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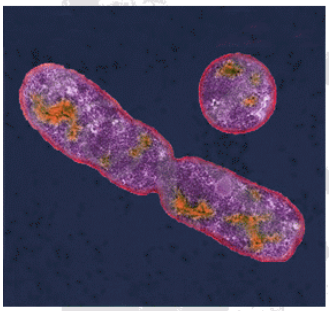
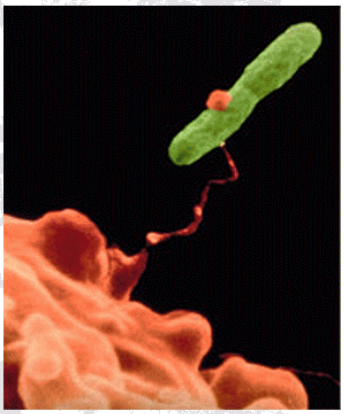
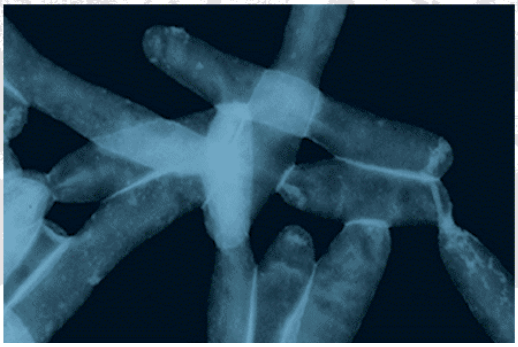
Legionella: Human Health Criteria Document







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I. Summary

This document was prepared to update information in the Environmental Protection Agency's (EPA) *Drinking Water Criteria Document on Legionella* (EPA 1985) and is intended to serve as an addendum to that report. Where appropriate, a summary of relevant information from the 1985 document is presented in each chapter of this addendum. For a more detailed description of information published before 1986, please refer to the 1985 document. This chapter presents a summary of the information contained in Chapters II through VII. Chapter VIII contains a discussion of research recommendations and Chapter IX lists references.

Legionella bacteria are aerobic gram-negative rods associated with respiratory infections. *Legionella pneumophila* was first recognized as a disease entity from a pneumonia outbreak at a 1976 Convention of the American Legion in Philadelphia. Of the 42 known species of *Legionella*, 18 have been linked to pneumonia infections in humans. The species *L. pneumophila* (particularly serogroups 1-6) has been accepted as the principal cause of human outbreaks of legionellosis, which includes both legionnaires' disease and Pontiac fever.

Legionella are ubiquitous in natural aquatic environments, capable of existing in waters with varied temperatures, pH levels, and nutrient and oxygen contents. They can be found in groundwater as well as fresh and marine surface waters. Their widespread survival in nature can be attributed to their relationships with other microorganisms in the environment. Symbiotic existence with algae and other bacteria, particularly in biofilms, increases the availability of nutrients. They also are able to infect protozoans and subsequently reproduce within these organisms. These relationships provide protection against adverse environmental conditions, including standard water disinfection techniques. Consequently, *Legionella* are also prevalent in anthropogenic waters such as potable water, cooling tower reservoirs, and whirlpools.

Aerosol-generating systems such as faucets, showerheads, cooling towers, and nebulizers aid in the transmission of *Legionella* from water to air. Human inhalation of contaminated aerosols leads to *Legionella* infections and disease outbreaks. Historically, many of the reported outbreaks were nosocomial (i.e., hospital-acquired), resulting from the adulteration of hospital potable water supplies, air conditioning systems, or cooling towers. Due to increased awareness of the disease, numerous community-acquired and travel-acquired outbreaks are now reported each year as well. However, most cases of legionnaires' disease are sporadic (i.e., non-outbreak related) and are acquired in the community.

Collection of *Legionella* from natural environmental samples, anthropogenic sources such as plumbing fixtures and potable water systems, and biological specimens is generally done by taking swab samples. These samples are typically concentrated by filtration, treated with an acid buffer, and isolated on a BCYE agar culture medium. An array of serological tests then are used to detect the bacteria. The most commonly used tests are direct and indirect immunofluorescence assays; however, new techniques are consistently being developed and improved upon as well.

The health effects of *Legionella* contamination have been studied in animals as well as in humans. Experimental studies on guinea pigs and other animals have been conducted to better understand human infection by *Legionella*, even though animals are not naturally infected by the bacteria. Infected animals hosting *Legionella* in their lungs experience impaired respiratory system performance as a result of the disease process. Clinical features include hypoxia, fever, seroconversion, and weight loss.

Legionella infection in humans occurs when bacteria are inhaled or aspirated into the lower respiratory tract and subsequently engulfed by enteric pulmonary macrophages. The bacteria rapidly reproduce within the macrophages and are eventually released when the host cell lyses. Recent research indicates that the ability of *Legionella* to infect certain strains of amoeba is a factor in their infection of human lung tissue, as the amoeba provides a habitat within the pulmonary system in which the bacteria can live and reproduce. Resistance to *Legionella* infection is mainly cell-mediated, although humoral immune responses may also play a role. Legionellosis in humans has typically been characterized as either an acute self-limiting, non-pneumonic condition known as Pontiac fever or a potentially fatal pneumonic condition known as legionnaires' disease. Timely treatment of legionnaires' disease is extremely important for a patient's recovery. Although erythromycin has historically been used to treat patients with legionnaires' disease, newer macrolides and quinolones are gaining acceptance as the first choice for treatment.

In terms of risk assessment, it is important to realize that the most prevalent source of *Legionella* transmission is potable water from large buildings, particularly hospitals. Thus, although *Legionella* are widely distributed in both natural and man-made water systems, transmission to humans from a water source results mainly from inhalation or aspiration of aerosolized contaminated potable water. Potential risks caused by *Legionella* in water supplies are not quantifiable by the measures of modern science. However, preventative and corrective actions have been discovered and implemented to protect the population, especially highly susceptible individuals (e.g., immunosuppressed people, certain hospital patients). The most effective measures of treatment have proven to be a combination of systemic sanitization of entire water systems (e.g., thermal

disinfection, hyperchlorination, copper-silver ionization) and focal disinfection of specific portions of those systems (e.g., UV light sterilization, instantaneous heating systems, ozonation). These treatment procedures are very useful in preventing the recolonization of *Legionella* in most water distribution systems.

Fresh, innovative methods of detection and treatment of *Legionella* in water supplies and sources are consistently being uncovered and tested. In addition, new medications are being developed to treat patients overcome with legionnaires' disease. Substantial advancements have been made since the 1985 report, and modern science presses on with goals of further understanding *Legionella* and legionnaires' disease and the eventual eradication of *Legionella* colonies in water distribution systems.

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II. General Information and Properties

A. History

In January 1977, Joseph McDade of the U.S. Centers for Disease Control (CDC) discovered a novel bacteria while investigating the unexplained pneumonia outbreak at the 1976 American Legion Convention in Philadelphia (Brenner 1987). Of those attending the convention, 221 became ill with pneumonia, and 34 of those affected died. The aerobic gram-negative bacteria isolated from infected post-mortem lung tissue and identified as the causative agent of this pneumonia outbreak was later called *Legionella pneumophila*, receiving the name *Legionella* to honor the stricken American legionnaires and *pneumophila* from the Greek word meaning "lung-loving" (Fang et al. 1989).

The symptoms exhibited in the 1976 outbreak were termed Legionnaires' disease. Humans can be affected by *Legionella* bacteria in two ways: (1) a potentially fatal multi-system disease involving pneumonia (legionnaires' disease) and (2) a self-limited influenza-like infection (Pontiac fever) (Hoge and Brieman 1991). Pneumonia occurs in approximately 95 percent of *Legionella* infections (Nguyen et al. 1991).

Subsequent to finding *L. pneumophila*, additional investigations ensued to determine whether prior undetected outbreaks had occurred. Research revealed five additional outbreaks of legionellosis (i.e., diseases caused by *Legionella*), which were attributed to *L. pneumophila.* The first occurred in 1965 at St. Elizabeth's Hospital in Washington, D.C. Eighty-one patients became ill with pneumonia, and 14 died (Lowry et al. 1993). The second pneumonia outbreak occurred in 1973 in Benidorm, Spain, and the third occurred in 1974 in the same hotel as the Philadelphia outbreak of 1976. In addition, two outbreaks of Pontiac fever occurred, one in Pontiac, Michigan, in 1968 and the other in 1973 in James River, Virginia. Aside from outbreaks, sporadic cases of legionellosis were detected in 1943, 1947, and 1959 (Brenner 1987).

Within two years of identifying *L. pneumophila*, the second species of *Legionella*, *L. micdadei*, was discovered (Dowling et al. 1992). In the following years, advances in growth and enrichment media, combined with clinical and environmental studies, allowed for the discovery of numerous species of *Legionella* (Brenner 1987).

B. Taxonomy

DNA-DNA hybridization studies, as well as unique cellular fatty acid composition, indicated that the bacteria causing the pneumonia outbreak of 1976 should be classified as a new species. At the First International Symposium on Legionnaires' Disease, held in 1978, the bacteria received the name *Legionella pneumophila* and became apart of the new family *Legionellaceae* (Bangsborg 1997, Brenner 1986).

The 1985 *Legionella* Criteria Document discusses the taxonomic approaches and diagnostic techniques used to classify *Legionella* species. Molecular techniques used include DNA hybridization, genomic DNA size comparison using (Guanine+Cytosine) content, oligonucleotide cataloguing of 16s rRNA, and plasmid analysis (EPA 1985). Comparison of bacterial DNA and the use of antigenic analysis of proteins and peptides are the best current methods to classify *Legionella* species, although some phenotypic characteristics (i.e., gram reactivity, cell membrane fatty acid and ubiquinone content, morphology, and growth on specific media) can be used to recognize bacteria at the genus level (Bangsborg 1997, Fang et al. 1989, Winn 1988).

Following the initial identification of *L. pneumophila* in 1977, numerous species have been discovered within the *Legionella* genus. The 1985 *Legionella* Criteria Document listed 22 species within the genus. Currently, the genus consists of 42 species, seven of which can be further divided into serogroups (Bangsborg 1997). The bacterial strains within a species that can be divided by serotype are genetically homologous (based on DNA hybridization experiments), but can be differentiated by specific reactivity to antibodies (EPA 1985). Eighteen of the 42 species of *Legionella* have been linked to patients with pneumonia (Bangsborg 1997). A majority of human infections (70-90%) have been caused by *L. pneumophila*, especially serogroups 1 and 6 (Lo Presti et al. 1997). Table II-1 is a compilation of species information.

