | NA | NA | S |
| *Bacteroides* phages | SA | SA | SA | NA | SA | NA | NA | NA | S |
| *Clostridium perfringens* | SA | SA | SA | SA | NA | NA | NA | NA | S |
| Enteric viruses | S | S | S | NR | NR | NA | NA | NA | S |
| *Giardia* cysts | S | S | SA | S | NR | NA | NA | NA | S |
| *Cryptosporidium* oocysts | S | S | SA | S | NR | NA | NA | NA | S |

Key: S: suitable; *: in distribution systems without residual disinfection; SA: suitable alternative; NR: not recommended; ISD: insufficient data; NA: not applicable

If one desires to calculate the risk of infection or illness from a waterborne pathogen, one must have quantitative information on the presence of infectious organisms. In the case of some pathogens, such as viruses, this means that a cultural method must be used, as detection of viral nucleic acid does not necessarily correlate with infectivity. A comparison of some of the features of methods used to detect viruses is shown in Table 4.

Table 4. Virus Detection Methods

| Method | Infectivity Test? | Detection Limit | Time Required |
|---|---|---|---|
| Electron microscopy | no | 100,000 – 1 million | <24 hours |
| ELISA | | | |
| viral antigens | no | 100,000 | < 2 hours |
| antiviral antibodies | yes | 100,000 | < 2 hours |
| Real-time (RT)PCR | no | 1-10 | < 8 hours |
| Plaque assay | yes | 1-10 | ≤ 21 days |

While the incentive for direct detection of pathogens to conduct risk assessments, obtain source water prevalence or occurrence data, or determine treatment level required to protect public health may be high, direct pathogen detection is seldom done on a routine basis. This is clear from the relatively limited data on pathogen occurrence in water. Some direct monitoring data are available for a few pathogens, namely, *Cryptosporidium* and *Giardia*, the culturable enteroviruses, and a few enteric bacteria. Direct detection of pathogens has been used for several nationwide occurrence studies, primarily in raw water, by several research groups or as part of the Information Collection Rule (USEPA, 1995), the Supplementary Surveys and in the near future, as part of the Long-Term- 2 Enhanced Surface Water Treatment Rule (USEPA, 2006). Some water utilities have established routine monitoring programs for these organisms, but these programs are conducted voluntarily and with relatively low monitoring frequency, typically once per month.

Clearly, there are a number of issues to consider and choices to make when establishing a program to monitor the microbiological quality of water. The purpose for which the monitoring is being conducted will dictate some of those choices, but consideration of available resources and personnel are also important. No one indicator or pathogen can provide all of the information that may be desired, and there are limitations associated with any choice that is made. Therefore, it is important to spend time carefully planning the project, by gathering the available information and seeking the advice of experts, before beginning any monitoring program.

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