Table II-1. Approved *Legionella* Species

Name	Implicated in Human Disease?
L. adelaidensis	No
L. anisa	Yes
L. birminghamensis	Yes
L. bozemanii * (Fluoribacter bozemanae) SG 1-2	Yes
L. brunensis	No
L. cherrii	Yes
L. cincinnatiensis	Yes
*L. dumoffii** (Fluoribacter dumoffii)	Yes

Name	Implicated in Human Disease?
L. erythra	No
L. fairfieldensis	No
*L. feelei** SG 1-2	Yes**
L. geestiana	No
*L. gormanii** (Fluoribacter gormanii)	Yes
L. gratiana	Yes
L. hackeliae SG 1-2*	Yes
L. israelensis	No
L. jamestowniensis	No
L. jordanis	Yes
L. lansingensis	Yes
L. londiniensis SG 1-2	No
L. longbeachae SG 1-2	Yes
*L. lytica**	Yes
L. maceachernii (Tatlockia maceachernii)	Yes
*L. micdadei** (Tatlockia micdadei)	Yes
L. moravica	No
L. nautarum	No
*L. oakridgensis**	Yes
L. parisiensis	Yes (Lo Presti et al. 1997)
L. pneumophila SG 1-16*	Yes
L. quateirensis	No
L. quinlivanii SG 1-16	Yes
L. rubrilucens	No
L. sainthelensi SG 1-2	Yes
L. santicrucis	Yes
L. shakespearei	No
L. spiritensis	No
L. steigerwaltii	No
L. tucsonensis	Yes
L. wadsworthii	Yes

Name	Implicated in Human Disease?
L. walteri sii	No
L. worsleiensis	No

Source: Bangsborg 1997, unless otherwise noted

SG = serogroup
* = species with experimentally documented ability to parasitize amoeba
** = causes Pontiac fever, but rarely pneumonia (Lo Presti et al. 1998)

An ongoing controversy about the taxonomy of the *Legionellaceae* family involves the single genus designation. Because several species have a unique phenotypic characteristic (blue white fluorescence in UV light) and very low DNA-DNA hybridization homology to *L. pneumophila*, two additional genera, Tatlockia and Fluoribacter, have been proposed by Garrity et al. and Brown et al. (see Table II-1 for the accepted and proposed names) (Bangsborg 1997). The new genera have not been accepted by the mainstream scientific community, but Bangsborg (1997) suggested that the classifications may be justified.

The method for determining whether two organisms are of the same genus and/or species is based on DNA-DNA hybridization studies of *Enterobacteriaceae* (Bangsborg 1997). Members of the same species are indicated by 70 percent or greater homology under optimal reaction conditions or 60 percent homology under stringent conditions; 25-60 percent homology indicates genus member status. The *Legionella* species in the proposed Tatlockia and Fluoribacter genuses share less than 25 percent DNA sequence homology with *L. pneumophila,* suggesting the need for new genera. However, many argue that the use of DNA-DNA hybridization is not an effective method to distinguish between genera, since the technology is most accurate for organisms more closely related. Furthermore, the species in dispute exhibit phenotypic characteristics present in the *Legionella* species. Finally, infection with these species results in the same human disease and is treatable with the same antibiotics as all other *Legionella* species (Bangsborg 1997).

Bangsborg (1997) examined the multi-genus argument by using crossed immuno-electrophoresis of proteins from *Legionella* species. Three rabbit antibody preparations, one against *L. pneumophila,* a second against *L. micdadei,* and a third against *L. bozemanii, L. dumoffii,* and *L. gormanii* were used on the electrophoresed proteins. Findings based on the antigenic profiles suggest that creating the *Tacklockia* and *Fluoribacter* genera is warranted. Further taxonomic investigation is necessary to clarify this debate.

Finally, identification of species isolates is another highly important taxonomic area of study, since determining sources of outbreaks is essential to public safety. Molecular methods have been used to identify individual isolates and will be discussed in Chapter VII, Analysis and Treatment of *Legionella*.

C. Microbiology, Morphology, and Ecology

All *Legionella* species appear as small rods, faintly staining gram-negative. They are unencapsulated, nonsporeforming, with physical dimensions from 0.3 to 0.9 m in width and from 2 to 20 m in length (Winn 1988). Most exhibit motility through one or more polar or lateral flagella. *Legionella* cell walls are unique from other gram-negative bacteria in that they contain significant amounts of both branched-chain cellular fatty acids and ubiquinones with side chains of more than 10 isoprene units. These bacteria are aerobic, microaerophillic, and have a respirative metabolism that is non-fermentative and is based on the catabolism of amino acids for energy and carbon sources (Brenner et al. 1984).

Ubiquitously found in nature, *Legionella* species exist primarily in aquatic environments, although some have been isolated in potting soils and moist soil samples (Fields 1996). *Legionella* can survive in varied water conditions, in temperatures of 0-63 °C, a pH range of 5.0-8.5, and a dissolved oxygen concentration in water of 0.2-15 ppm (Nguyen et al. 1991).

Even though *Legionella* are ubiquitous in nature, they have specific growth requirements for culturing. The 1985 EPA *Legionella* Criteria document provides a detailed explanation of the process of determining appropriate growth media to sustain *Legionella* bacterial growth. A typical media used to grow *Legionella* is charcoal yeast extract (BCYE) agar supplemented with -ketoglutarate, L-cysteine, iron salts, and buffered to pH 6.9 (EPA 1985). Bangsborg (1997) also provides information about *Legionella* growth mediums. The BCYE agar can be further supplemented with antibacterial agents to suppress microflora (cefamandole and vancomycin to inhibit gram-positive bacteria and polymyxin B to inhibit gram-negative bacteria), antifungal agents (anisomycin for yeast), and inhibitors (glycine) (Nguyen et al. 1991). However, some antibiotics can be detrimental to *Legionella* growth. For example, cefamandole can inhibit *L. micdadei* and several strains of *L. pneumophila* (Winn 1993). In addition, pretreatment of respiratory tract specimens with acid before culturing can be very useful in selecting for *Legionella,* since these bacteria exhibit acid resistance, unlike most other bacteria (Nguyen et al. 1991). Optimal temperatures for culturing are 35-37°C (EPA 1985). Bacterial growth

can be enhanced in a culturing environment with a CO_2 concentration from 2.5- 5 percent, but not in excess of 8-10 percent, which can be inhibitory (EPA 1985, Winn 1993).

D. Symbiosis in Microorganisms

Experiments have demonstrated that *Legionella* in sterile tap water show long-term survival but do not multiply, whereas *Legionella* in non-sterile tap water survive and multiply (Surman et al. 1994). Furthermore, *Legionella* viability is maintained when they are combined with algae in culture, whereas *Legionella* viability decreases once the algae are removed (Winn 1988). *Legionella* proliferation is dependent on their relationships with other microorganisms.

The first evidence that *Legionella* share a symbiotic relationship with other microorganisms came with the discovery of *L. pneumophila's* co-existence in an algal mat from a thermally polluted lake (EPA 1985). In contrast, *Legionella* survive almost entirely as parasites of single-celled protozoa (Fields 1996). This relationship first became apparent to Rowbotham in 1980, with the demonstration of *L. pneumophila's* ability to infect two types of amoeba, *Acanthamoeba* and *Naegleria* (EPA 1985). Currently, *Legionella* can infect a total of 13 species of amoebae and two species of ciliated protozoa (Fields 1996). Table II-1 indicates species of *Legionella* that have been shown experimentally to infect amoeba.

Legionella also can multiply intra-cellularly within protozoan hosts (Vandenesch et al. 1990). *Legionella* strains that multiply in protozoa have been shown to be more virulent, possibly due to increased bacterial numbers (Kramer and Ford 1994). The ability to infect and proliferate within hosts provides *Legionella* with protection from otherwise harmful environmental conditions. Therefore, they survive in habitats with a greater temperature range, are more resistant to water treatment with chlorine, biocides and other disinfectants, and survive in dry conditions if encapsulated in cysts. Enhanced resistance to water treatment has major implications for disease transmittance and water treatment procedures.

Legionella also grow symbiotically with aquatic bacteria attached to the surface of biofilms (Kramer and Ford 1994). Biofilms provide the bacteria with protection from adverse environmental conditions (including during water disinfection) and nutrients for growth. The concentration of *Legionella* in biofilms depends upon water temperature; at higher temperatures, they can more effectively out compete other bacteria. *Legionella* have been found in biofilms in the absence of amoeba (Kramer and Ford 1994). Because biofilms colonize

drinking water distribution systems, they provide a habitat suitable for *Legionella* growth in potable water, which can lead to human exposure.

III. Occurrence

Because routine culturing for *Legionella* in the environment is not a common practice, the occurrence of these bacteria is often indicated by outbreaks or sporadic cases of legionellosis (i.e., any disease caused by *Legionella*). Therefore, this chapter considers the worldwide occurrence or incidence of legionellosis (Section A) and outbreaks of legionellosis (Section E) as well as the occurrence of *Legionella* bacteria in water (Sections B), soil (Section C), and air (Section D). Environmental factors influencing *Legionella* survival are discussed in Section F.

A. Worldwide Distribution

Legionellosis has been reported to occur in North and South America, Asia, Australia, New Zealand, Europe, and Africa (Edelstein 1988). The true incidence of legionellosis is difficult to determine because identification of cases requires adequate surveillance. Research suggests that legionnaires' disease is under reported to national surveillance systems (Marston et al. 1994; Edelstein 1988). Its recognition depends on physician awareness of the disease and resources available to diagnose it.

Although legionellosis is widely distributed geographically throughout the world, most cases have been reported from the industrialized countries. The ecological niches that support *Legionella* (complex recirculating water systems and hot water at 35-55°C) are not as common in developing countries, so the incidence of legionellosis may be comparatively low in these countries (Bhopal 1993). However, most geographical variation in the incidence of legionellosis is probably artifact due to differences in definitions, diagnostic methods, surveillance systems, and data presentation (Bhopal 1993).

The 1985 *Legionella* Criteria Document focused mainly on the distribution of legionellosis in the United States because, at that time, national surveillance data for the United States were available from the Centers for Disease Control (CDC), whereas surveillance programs in many other countries had not yet been developed. Surveillance in England and Wales began in 1979, but these data were not included in the 1985 report. Since 1985, many European countries as well as Australia and New Zealand have implemented surveillance programs to monitor the occurrence of legionellosis. Recent findings of national surveillance programs are summarized below.

United States

The CDC first began collecting data on the occurrence of legionellosis in 1976. The 1985 *Legionella* Criteria Document provides a detailed summary of the occurrence and distribution of legionellosis in the United States through 1983. Data regarding the occurrence of legionellosis in the United States reported to CDC from 1984-1996 are summarized in Table III-1 and in Figure III-1. In the United States, the number of cases per million population rose from 3.5 in 1984 to a peak of 6.3 in 1994 and then began to decline to 4.7 in 1996.

An analysis of data reported to the CDC during the period 1980-1989 examined 3,524 confirmed cases of legionnaires' disease in the United States (Marston et al. 1994). Disease rates did not vary by year, but rates were higher in northern states and during the summer. *L. pneumophila*, serogroup 1, constituted 71.5 percent of the fully identified isolates of *Legionella*. Risk factors for morbidity and/or mortality included older age, male gender, African-American ethnicity, smoking, nosocomial acquisition of the disease, immunosuppression, end-stage renal disease, and cancer (see Chapter VII, Section D for further discussion of risk factors).

Marston et al. (1994) also concluded that legionnaires' disease is under reported to the CDC. They cite two studies in which diagnostic tests for legionellosis were routinely performed; *Legionella* infections accounted for 3.4 and 4.6 percent of community-acquired pneumonia cases requiring hospitalization. By projecting this proportion to the estimated total number of community-acquired pneumonia cases in the United States annually (500,000 cases), they estimate that there would be 17,000-23,000 cases of legionnaires' disease leading to hospitalization annually. However, fewer than 500 cases of legionnaires' disease are reported to the CDC annually; therefore, the surveillance system detects fewer than 5 percent of *Legionella* pneumonia cases in the United States.

Table III-1. Summary of Reported Cases of Legionellosis in the United States, 1984-1996

Year	Number of Cases	Cases per Million Population
1984	750	3.5
1985	830	3.7
1986	980	4.3
1987	1,038	4.3

1988	1,085	4.4
1989	1,190	4.8
1990	1,370	5.5
1991	1,317	5.3
1992	1,339	5.3
1993	1,280	5.0
1994	1,615	6.3
1995	1,241	4.8
1996	1,198	4.7

Sources: CDC 1994, CDC 1996, CDC 1997b

Figure III-1. Summary of Reported Cases of Legionellosis in the United States, 1984-1996

1984	3.5
1985	3.7
1986	4.3
1987	4.3
1988	4.4
1989	4.8
1990	5.5
1991	5.3
1992	5.3
1993	5
1994	6.3
1995	4.8
1996	4.7

Sources: CDC 1994, CDC 1996, CDC 1997b

United Kingdom

Legionnaires' disease is not a statutorily notifiable disease in England and Wales; therefore, cases are reported on a voluntary basis. The National Surveillance Scheme for Legionnaires' Disease for residents of England and Wales was set up in 1979 by the Public Health Laboratory Service (PHLS) Communicable Disease Surveillance Centre (CDSC), and data have been collected each year since. In addition, the PHLS CDSC obtains information about cases of legionnaires' disease in residents of England and Wales that are the result of travel, either abroad or in the United Kingdom, from the European Surveillance Scheme for Travel-Associated Legionnaires' Disease, which was established in 1987. Data on the occurrence of legionnaires' disease in residents of England and Wales in 1996 were reported in the Communicable Disease Report (Joseph et al. 1997) and are summarized below.

In 1996, 201 cases of legionnaires' disease were reported to the PHLS CDSC (Joseph et al. 1997). The number of cases associated with various sources of infection were: 101 (50%) cases resulting from travel, either abroad or in the United Kingdom; two (1%) hospital-acquired cases; and 98 (49%) community-acquired cases. The number of cases linked to outbreaks or clusters was 55 (27%), and the remaining 146 cases (73%) were reported as single cases. Six outbreaks were associated with industrial sites, and nine outbreaks or clusters were associated with travel.

A total of 3,005 cases of legionnaires' disease in residents of England and Wales were reported during the period 1980-1996 (Joseph et al. 1997). Overall, travel and community cases each accounted for 46 percent, and hospital-acquired infections accounted for the remaining 8 percent. The annual totals of reported cases fell between 1989 and 1991, following a peak of 279 cases reported in 1988. Since 1993, the annual totals have been increasing; there was a sharp increase in the number of cases of legionnaires' disease reported in 1996 (201 cases) compared to 160 in 1995. The 201 cases reported in 1996 was the highest total recorded since 1989. Cases associated with travel abroad accounted for the second highest number of travel cases reported since 1980, and community-acquired cases the largest since 1989. In contrast, the number of hospital-acquired cases was lower in 1996 than in any of the previous years.

Legionnaires' disease has been a notifiable disease in Scotland since 1988 (Joseph et al. 1997); data are reported to the Scottish Centre for Infection and Environmental Health (SCIEH). The most recent data available are for 1996, which were summarized in the Communicable Disease Report (Christie 1997).

In 1996, 24 cases of legionnaires' disease in residents of Scotland were reported to SCIEH (Christie 1997). A total of 15 cases resulted from travel, 13 from travel outside the UK and two from travel within the

UK. The travel cases were associated with three clusters and two linked groups (cases linked to the same accommodation but who became ill more than six months apart). There were no cases of hospital-acquired infection in 1996. Two cases may have been associated with workplace exposure (article does not specify occupation). The remaining seven cases were presumed by the author to have been acquired in the community. The total number of cases reported in Scotland in 1996 (24) is 14 fewer than in 1995.

Europe

Since 1993, 24 collaborating European countries have been submitting information on cases of legionnaires' disease in Europe through completion of the annual reporting forms prepared by the PHLS CDSC in London. The annual results for 1996 were reported in the Weekly Epidemiological Record (Anonymous 1997b) and are summarized below.

In 1996, 1,566 cases of legionnaires' disease were reported in 24 European countries including England, Wales, and Scotland (Anonymous 1997b). The number of cases as well as the rate of infection for each of the 24 countries is shown in Table III-2. Four countries reported more than 100 cases each: Spain, 430; France, 294; England and Wales, 200; and Germany (North and South-East), 181. The highest rates of infection (per million) occurred in Germany (30.17), Croatia (16.00), Denmark (14.40), Spain (11.03), Greece (7.00), and France (5.25). In all other countries, the rate of infection was less than 5.00 per million population.

In 1996, there were nearly 300 more cases than in 1995 and nearly 400 more cases than in 1994 (Anonymous 1997b). The increase was attributed mainly to a large community outbreak in Spain in 1996. In addition, the average European rate of 4.45 cases per million population in 1996 reflected an increase of almost 1 case per million population from 1995.

Table III-2. Legionnaires' Disease in 24 European Countries in 1996

Country	Cases	Population (millions)	Rate per Million Population
Austria	20	8	2.50
Belgium	16	10	1.60
Croatia	24	1.5	16.00
Czech Republic	12	10.5	1.14

Country	Cases	Population (millions)	Rate per Million Population
Denmark	72	5	14.40
England and Wales	200	52	3.85
Finland	18	5	3.60
France	294	56	5.25
Germany (North and South-east)	181	6	30.17
Greece	7	1	7.00
Ireland	0	3.5	0.00
Italy	84	57	1.47
Malta	0	0.4	0.00
Netherlands	40	15.5	2.58
Northern Ireland	0	1.6	0.00
Norway	1	4.3	0.23
Portugal	16	10	1.60
Russian Federation (Moscow)	45	10	4.50
Scotland	24	5	4.80
Slovakia	3	5	0.60
Spain	430	39	11.03
Sweden	40	9	4.44
Switzerland	26	7	3.71
Turkey	13	30	0.43
Total	**1,566**	**352.3**	**4.45**

Source: Anonymous 1997b

The distribution of cases between various sources of infection were: 16 percent of cases resulting from travel; 6 percent hospital-acquired cases; 40 percent community-acquired cases; and 38 percent of unknown origin (Anonymous 1997b). The proportion of community-acquired cases rose from 16 percent in 1994 and 21 percent in 1995 to 40 percent in 1996 largely due to a decline in the proportion of cases from unknown origin, which represented 55 percent in 1994, 50 percent in 1995, and 38 percent in 1996.

In 1996, individual European countries detected 22 outbreaks: two linked to hospitals, eight to the community, and 12 to travel (Anonymous 1997b). This distribution represents a decline in nosocomial outbreaks and a rise in community outbreaks in comparison to 1995 data. The number of outbreaks and the

number of cases linked to outbreaks may be largely under reported because many countries are still unable to provide any epidemiological data associated with the cases of legionnaires' disease they diagnose. For example, the European Surveillance Scheme for Travel-Associated Legionnaires' Disease detected around 20 travel-related outbreaks, whereas individual countries detected only 12 travel-related outbreaks. It also is likely that many industrial-related community outbreaks remain undetected in countries without enhanced surveillance.

The majority of European cases (75%) reported in 1996 were caused by *L. pneumophila*, serogroup 1 (Anonymous 1997b). *L pneumophila* of other or undetermined serogroups accounted for 18 percent, and the remaining 7 percent were attributed to other or unknown *Legionella* species.

Australia

The Communicable Diseases Network Australia New Zealand collects data on cases of legionellosis in Australia and New Zealand as part of the National Notifiable Diseases Surveillance System. There have been 1,041 notifications of legionellosis in Australia since 1991, with similar numbers of cases reported each year (Anonymous 1997a). Since 1995, 255 notifications provided species identification. The majority of cases were caused by *L. pneumophila* (41%); however, at least 22 percent of legionellosis cases were attributed to *L. longbeachae* (2 percent of cases attributed to other species, and 35 percent of cases not speciated). The report suggests that these data indicate a microbiological difference in the incidence of legionellosis in Australia because *L. pneumophila* has been reported as responsible for at least 90 percent of legionellosis infections in other countries (Anonymous 1997a).

B. Occurrence in Water

The 1985 *Legionella* Criteria Document states that *Legionella* are widely distributed in the aqueous environment in the United States and, apparently, wherever they are sought (EPA 1985). Since 1985, research has revealed that *Legionella* thrive in biofilms, and interaction with other organisms in biofilms is essential for their survival and proliferation in aquatic environments (Kramer and Ford 1994, Yu 1997, Lin et al. 1998a). *Legionella* survival is enhanced by symbiotic relationships with other microorganisms; sediment within biofilms stimulates the growth of these commensal microflora, which stimulate the growth of *Legionella* (see Section F in this chapter for further discussion of symbiotic microorganisms). This section considers the specific occurrence of *Legionella* in natural water bodies (surface water and groundwater) as well as man-made waters (e.g., potable water, cooling towers, whirlpools, etc.).

1. Natural Surface Water

Legionella are considered to be ubiquitous in the aqueous environment, although few studies examine natural nonepidemic surface waters for their presence. The 1985 *Legionella* Criteria Document cited several studies that clearly demonstrate the widespread occurrence of *Legionella* from natural surface freshwater sources (e.g, lakes and streams) in the United States. At the time of the 1985 report, there was little evidence that the marine environment is a normal habitat for *Legionella* although they had been isolated from estuarine waters in Puerto Rico (EPA 1985). More recent studies indicate that *Legionella* are fairly common in marine waters (Ortiz-Roque and Hazen 1987, Palmer et al. 1993).

Ortiz-Roque and Hazen (1987) investigated the occurrence of *Legionella* at twenty-six sampling sites in Puerto Rico (16 marine, 8 freshwater, and 2 estuarine). *L. pneumophila* was the most abundant species at all sites, with highest densities reported for sewage-contaminated coastal waters. *L. pneumophila* was found in densities several orders of magnitude higher than those in corresponding natural aquatic habitats in the United States, which the researchers attributed to the presence of higher concentrations of organic matter in the water. Several other species were widely distributed at all sites, including *L. bozemanii*, *L. dumoffii*, *L. gormanii*, *L. longbeachae*, and *L. micdadei*. The study notes the occurrence of *Legionella* in water samples taken from epiphytic rain forest plants, which further demonstrates the ubiquitous nature of these organisms in natural surface water.

Palmer et al. (1993) studied the occurrence of *Legionella* in ocean water as part of an investigation of their presence in raw and treated sewage and nearby receiving waters in California. Ocean-receiving water located five miles offshore from where treated sewage was discharged contained *Legionella*; however, ocean water between the discharge site and coastal bathing beaches was negative. The presence of *Legionella* at a nearby beach swimming area was attributed to surface runoff from a flood control channel and river, which tested positive for *Legionella*.

2. Groundwater

The 1985 *Legionella* Criteria Document reported that no studies had documented the occurrence of *Legionella* in groundwater (EPA 1985). Recognizing the need for data on the occurrence of *Legionella* in groundwater, the U.S. EPA and the American Water Works Association Research Foundation (AWWARF) sponsored a study in which untreated groundwater samples from 29 public water supply system wells were

analyzed for the presence of *L. pneumophila* (Lieberman et al. 1994). A variety of hydrogeologic settings were represented by the wells selected. Samples positive for *L. pneumophila* were collected from six (21%) of the sampling sites. In contrast, Campo and Apraiz (1988) sampled water coming from wells in Spain that were not subject to disinfection; of the 29 samples from eight wells, none were positive for *Legionella*.

3. Man-Made Waters

As noted previously, *Legionella* thrives in biofilms. Because bacteria in biofilms are relatively resistant to standard water disinfection procedures, *Legionella* are able to enter and colonize potable water supplies (Kramer and Ford 1994, Lin et al. 1998a). Artificial aquatic habitats (e.g., components of water distribution systems and cooling towers) are believed to function as amplifiers or disseminators of *Legionella* present in potable water (EPA 1985). The 1985 *Legionella* Criteria Document clearly establishes that these bacteria occur in a variety of man-made water sources, including components of internal plumbing systems (e.g., faucets and showerheads), cooling towers, respiratory-therapy equipment, humidifiers, and whirlpools.

Potable Water Supplies and Distribution Systems

In 1980, British investigators first demonstrated that plumbing fixtures in potable water systems contained *Legionella* (EPA 1985). The 1985 *Legionella* Criteria Document provides extensive evidence of *Legionella* occurrence in a variety of plumbing equipment, including faucets, shower heads, hot water tanks, and water storage tanks. Since that time, numerous studies have continued to document the occurrence of *Legionella* in components of potable water distribution systems; these studies are summarized in Table III-3.

As awareness of the ecology and epidemiology of *Legionella* has increased, attention has shifted from heat-exchange units, such as cooling towers, to potable water distribution systems as sources of human exposure and infection. The 1985 *Legionella* Criteria Document notes the

Table III-3. Occurrence of *Legionella* Bacteria in Potable Water Supplies and Distribution Systems

Setting	Year	Location	Species (Serogroup)	References
community, hospitals, hotels, residential	1987	Alicante, Spain	*L. pneumophila* (serogroups 1,8,6)	Campo and Apraiz 1988
community	1986-1987	North West England	*L. pneumophila*	Jones and Ashcroft 1988
community	1985-1987	England	*L. pneumophila* (serogroup 1)	Colbourne et al. 1988, Colbourne and Dennis 1989
community	NS	NS	NS	Hsu 1986
community	NS	Columbus, Ohio	*L. pneumophila* (serogroup 1)	Voss et al. 1985
community	NS	Columbus, Ohio	*L. pneumophila* (serogroup 1)	Voss et al. 1986
community	NS	Adelaide, Australia	proposed name: *L. waltersii*	Benson et al. 1996
community	NS	Pittsburgh, Pennsylvania	*L. pneumophila* (serogroups 1,3,4-6,12)	Stout et al. 1992a
community	NS	U.S. Virgin Islands	*L. pneumophila* (serogroups 1-6) *L. micdadei* *L. gormanii*	Broadhead et al. 1988
hospitals	1994-1995	Allegheny County, Pennsylvania	*L. pneumophila* (serogroups 1,3,5)	Goetz et al. 1998
hospital	1993-1994	Taiwan	*L. pneumophila* (serogroup 1)	Pan et al. 1996
hospital	1990-1992	England and Scotland	*L. pneumophila* (serogroups 1,4 6)	Liu et al. 1993
hospital	1990	Halifax, Nova Scotia, Canada	*L. pneumophila* (serogroups 1,5)	Bezanson et al. 1992
hospital	1986-1990	Halifax, Nova Scotia, Canada	*L. pneumophila* (serogroup 1)	Marrie et al. 1992
hospital	1989	Stanford University Medical Center, California	*L. dumoffii*	Lowry et al. 1991
hospital and hotel	1985-1987	Lower Saxony, Germany	*L. pneumophila* (serogroups 1-6,9,10) *L. dumoffii* *L. anisa*	Habicht and Müller 1988
hospital	1984-1986	Brussels, Belgium	*L. pneumophila* (serogroups 6,10)	Ezzeddine et al. 1989
hospital	1985	London, England	*L. pneumophila* (serogroups 1,4)	Oppenheim et al. 1987
hospital	1984-1985	Dublin, Ireland	*L. pneumophila* (serogroups 3,5,6)	Haugh et al. 1990

Setting	Year	Location	Species (Serogroup)	References
hospital	1984-1985	Torino, Italy	*L. pneumophila* (serogroup 1)	Moiraghi Ruggenini et al. 1989
hospitals	1983	Canada	*L. pneumophila* (serogroups 1,3) *L. dumoffii*	Tobin et al. 1986
hospital	1982-1983	NS	*L. pneumophila*	Stout et al. 1985b
hospital	1982	France	*L. anisa*	Bornstein et al. 1985
hospital	1981	Pittsburgh, Pennsylvania	*L. pneumophila* (serogroup 1)	Stout et al. 1982
hospitals	1980-1981	Chicago, Illinois Los Angeles, California	*L. anisa*	Gorman et al. 1985
hospital	NS	Germany	*L. pneumophila*	Botzenhart et al. 1986
hospital	NS	England	*L. pneumophila* (serogroup 1)	Ribeiro et al. 1987
hospital	NS	Duesseldorf, Germany	*L pneumophila* (serogroups 1,6)	Hell 1989
hospital	NS	Quebec, Canada	*L. pneumophila* (serogroups 1-6,8) *L. longbeachae* (serogroups 1,2) *L. micdadei*	Alary and Joly 1992
hospitals	NS	Sao Paulo, Brazil	*L. pneumophila* (serogroups 1,6)	Pellizari and Martins 1995
hotel, residential, and industrial	NS	Bangladesh	*L. pneumophila*	Hossain and Hoque 1994
hotel	1986	Greece	*L. pneumophila* (serogroups 1,8)	Alexiou et al. 1989
laboratory	1989	Detroit, Michigan	NS	Paszko-Kolva et al. 1991
residential	1989-1991	Finland	*L. pneumophila*	Zacheus and Martikainen 1994
residential	1982-1983	Chicago, Illinois	*L. pneumophila* (serogroups 1-6)	Arnow et al. 1985
residential	NS	Germany The Netherlands Austria	*L. pneumophila*	Tiefenbrunner et al. 1993
residential	NS	South-eastern Germany	*L. pneumophila* (serogroups 1,3,6,10)	Lück et al. 1993
residential and institutional	NS	South Africa	NS	Augoustinos et al. 1995

Setting	Year	Location	Species (Serogroup)	References
residential	NS	Pittsburgh, Pennsylvania	*L. pneumophila*	Stout et al. 1992b
residential	NS	Pittsburgh, Pennsylvania	*L. pneumophila*	Lee et al. 1988
residential	NS	Vermont	*L. pneumophila*	Witherell et al. 1988
NS	1987-1988	New South Wales, Australia	NS	Hedges and Roser 1991

NS = not specified

presence of *Legionella* in water distribution systems of hospitals, hotels, clubs, public buildings, homes, and factories; recent studies confirm that these systems continue to be a major source of *Legionella* exposure (see Table III-3 for examples).

The 1985 *Legionella* Criteria Document stated that no isolations of *Legionella* had been reported from the extramural components of community water distribution systems. Based on indirect evidence, water in distribution systems was believed to be contaminated with *Legionella* infrequently and with low numbers of organisms (EPA 1985). At that time, *Legionella* were thought to be introduced into the distribution systems through cross connections with equipment such as cooling towers, evaporative condensers, lawn sprinkling equipment, and hoses (EPA 1985). Since 1985, studies have shown that *Legionella* are present in all segments of community water supplies, including treatment facilities (Campo and Apraiz 1988, Colbourne and Dennis 1989, Colbourne et al. 1988, Voss et al. 1986).

Cooling Towers

The first outbreak of Pontiac fever in 1968 was later linked to the presence of *Legionella* in a defective evaporative condenser in a county health department building (EPA 1985). The 1985 *Legionella* Criteria Document notes numerous outbreaks of legionellosis that have been linked to cooling towers and evaporative condensers in hospitals, hotels, and public buildings, clearly establishing these water sources as habitats for *Legionella*. Table III-4 summarizes more recent studies that document the continued presence of *Legionella* in cooling towers and evaporative condensers.

Whirlpools and Spas

Whirlpools and spas serve as an ideal habitat for *Legionella* because they are maintained at temperatures ideal for their growth (Hedges and Roser 1991). In addition, organic nutrients suitable for bacterial growth often accumulate in these waters. Whirlpools and spas can produce

Table III-4. Occurrence of *Legionella* Bacteria in Cooling Towers

Setting	Year	Location	Species (Serogroup)	References
commercial	NS	NS	*L. pneumophila* (serogroups 1,3,5,6,10)	Kusnetsov et al. 1993
commercial	NS	São Paulo, Brazil	*L. pneumophila* (serogroups 1,6) *L. bozemanii*	Pellizari and Martins 1995
commercial	NS	NS	NS	Cappabianca et al. 1994
commercial	NS	San Juan, Puerto Rico	*L. pneumophila* (serogroups 1-6) *L. bozemanii* *L. micdadei* *L. gormanii* *L. dumoffii*	Negron-Alvira et al. 1988
hospital	1993-1994	Taiwan	*L. pneumophila* (serogroup 1)	Pan et al. 1996
hospital	1985	Singapore	*L. pneumophila* (serogroups 1,4)	Nadarajah and Goh 1986
hotels, universities, hospitals	1983	Canada	*L. pneumophila* (serogroups 1,4,6)	Tobin et al. 1986
industrial	1980-1981	Jamestown, New York	*L. anisa*	Gorman et al. 1985
industrial	NS	Bangladesh	*L. pneumophila*	Hossain and Hoque 1994
sewage treatment plant	NS	Adelaide, Australia	informal name: *L.* genomospecies 1	Benson et al. 1996
NS	1993	Fall River, Massachusetts	*L. pneumophila* (serogroup 1)	Keller et al. 1996
NS	1988-1991	Adelaide, Australia	*L. pneumophila* (serogroups 1-14) *L. anisa* *L. rubrilucens*	Bentham 1993
NS	1988-1991	United States	NS	Shelton et al. 1994
NS	1987-1988	New South Wales, Australia	NS	Hedges and Roser 1991
NS	1987	Singapore	*L. pneumophila* (serogroups 1,5,7,8) *L. dumoffii*	Meers et al. 1989
NS	1983-1987	Israel	NS	Shuval et al. 1988
NS	NS	South Africa	NS	Grabow et al. 1991

Setting	Year	Location	Species (Serogroup)	References
NS	NS	Japan	*L. pneumophila* (serogroups 1,3,6)	Ikedo and Yabuuchi 1986

NS = not specified

water droplets of respirable size that have the potential to transmit Legionella to humans (Jernigan 1996). The 1985 *Legionella* Criteria Document notes two outbreaks resulting from the presence of *Legionella* in whirlpools, one involving a therapeutic whirlpool and another involving a recreational whirlpool (EPA 1985). Recent studies document the continued presence of *Legionella* in whirlpools. Hsu et al. (1986) detected *Legionella* in 5 of 140 (13%) spa whirlpool samples. Hedges and Roser (1991) tested spas in New South Wales, Australia and found that 11 of 43 (26%) contained *Legionella*. In addition, several spa filters were found to have higher *Legionella* counts than the water contained in the pool, suggesting that spa filters can act as protective reservoirs or niches for *Legionella*. Fallon and Rowbotham (1990) also isolated *Legionella* from whirlpool water and filters while investigating a large outbreak of legionellosis at a leisure complex in Scotland. Jernigan et al. (1996) isolated *Legionella* from the sand filter in a cruise ship whirlpool spa following an outbreak of legionnaires' disease among cruise ship passengers.

Other related sources of *Legionella* include spring water spas and saunas. Spring water therapy is medicinally accepted in many European countries and often involves aerosol exposure or bathing in certain spring waters (thermal or non-thermal). During an epidemiologic survey of spa waters in France, 15 different *Legionella* species were isolated, including a species that had never before been identified, *L. gratiana* (Bornstein et al. 1989a, 1989b). Den Boer et al. (1998) reported a case of legionnaires' disease linked to an air-perfused footbath at a sauna in The Netherlands that was found to be contaminated with *L. pneumophila*.

Wastewater

The 1985 *Legionella* Criteria Document reports only a few instances of *Legionella* isolation from wastewater (EPA 1985). The 1985 document notes the difficulty of isolating *Legionella* from wastewater because it contains so many other microorganisms. Palmer et al. (1993) conducted an extensive study to determine whether *Legionella* were present in the influent of a major metropolitan sewage treatment plant and to determine how well the bacteria could survive the different stages of sewage treatment. They found that *Legionella* were always present in all phases of the sewage treatment process, including the secondary effluent that was discharged through an ocean outfall. They also noted that population numbers did not significantly decline in different stages of the treatment process.

In a later study, Palmer et al. (1995) examined tertiary treated (including chlorination) sewage effluents that are used as reclaimed water and aerosols obtained from above a secondary sewage treatment basin for the presence of *Legionella*. The bacteria were detected in samples of reclaimed water at all four sites tested using two detection methods: polymerase chain reaction and direct fluorescent antibody (see Chapter 7, Section A for explanation of detection methods). The researchers noted that they were not able to culture *Legionella* obtained from any of the reclaimed water samples, suggesting that chlorine may injure *Legionella* and cause them to enter a viable but nonculturable state. *Legionella* were detected in the air obtained from above secondary treatment (activated sludge) aeration tanks at one site using polymerase chain reaction, direct fluorescent antibody, and plate culture.

C. Occurrence in Soil

The 1985 *Legionella* Criteria Document reported that *Legionella* had been isolated from mud and sandy, moist soil on the edge of streams containing the bacteria. The 1985 document noted a lack of data indicating soil is involved in the transmission of *Legionella* to humans although excavations and other soil disturbances had been associated with some *Legionella* epidemics. At that time, *Legionella* had only been from mud or moist soil (EPA 1985). More recently, one species, *L. longbeachae*, was shown to inhabit and thrive in soil (Steele et al. 1990). Following an outbreak of legionellosis due to *L. longbeachae* in South Australia in 1988 and 1989, Steele et al. (1990) analyzed a number of water and soil samples to find the source of the organism. *L. longbeachae* was not isolated from any of the water samples or natural soil samples; however, the bacteria was isolated from three samples of potting soil mixes and from soil surrounding two potted plants. *L. longbeachae* was able to persist for seven months in two potting mixes stored at room temperature. The researchers concluded that the isolation and prolonged survival of *L. longbeachae* in potting mixes suggest that soil rather than water is the natural habitat of this species and may be a source of human exposure.

D. Occurrence in Air

As discussed in Sections B and C of this chapter, the natural habitat for *Legionella* appears to be aquatic bodies and perhaps, for *L. longbeachae*, soil. However, *Legionella* can be found in air as part of aerosols. The 1985 *Legionella* Criteria Document establishes aerosolization as an important component of *Legionella* transmission from the aquatic environment to the human respiratory system (see Chapter VI, Section C.2 for further discussion of transmission to humans). At the time of the 1985 report,

aerosol-generating systems that had been linked to disease transmission included cooling towers, evaporative condensers, plumbing equipment (e.g., faucets, showerheads, hot water tanks), humidifiers, respiratory-therapy equipment (e.g., nebulizers), and whirlpool baths (EPA 1985). Studies published after the 1985 report have confirmed the presence of *Legionella* in aerosols from several of these systems (Bollin et al. 1985, Seidel et al. 1987).

In most cases, disease outbreaks resulting from *Legionella* aerosolization have involved indoor exposure and outdoor exposure to within 200 meters. However, Addiss et al. (1989) describe an outbreak that occurred in Wisconsin in which aerosolized *L. pneumophila* from an industrial cooling tower was disseminated at least one mile (1.6 km) and perhaps up to two miles (3.2 km). Meteorological conditions that suppress vertical mixing and favor horizontal transport of aerosols (e.g., fog, high humidity, and cloud cover) occurred before and intermittently during the outbreak and presumably contributed to the lengthy transport.

E. Specific Disease Outbreaks

Legionellosis can occur as sporadic cases or as outbreaks. The majority of cases of legionnaires' disease are sporadic rather than outbreak related (Stout et al. 1992a). The study of outbreaks caused by *Legionella* has yielded essential information about these bacteria and the illnesses they cause. Early outbreaks illustrated the clinical course of legionnaires' disease and Pontiac fever. Subsequently, epidemics provided information regarding the sources of human exposure, risk factors for the development of disease, and the efficacy of treatment options.

Legionellosis outbreaks have been attributed most frequently to exposure to contaminated cooling towers, potable water, or components of water distribution systems. Outbreaks of legionellosis caused by contaminated cooling towers can be explosive with numerous cases over a short period of time (e.g., Addiss et al. 1989, Fiore et al. 1998, Gecewicz et al. 1994, O'Mahoney et al. 1990). Legionellosis outbreaks due to contaminated water or water distribution systems tend to be more insidious and may only be revealed after active surveillance is introduced (e.g., Brady 1989, Colville et al. 1993, Goetz et al. 1998, Guiget et al. 1989, Hanrahan et al. 1987, Helms et al. 1988, Le Saux et al. 1989, Meenhorst et al. 1985, Schlech et al. 1985, Struelens et al. 1992).

Establishing the source of *Legionella* bacteria causing a legionellosis outbreak can be problematic due to their ubiquitous nature in the environment. Epidemiologic investigations of outbreaks often rely on multiple molecular subtyping techniques to match clinical isolates of *Legionella* with isolates from environmental samples (Johnston et al. 1987, Mamolen et al. 1993, Struelens et al. 1992, Whitney et al. 1997). Detection of *Legionella* in environmental and biological samples is discussed further in Chapter VII.

Outbreaks of legionellosis typically are categorized as nosocomial (i.e., hospital-acquired), travel-acquired, or community-acquired. Table III-5 summarizes outbreaks of legionellosis that have been reported since 1985, including the type of outbreak, the setting in which the outbreak occurred, the source of the outbreak, the number of individuals affected, the species implicated, and the location and time of the outbreak. In addition, specific characteristics and features of the various types of outbreaks are described below.

1. Nosocomial Outbreaks

Studies have linked nosocomial legionellosis to air conditioning systems and cooling towers; however, numerous studies demonstrate the importance of hospital potable water supplies as a source of nosocomial infections (see Table III-5 for examples). *L. pneumophila* has most commonly been implicated as the causative agent in hospital-acquired legionellosis outbreaks (see Table III-5 for examples).

2. Outbreaks Among Travelers

Travelers are usually exposed to *Legionella* via contaminated hotel potable water or contaminated whirlpool spas at hotels, resorts and cruise ships (see Table III-5 for examples). Two reported outbreaks resulted from exposure to *Legionella*-contaminated water in decorative fountains (Fensterheib et al. 1988, Hlady et al. 1993). As with nosocomial legionellosis outbreaks, the most commonly implicated species is *L. pneumophila* (see table III-5 for examples).

Among U.S. residents, travel-associated legionellosis outbreaks are extremely difficult to detect, and extensive case investigations often are required. The European Surveillance Scheme for Travel Associated Legionnaires' Disease has greatly enhanced detection of travel-associated outbreaks in

European cities because individual cases are entered into a centralized database, which is then searched for other cases linked to the same place of accommodation (Joseph et al. 1997).

Table III-5. Occurrence of Legionellosis Outbreaks

Setting	Dates	Location	Source	# Affected	Species (Serogroup)	References
Nosocomial						
hospital	1996	Arizona	hot water distribution system	8	*L. pneumophila* (serogroups 6,10)	Kioski et al. 1997
hospital	January-June, 1996	Ohio	hot water distribution system	2	*L. pneumophila* (serogroup 1)	Kioski et al. 1997
hospital	July 2-12, 1995	Franklin County, Pennsylvania	cooling towers and rooftop air samples	22	*L. pneumophila* (serogroup 1)	Fiore et al. 1998
hospital	January, 1985-April, 1993	Innsbruck University Hospital, Austria	hot water system	14	*L. pneumophila* (serogroup 1)	Prodinger et al. 1994
hospital	March, 1992	Albany Medical Center, New York	potable water system used in nasogastric tubes	2	*L. pneumophila* (serogroup 6)	Venezia et al. 1994
hospital	February-March, 1992	Providence, Rhode Island	potable water	2	*L. pneumophila*	Mermel et al. 1995
hospital	March, 1983-September, 1991	Ontario, Canada	tap water, shock absorbers within water pipes	13	*L. pneumophila* (serogroup 1)	Memish et al. 1992
hospital	December, 1990-February, 1991	Varnamo, Sweden	hot water supply	31	*L. pneumophila* (serogroup 1)	Darelid et al. 1994
hospital	June-October, 1990	Glasgow Royal Infirmary, Scotland	fire hydrants connected to main water supply	3	*L. pneumophila* (serogroup 1)	Patterson et al. 1994
hospital and community	1988-1990	São Paulo, Brazil	NS	5	*L. pneumophila* (serogroups 1)	Levin et al. 1993

Setting	Dates	Location	Source	# Affected	Species (Serogroup)	References
hospital	June-August, 1989	NS	cooling towers	3	*L. pneumophila* (serogroup 1)	Shelton et al. 1994
hospital	May, 1989	Stanford University Medical Center	bath water	3	*L. dumoffii*	Lowry et al. 1991
hospital	July, 1988-April, 1989	Nottingham, England	domestic hot water system	12	*L. pneumophila* (serogroup 1)	Colville et al. 1993
hospital	1984-1988	Atlanta, Georgia	nebulizer and water system	13	*L. pneumophila* (serogroup 3)	Mastro et al. 1991
hospital	1977-1988	Charlottesville Virginia	study suggests potable water	16	*L. micdadei*	Doebbeling et al. 1989
hospital	October, 1985-September, 1987	Brussels, Belgium	water system	32	*L. pneumophila* (serogroup 1)	Struelens et al. 1992
hospital	September, 1985-February, 1986	Paris, France	shower supply and water tank	4	*L. pneumophila* (serogroup 1)	Meletis et al. 1987
hospital	October-December, 1985	Glasgow Royal Infirmary, Scotland	cooling tower	16	*L. pneumophila* (serogroup 1)	Winter et al. 1987
hospital	December, 1984-December, 1985	Manitoba, Canada	water system, renal transplant unit sink	6	*L. pneumophila* (serogroup 1)	Le Saux et al. 1989
hospital	August, 1982-December, 1985	Columbus, Ohio	potable water, showers	7	*L. pneumophila*	Brady 1989
hospital	January, 1983-December, 1985	Berlin, Germany	water supply system	35	*L. pneumophila*	Ruf et al. 1988
hospital	April 16-May 16, 1985	District General Hospital, Stafford, England	air conditioning unit	68 confirmed 35 suspected	*L. pneumophila*	Anonymous 1985 Dennis 1991 O'Mahony et al. 1990
hospital	May-September, 1984	University of Utah School of Medicine, Utah	cooling tower	4	*L. pneumophila* (serogroup 1)	Johnston et al. 1987

Setting	Dates	Location	Source	# Affected	Species (Serogroup)	References
hospital	June-July, 1984	Halifax, Nova Scotia	shower heads, faucets, ac filter	8	*L. pneumophila* (serogroup 1)	Martin et al. 1988
hospital	1983	NS	hot water	NS	*L. pneumophila*	Palmer 1986
hospital	August, 1978-November, 1983	Leiden University Hospital, The Netherlands	hot potable water system	21	*L. pneumophila* (serogroups 1,10)	Meenhorst et al. 1985
hospital	June 27-August 25, 1983	Rhode Island	water in cooling tower	15	*L. pneumophila* (serogroup 1)	Garbe 1985
hospital	November, 1982-March, 1983	Paris, France	water supply	47	*L. pneumophila* (serogroup 1)	Guiguet et al. 1987
hospital	February-September, 1982	Upstate New York	potable water, showers, and water system	7 confirmed 4 suspected	*L. pneumophila* (serogroup 1)	Hanrahan et al. 1987
hospital	1981	Iowa City, Iowa	hot and cold water systems	16	*L. pneumophila* (serogroup 1)	Helms et al. 1988
hospital	1981	Paris, France	hot water system	6	*L. pneumophila* (serogroup 1)	Neill et al. 1985
hospital	NS	Quebec City	distilled water	5	*L. dumoffii*	Joly et al. 1986
hospital	NS	NS	hot water supply system	19	*L. pneumophila* (serogroup 1) *L. anisa*	Bornstein et al. 1986
rehabilitation center	NS	Germany	potable water	11	*L. pneumophila* (serogroup 1)	Nechwatal et al. 1993
renal transplant unit	June, 1989-March, 1990	São Paul, Brazil	potable water system	8	*L. pneumophila* (serogroup 1)	Levin et al. 1991
Travel-Acquired						
cruise ship	July-August, 1994	Cruise ship to Bermuda	whirlpool	14	*L. pneumophila* (serogroup 1)	Guerrero et al. 1994 Guerrero and Filippone 1996

Setting	Dates	Location	Source	# Affected	Species (Serogroup)	References
cruise ships	April, 1994	New York City to Bermuda	whirlpool spas and aerosols	16 confirmed 34 probable	*L. pneumophila* (serogroup 1)	Jernigan et al. 1996
hotel	September-October, 1996	Mamora Bay, Antiqua	solar powered hot water system	3	*L. pneumophila* (serogroup 1)	Joseph et al. 1997
hotel	May 1996	Minorca, Spain	hot water system	4-5	*L. pneumophila* (serogroup 1)	Joseph et al. 1996, 1997
hotel	May-August, 1995	Kusadasi, Turkey	water supply	7	*L. pneumophila* (serogroup 1)	Anonymous 1995a, 1995b
hotel	January 6-February 2, 1992	Orlando, Florida	decorative fountain	5	*L. pneumophila* (serogroup 1)	Hlady et al. 1993
hotel	1986-1990	Ischia Island, Naples, Italy	hot-water supply	6	NS	Castellani Pastoris et al. 1992
hotel	1988	Santa Clara County, California	fountain in lobby	34	*L. anisa*	Fenstersheib et al. 1990
hotel	August-September, 1987	Yugoslavia	NS	15	NS	Anonymous 1988
hotels	1973-1987	Northern Italy	potable water	117	*L. pneumophila* (serogroups 1,3,4)	Passi et al. 1990
hotel	1979-1982	U.S. Virgin Islands	potable water system	27	*L. pneumophila* (serogroup 1)	Schlech et al. 1985
leisure complex	1988	Lochgoilhead, Scotland	whirlpool and filter	NS	*L. micdadei*	Fallon and Rowbotham 1990
leisure complex	January-March, 1995	Northwest England	whirlpool	8 confirmed 32 possible	*L. micdadei*	Newton et al. 1996
ski lodges	October, 1987	Vermont	water sources, whirlpool spa	17	*L. pneumophila* (serogroup 1)	Mamolen et al. 1993
ski resort	January, 1991	Vermont	hot tub	6	*L. pneumophila*	Thomas et al. 1993

Setting	Dates	Location	Source	# Affected	Species (Serogroup)	References
Community-Acquired						
artesian well construction site	October, 1990	Apulia, Italy	groundwater	2	*L. pneumophila*	Miragliotta et al. 1992
business district	April 11-20, 1992	Fairfield, Sydney, Australia	not determined	26	*L. pneumophila* (serogroup 1)	Levy et al. 1994
coal mine	1979-1982	South Wales, UK	open pit pond	3	*L. pneumophila*	Davies et al. 1985
commercial building (BBC)	April, 1988	London, England	cooling systems	NS	*L. pneumophila* (serogroup 1)	Dennis 1991
community	September 11-October 18, 1996	Alcala de Henares, Spain	cooling towers, water storage tanks	49 confirmed 197 possible	*L. pneumophila* (serogroup 1)	Anonymous 1996
community	September-November, 1991	Chorley, United Kingdom	cooling tower	11	*L. pneumophila*	Peiris et al. 1992
community	1988-1990	São Paulo, Brazil	NS	3	*L. pneumophila* (serogroups 1,5)	Levin et al. 1993
community	May, 1987-June, 1989	South Australia	potting soils, mixes	30	*L. longbeachae*	Steele et al. 1990
community (Piccadilly Circus)	January-February, 1989	London, England	cooling towers	33 confirmed 10 suspected	*L. pneumophila* (serogroup 1)	Watson et al. 1994
community	May 30, 1986 and August 27-October 27, 1986	Gloucester, England	wet cooling towers	15	*L. pneumophila* (serogroup 1)	Hunt et al. 1991
community	August 10-29, 1986	Sheboygan, Wisconsin	industrial cooling tower	29	*L. pneumophila* (serogroup 1)	Addiss et al. 1989
grocery store	October 10-November 13, 1989	Bogalusa, Louisiana	mist machine, aerosols	33	*L. pneumophila* (serogroup 1)	Mahoney et al. 1992

Setting	Dates	Location	Source	# Affected	Species (Serogroup)	References
home and butcher shop	September 1986	Italy	shower and condensation water	3	*L. pneumophila* (serogroup 1)	Castellani Pastoris et al. 1988
hospital	July 2-12, 1995	Franklin County, Pennsylvania	cooling towers and rooftop air samples	22	*L. pneumophila* (serogroup 1)	Fiore et al. 1998
hot spring	1986	France	spring water system	5	*L. pneumophila* (serogroups 1,3)	Bornstein et al. 1989a
hotel	April 22-27, 1993	Sydney, Australia	cooling towers	4	*L. pneumophila* (serogroup 1)	Bell et al. 1996
industrial	October, 1988	Lostock, England	water cooling system	57	NS	Anonymous 1989
industrial estate	1996-1997	Northamptonshire Enlgand	cooling towers	20	*L. pneumophila* (serogroup 1)	Joseph et al. 1997
industrial foundries	October-November, 1996	West Midlands, England	cooling tower	7	*L. pneumophila* (serogroup 1)	Joseph et al. 1997
industrial plant	June-August, 1994	Birmingham, England	cooling towers	8	*L. pneumophila* (serogroup 1)	Joseph et al. 1995
industrial plants	July, 1987	NS	potable water	3	*L. pneumophila* (serogroup 1)	Muraca et al. 1988
locker room	May 15-17, 1982	Michigan	whirlpool aerator	14	*L. pneumophila* (serogroup 6)	Mangione et al. 1985
nursing home	1994	Ontario, Canada	water system	10	*L. sainthelensi* (serogroup 1)	Tang et al. 1995
nursing home	1994	Ontario, Canada	water system	9	*L. sainthelensi* (serogroup 1)	Tang et al. 1995
nursing home	December, 1990	Nagasaki, Japan	not determined	2	*L. pneumophila* (serogroup 1)	Maesaki et al. 1992

Setting	Dates	Location	Source	# Affected	Species (Serogroup)	References
office building	January-February, 1990	Christchurch, New Zealand	cooling tower	4 confirmed 3 suspected	*L. pneumophila* (serogroup 1)	Mitchell et al. 1991
office building	April, 1984	New York City, New York	cooling tower	86	*L. pneumophila* (serogroup 1)	Friedman et al. 1987
plastics factory	October-November, 1996	Trent, England	unregistered cooling tower	2	NS	Joseph et al. 1997
plastics factory	1996	Wales	cooling towers	4	*L. pneumophila* (serogroup 1)	Joseph et al. 1997
plastics factory	August, 1996	Yorkshire, England	water from an uncovered outdoor tank	2	*L. pneumophila* (serogroup 1)	Joseph er al. 1997
police HQ building	October, 1985	United Kingdom	air conditioning system	6	*L. pneumophila* (serogroup 1)	O'Mahony et al. 1989
power station	September-October, 1981	United Kingdom	small capacity cooling towers	3 confirmed 2 suspected	*L. pneumophila* (serogroup 1)	Morton et al. 1986
prison	August-September, 1993	Michigan	cooling towers	17	*L. pneumophila* (serogroup 1)	Gecewicz et al. 1994
recycling plant	June, 1994	South England	cooling tower	5	*L. pneumophila* (serogroup 1)	Joseph et al. 1995
retail store	September 29-October 22, 1996	Southwestern Virginia	whirlpool spa display	23	*L. pneumophila*	Hershey et al. 1997
retail store	May-June, 1986	Maryland	not determined	27	*L. pneumophila* (serogroup 1)	Redd 1990
retirement home	June 10-July 22, 1988	Los Angeles, California	evaporative condenser and potable water	6	*L. pneumophila* (serogroup 1)	Breiman et al. 1990

Setting	Dates	Location	Source	# Affected	Species (Serogroup)	References
sauna	December, 1992-January, 1996	The Netherlands	hot water system	6	*L. pneumophila*	Den Boer et al. 1998
town building	July August, 1993	Fall River, Massachusetts	cooling towers	11	*L. pneumophila* (serogroup 1)	Gecewicz et al. 1994 Keller et al. 1996
town building	August -October, 1993	Rhode Island	cooling towers	17	*L. pneumophila* (serogroup 1)	Gecewicz et al. 1994 Whitney et al. 1997
Unknown						
NS	March-April, 1993	Georgia and Florida	NS	1 confirmed 24 suspected	NS	Anonymous 1993
NS	1986-1996	Singapore	cooing towers, fountains, spa pools	22 confirmed 236 presumed	*L. pneumophila*	Heng et al. 1997

NS = not specified

3. Community Outbreaks

Cooling towers and potable water are the most common causes of community outbreaks of legionellosis (see Table III-5 for examples). Other less common sources reported include a whirlpool spa display at a retail store (Hershey et al. 1997) and a grocery store mist machine (Mahoney et al. 1992). Some outbreaks involve residential exposure (e.g., Breiman et al. 1990, Tang et al. 1995), whereas others involve exposure at the workplace (e.g., Anonymous 1989, Dennis 1991, Joseph et al. 1997, Joseph et al. 1995, Muraca et al. 1988). Community-acquired outbreaks have often been associated with urban rather than rural areas (Joseph et al. 1997), which is not surprising given the increased availability of artificial water bodies in urban areas. As with nosocomial and travel outbreaks, *L. pneumophila* is the species most commonly implicated in community-acquired outbreaks.

As noted previously, the vast majority of cases of legionnaires' disease are community-acquired sporadic (i.e., non-outbreak related) (Stout et al. 1992a). Straus et al. (1996) studied 146 adults diagnosed with having nonepidemic, community-acquired legionnaires disease and the possible link to residential potable water. *Legionella* was isolated from water in six percent of case patients homes (1-8 sites per home) compared to three percent of control patients homes. The researchers suggest that transmission of *Legionella* from domestic water may have occurred in more instances than the study results indicate, since sampling occurred as much as six weeks after a patient's illness.

F. Environmental Factors Affecting *Legionella* Survival

1. Symbiotic Microorganisms

Legionella can only exist on artificial cultured media in very specific conditions and under particular temperature, pH, and nutritional requirements. Nevertheless, they survive in an extremely wide range of conditions in natural and man-made aquatic habitats. Their survival is enhanced by symbiotic relationships with other microorganisms such as protozoa, algae, and other bacteria, which provide them with advantages in the natural environment as well as in anthropogenic potable water distribution systems. *Legionella* have the unique ability to multiply within protozoan cells, which helps

them survive over a wide temperature range and resist the effects of chlorine, biocides, and other disinfectants.

Amoebae and Other Protozoa

Many species of *Legionella* can infect amoebae and other protozoa and subsequently reproduce within these protozoans. *Legionella* have been found to infect and incorporate themselves into at least 13 species of amoebae including *Acanthamoeba*, *Hartmanella*, *Valkampfia*, and *Naegleria*, and two strains of ciliates, *Tetrahymena* and *Cyclidium* (Lee and West 1991, Paszko-Kolva et al. 1993, States et al. 1989, Kramer and Ford 1994, Henke and Seidel 1986, Fields 1996, Vandenesch et al. 1990). Further, a study by Vandenesch et al. (1990) illustrated that *L. pneumophila* can infect and reproduce within the amoeba *Acanthamoeba,* even when the ratio of *Legionella* cells to amoebae is low. Various species of *Legionella* have been detected recently that are able to grow intracellularly in protozoan cells even though they have never been capable of growth on standard *Legionella* media. These organisms have been called LLAP (*Legionella*-like amoebal pathogens) organisms, and they have the ability to infect and propagate in many mammalian and protozoan cells (Fields 1996). After the bacteria are phagocytosed by amoebae, they multiply within their vesicles and remain encapsulated in the cysts until the vesicles and/or amoeba rupture (States et al. 1989). Because *Legionella* replicate rapidly intracellularly within protozoan hosts for prolonged periods of time, amoebic vesicles can contain hundreds of *Legionella* cells at once (Berk et al. 1998, Lee and West 1991). In addition, replication within protozoa can contribute to enhanced virulence of *Legionella* (Kramer and Ford 1994).

The fact that *Legionella* have the ability to infect and grow in protozoa is extremely critical to their maintenance and survival. Not only can they multiply quickly within protozoan cells, but they also obtain protection from disinfectants and other adverse environmental conditions. For example, *Legionella* caught in encysted protozoa have demonstrated better resistance to chlorine than *E. coli*, a common indicator of water quality (Paszko-Kolva et al. 1993, States et al. 1989, Kramer and Ford 1994). *Legionella* trapped in the amoeba *A. polyphaga* have been shielded from the effects of exposure to 50 mg/L of free chlorine (Paszko-Kolva et al. 1993, Fields 1996). Intracellularly grown *Legionella* are also more resistant to biocides, chemical disinfection, and other physical stresses than *Legionella* grown on cultured media. Because protozoa ingest virulent strains of *L. pneumophila*, they also augment growth

of the bacteria in cooling towers and other epidemic sources (Barbaree et al. 1986). In addition, encapsulation in cysts allows *Legionella* to survive in the dry conditions of an aerosol for extended time periods, thus allowing the bacteria to persist, disperse, and infect human hosts (Fields 1996).

Algae and Other Bacteria

Certain algae such as the cyanobacterium *Fischerella* and the green algae *Scenedesmus*, *Chlorella*, and *Gleocystis* have fostered the growth of *Legionella*, but only in the presence of light (Lee and West 1991, Kramer and Ford 1994, States et al. 1987, Henke and Seidel 1986, Paszko-Kolva et al. 1993). States et al. (1987) found that the highest incidence of *Legionella* multiplication came from samples gathered from zones affected by the accumulation of algal materials and leaf litter. *Legionella* growth is further supplemented by their utilization of the nutrients supplied by the decomposition and excretion of algae, as well as decaying organic matter from leaf litter (States et al. 1987).

Legionella also have formed colonies in media deficient in cysteine or iron salts, which they require for growth. The colonies have been found around strains of common aquatic bacteria such as *Flavobacterium*, *Pseudomonas*, *Alcaligenes*, and *Acinetobacter*, which are presumed to provide these nutrients (Lee and West 1991, Paszko-Kolva et al. 1993, Kramer and Ford 1994, Stout et al. 1985a). *Legionella* have also been found attached to the surface of biofilms in water systems (Kramer and Ford 1994). Biofilms are encased microcolonies made up of bacterial cells and attached to a conglomerate of polysaccharides. They trap nutrients for growth and provide a protective layer for many microbes. *Legionella* survive in these biofilms via nutritional symbiosis with other inhabiting organisms (Kramer and Ford 1994).

2. Water Temperature

Legionella exhibit the ability to survive in an incredibly wide range of temperatures. As a lower limit, Bentham (1993) observed growth at a water temperature of 16.5°C. The highest water temperature of a sample cultivated by Botzenhart et al. (1986) was 64°C, while Henke and Seidel (1986) claimed *Legionella* to be a "thermoresistant" organism, exhibiting survival in natural warm waters of up

to 60°C and artificially heated waters of 66.3°C. Optimum temperatures for *Legionella* reproduction range from 32 to 45°C (Vickers 1987, Kramer and Ford 1994).

Nevertheless, temperature has a formidable effect on the persistence and dissemination of *Legionella* in aquatic habitats. While *Legionella* populations seem to be controlled by extremely low temperatures, they are enhanced by heat and elevated temperatures found in areas like whirlpools, hot springs, and blast zones (Henke and Seidel 1986, Lee and West 1991, Verissimo et al. 1991). Colbourne and Dennis (1989) contend that although *Legionella* are not thermophilic, they exhibit thermo-tolerance at temperatures between 40 and 60°C, which gives them a survival advantage over other organisms competing in man-made warm water systems. Although temperatures between 45 and 55°C are not optimal for *Legionella*, these temperatures enable them to reach higher concentrations than other bacteria commonly found in drinking water, thus providing *Legionella* with a selective advantage over other microbes (Kramer and Ford 1994). *Legionella* were found in natural surface waters of Puerto Rico in densities several orders of magnitude higher than those in corresponding natural habitats in the United States (Ortiz-Roque and Hazen 1987) although these differences may be due to factors other than temperature (e.g., increased nutrient availability). In contrast, the distribution and abundance of *Legionella* in south-eastern Australia is comparable to the United States and Europe (Hedges and Roser 1991).

3. Other Factors

Although interaction with other microorganisms and water temperature are the most significant and evident factors affecting *Legionella* growth and survival, there are a few other factors, such as sediment and metals content, that are notable influences as well. These factors are usually amplified by ideal water temperature or coexisting environmental microflora.

Stout et al. (1985a) tested different external influences of *Legionella* growth and sustenance. The results indicated that growth of *L. pneumophila* declined in the absence of environmental microflora such as algae and amoebae. The results also showed that as the amount of sediment increased, so did the population of *L. pneumophila*. This was largely attributed to the fact that the scale, or mineral deposits, and detritus, or decaying plant matter, that make up sediment, are used by *Legionella* organisms as a

major source of nutrients. In this study, the greatest effect on *Legionella* growth and survival was caused by the presence of both sediment and other microbes. The researchers theorized that the sediment stimulates the growth of environmental microorganisms, which prompts the growth of *Legionella* that rely on their environmental by-products and availability as hosts (Stout et al. 1985a).

States et al. (1987) noted that *Legionella* growth was more evident at the corners and bottoms of tanks, sedimentation basins, and reservoirs than anywhere else due to the excess sediment and scale in those areas. It follows that total organic carbon and turbidity are also factors that motivate *Legionella* growth since these influences are found in water zones rich in sediment. Vickers et al. (1987) studied the design of water distribution systems and concluded that vertical tanks were more prone to *Legionella* growth due to thicker accumulation of sediment at the bottom of the tank. Also, greater amounts of scale and sediment in older tanks may contribute to increased growth of *Legionella*. Sediment is important to *Legionella* growth because it provides essential nutrients, aids in the growth of other coexisting microflora, and shelters the organism as well (Vickers et al. 1987).

Changes in water pressure and flow rates of water distribution systems may cause disruption of the biofilm, resulting in increased concentrations of Legionella in water supplies (Kramer and Ford 1994). Mermel et al. (1995) remarked that repressurization of potable water upon completion of a construction project may lead to increased concentration of *Legionella* in the water. They noted that this phenomenon could occur in the absence of construction (i.e., any situation in which the water pressure is changed). Straus et al. (1996) reported that recent residential plumbing repair is an independent risk factor for community-acquired legionnaires' disease.

Water hardness is determined primarily by the amount of calcium and magnesium in scale deposits. *Legionella* have been found to flourish in areas where these metallic cations are present (Vickers et al. 1987). Low levels of iron, zinc, and vanadium also may stimulate the growth of *Legionella* (Kusnetsov 1993, States et al. 1987, Stout et al. 1992b), while higher concentrations of metals like copper, iron, manganese, and zinc may actually be toxic (Kusnetsov 1993).

G. Summary

Cases of legionellosis have been documented throughout the world; however, the true incidence of the disease is unknown due to inadequate surveillance. Geographical variation in the incidence of legionellosis has been attributed to differences in definitions, diagnostic methods, surveillance systems, and data presentation. National surveillance programs currently are conducted in the United States, 24 European countries (including England), and Australia and New Zealand. In the United States, the number of cases per million population rose from 3.5 in 1984 to a peak of 6.3 in 1994 and then began to decline to 4.7 in 1996. In England and Wales, annual totals declined briefly after a peak in 1988 but have been increasing since 1993.

Legionella are widely distributed in the aqueous environment, including both natural water bodies (surface water and groundwater) and man-made waters (e.g., potable water, cooling towers, whirlpools, etc.). The presence of *Legionella* has been documented in fresh surface water sources (e.g, lakes and streams), estuarine and marine surface water sources, and groundwater. *Legionella* thrive in biofilms, and interaction with other organisms in biofilms is essential for their survival and proliferation in aquatic environments. Bacteria in biofilms are relatively resistant to standard water disinfection procedures, and therefore, *Legionella* are able to enter potable water supplies. *Legionella* find niches suitable for survival and growth in artificial aquatic habitats (e.g., internal plumbing systems, cooling towers, respiratory-therapy equipment, humidifiers, and whirlpools), which function as amplifiers or disseminators of these bacteria.

Although water has been the most documented source of *Legionella* in the environment, these bacteria have been isolated from mud, moist soil, and potting soil. *Legionella* can be transmitted from water to air by aerosol-generating systems such as cooling towers, evaporative condensers, plumbing equipment (e.g., faucets, showerheads, hot water tanks), humidifiers, respiratory-therapy equipment (e.g., nebulizers), and whirlpool baths. Inhalation of *Legionella*-contaminated aerosols is an important source of human exposure and infection.

Human exposure to *Legionella*-contaminated sources can result in outbreaks of legionellosis. Outbreaks can be categorized as nosocomial (i.e., hospital-acquired), travel-acquired, or community-acquired. Nosocomial outbreaks have been linked to hospital potable water supplies as well as air conditioning systems and cooling towers. Travelers are usually exposed to *Legionella* in contaminated

hotel potable water or contaminated whirlpool spas. Community outbreaks are caused by exposure to the widest variety of sources, but potable water and cooling towers are the most common. *L. pneumophila* has most frequently been implicated as the causative agent for all three types of outbreaks. The majority of cases of legionnaires' disease, however, are community-acquired sporadic (i.e., non-outbreak related).

The growth and survival of *Legionella* in the environment is enhanced by their ability to form symbiotic relationships with other microorganisms. *Legionella* are able to infect and multiply intracellularly within at least 13 species of amoebae, allowing them to survive over a wider range of environmental conditions and resist the effects of chlorine, biocides, and other disinfectants. Because *Legionella* replicate rapidly intracellularly within these protozoan hosts, often for prolonged periods of time, a single amoebic vesicle can contain hundreds of *Legionella*. Relationships with certain algae and bacteria in biofilms also foster the growth of *Legionella*, presumably due to the increased availability of nutrients and resistence to disinfection. Other factors influencing the survival of *Legionella* in the environment include water temperature, presence of sediment, and metal content.

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IV. Health Effects in Animals

A. Laboratory Studies

Although *Legionella* are widely distributed in the environment, there are no reports of their isolation from naturally infected animals, and they are considered to be strictly human pathogens. As discussed in detail in the 1985 *Legionella* Criteria Document, there is considerable serological evidence that exists to support exposure or possible subclinical infection in animals, such as horses, cattle, sheep, swine, nonhuman primates, goats, dogs, and protozoa. It should be noted, however, that controversy exists in the applicability of the utilization of titer criteria in animals, an evaluation method which is established for measurement of antibody levels in humans.

Animals have been primarily used as hosts for the isolation of the *Legionella*, models for the study of the disease process in human legionellosis, models for the study of the virulence of various *Legionella* species, as well as for the testing of new diagnostic techniques, immunological responses, and possible therapeutic approaches. Guinea pigs have been studied extensively due to similarities between the natural legionnaires' disease in humans, and the experimental disease in guinea pigs. Other species including rats, gerbils, mice, hamsters, rabbits, nonhuman primates and embryonated hens' eggs have also been utilized for study of infection by *Legionella*.

Experimental routes of exposures have been primarily respiratory, including small particle aerosols, intranasal instillation, nose-only inhalation and intratracheal injections. Infections have also been induced by ingestion (drinking water and gastric intubation) and intraperitoneal injection routes.

Clinical Features and Symptomatology in Guinea Pigs

The disease process, following an inhalation exposure of *L. pneumophila* in guinea pigs, has been characterized by investigators as fever for several days, bacteremia, and fibrinopurulent pneumonia with congestion and eventually consolidation (Baskerville 1984, Davis et al. 1983c). The most striking clinical symptoms are fever and weight loss (Twisk-Meijssen et al. 1987). In fact, weight loss, fever and seroconversion are considered to be the only dependable clinical criteria of aerosol infection (Berendt et

al. 1980). Clinical symptoms and mortality are dose-dependent in nature, and are discussed at length in the 1985 *Legionella* Criteria Document. The defense mechanism initially involves resident alveolar macrophages and polymorphonuclear cells (PMNs), followed by the presence of immunospecific antibodies (Davis et al. 1983b). The multiplication of the *Legionella* in recruited macrophages results in destruction of the macrophages and the release of toxic products in the lungs of susceptible animals. The alveolar membrane integrity is destroyed, and serum proteins, together with PMNs, macrophages, bacteria, and debris, fill the air sacs producing hypoxia and impairing respiratory function.

In contrast, intraperitoneal exposure in guinea pigs to *L. pneumophila* causes a diffuse fibrinopurulent peritonitis involving the liver and spleen (Chandler et al. 1979c, Hambleton et al. 1982). In addition, foci of inflammation and necrosis may also be found in the lungs, lymph nodes, pancreas, and heart. The histological features of the pneumonitis induced by intraperitoneal inoculation are quite different from those observed in animals infected by the aerosol route; lesions are more focal, the interstitium is more strongly involved, and necrosis and fibrin in the alveolar exudate is minimal. It was also noted by Hambleton et al. (1982) that extrapulmonary symptoms, such as diarrhea, kidney or liver failure, or neurologic disturbances, that are observed with intraperitoneal infections are seldom observed in guinea pigs infected by the aerosol route. Biochemical changes observed in guinea pigs infected by the intraperitoneal route include hyponatremia, striking changes in serum trace metals, amino acids and proteins, changes in liver enzymes indicating hepatic necrosis, and evidence of leukocytosis followed by leukopenia (Hambleton et al. 1982).

Guinea pigs infected with *L. pneumophila* by an oral route of exposure demonstrate a febrile disease with mild pneumonitis and splenitis (Katz and Matus 1984). In one study, subacute exposure to *L. pneumophila* in drinking water over a period of 17 days did not cause clinical illness, and none of the guinea pigs seroconverted (Conner and Gilbert 1979).

Other Animal Models

Rats have also been used as models for *L. pneumophila* infection. Winn et al. (1982) found that acute pneumonia occurred in both rats and guinea pigs; however, the rats appeared to be more resistant to lethal infection and extrapulmonary inflammatory lesions. Davis et al. (1983a) also demonstrated a

milder illness in rats similarly exposed. Exposure of marmosets to small-particle aerosols of *L. pneumophila* produces acute fibrinopurulent pneumonia like that observed in guinea pigs (Baskerville et al. 1983b). Rhesus monkeys are less susceptible than the marmosets or guinea pigs, and the pulmonary lesions are less severe.

Mice have also been used as models for *Legionella* infection. Fitzgeorge et al. (1983) found that Porton mice were highly resistant to aerosol infection with *L. pneumophila*; mice remained healthy and did not develop antibodies. ICR mice infected by intraperitoneal injection had a moderate to low susceptibility to infection by *Legionella*, and Mongolian gerbils were found to be highly susceptible (Patton et al. 1979). A tabular summary of the dose responses of various animals to experimental *L. pneumophila* infection provided in the 1985 *Legionella* Criteria Document aids in emphasizing that there is great species variation in susceptibility to *Legionella* infection.

LD_{50} Data

LD_{50} data and median 50% infection doses (ID_{50}) have been documented in guinea pigs exposed to *L. pneumophila* by the aerosol route:

- ID_{50} of <129 bacteria with an LD_{50} of $1.4x10^5$ organisms (Berendt et al. 1980)
- LD_{50} in the range of 500-5000 retained CFU and a fever production ID_{50} dose of 20 CFU (Huebner et al. 1984)
- retained LD_{50} of 10^4 (Baskerville 1984)

Long-Term Effects

The long-term effects of *Legionella*-induced pneumonia are pulmonary fibrosis and functional impairment of the lung. Studies in surviving guinea pigs, Rhesus monkeys and marmosets exposed to aerosol infections of *L. pneumophila* have shown that alveolar fibrosis, cellular infiltration of alveolar walls, and blockage of some terminal airways are common features 10 days after exposure, and were still present in guinea pigs after one month (Baskerville et al. 1983a). The infecting organism did not persist in the lungs, and pulmonary abscesses did not develop. In Syrian hamsters intratracheally infected with

L. pneumophila, the alveolar response to the infection was still prominent after 90 and 180 days in some lungs, and the severity of the inflammation was correlated with a persistent restrictive defect in lung elasticity (Parenti et al. 1989).

B. Summary

Although animals are not naturally infected by *Legionella*, their use as models for the study of human legionellosis is beneficial in understanding the etiology of its clinical manifestations. Experimental studies of legionellosis in animals, particularly guinea pigs exposed by the respiratory route of infection, provide useful information on human legionellosis due to the close similarities of these diseases. These similarities are discussed in detail in the 1985 *Legionella* Criteria Document.

The disease process in the lungs of susceptible animals is characterized by multiplication of the *Legionella* in recruited macrophages; destruction of the macrophages eventually results in hypoxia and impaired respiratory function. Clinical features include weight loss, fever and seroconversion. The LD_{50} for guinea pigs exposed to *L. pneumophila* by the aerosol route is somewhat less than 10^5 cells. The long-term effects of *Legionella*-induced pneumonia are pulmonary fibrosis and functional impairment of the lung.

There are varying degrees of susceptibility to *Legionella* infection among animal species. In comparison to guinea pigs, which have been studied extensively, rats, monkeys, marmosets and mice are more resistant to infection by *Legionella* aerosols. Gerbils are highly susceptible to infection by the intraperitoneal route.

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V. Health Effects in Humans

Legionellosis in humans has typically been characterized as either a non-pneumonic condition known as Pontiac fever or a pneumonic condition known as legionnaires' disease. This chapter summarizes new information available since publication of the 1985 *Legionella* Criteria Document on legionellosis in humans, specifically symptoms and clinical manifestations, clinical laboratory findings, mechanism of action, immunity, chronic conditions, and treatment.

A. Symptoms and Clinical Manifestations

Pontiac fever is described as an acute, self-limiting illness with "flu-like" symptoms. The illness is characterized by an attack rate of greater than 90 percent of exposed persons and an incubation period ranging from 24 to 48 hours (Nguyen and Yu 1991, Roig et al. 1994). The symptoms include fever, chills, headache, myalgia, and malaise (Muder et al. 1989, Nguyen and Yu 1991). The illness typically resolves without complications within two to five days (Muder et al. 1989). Upper or lower respiratory tract symptoms have not been associated with this illness. No additional information on Pontiac fever was located.

Since publication of the 1985 *Legionella* Criteria Document, the course of legionnaires' disease has been more precisely defined (Davis and Winn 1987, Ampel and Wing 1990; Nguyen et al. 1991, Stout and Yu 1997). The incubation period for legionnaires' disease is two to ten days, although incubation periods exceeding ten days have been reported (WHO 1990). Malaise, myalgia, anorexia, and headache typically occur within 48 hours. These symptoms are usually accompanied by a rapidly rising fever that frequently reaches 39°C or 40°C. Chills may also occur with the fever. A dry cough is typically present in the early stages of the illness. Although the cough may become productive with minimally or moderately purulent sputum within several days, hemoptysis is rarely observed. Other common early features of the illness include neurologic abnormalities (e.g., confusion, disorientation, lethargy) and gastrointestinal symptoms (e.g., nausea, vomiting, watery diarrhea). As the illness progresses, chest pain (often pleuritic), dyspnea, and respiratory distress may be observed.

Frequencies of these common symptoms vary. Table V-1 summarizes frequencies for these symptoms based on estimates provided in two review articles. An important point to note is that the clinical features described for legionnaires' disease do not distinguish it from other bacterial pneumonias (Roig et al. 1994). Recent studies have shown that symptoms initially thought to occur with greater frequencies in patients with legionnaires' disease are actually not distinctive. For example, Edelstein (1993) reported that diarrhea, which has historically been considered a distinctive feature of legionnaires' disease, occurred with similar frequency in patients with legionnaires' disease (0-25%) compared to patients with pneumonias caused by other agents (3-36%). Similarly, bradycardia and neurologic abnormalities have been "discredited" as distinctive features (Roig et al. 1994, Stout and Yu 1997). Edelstein (1993) concluded that prospective comparative studies have demonstrated that no one clinical feature can be used to distinguish legionnaires' disease from pneumonia caused by other agents.

Extrapulmonary diseases resulting from legionnaires' disease are rare, but have been reported with increasing frequency since publication of the 1985 *Legionella* Criteria Document. Infections in which *Legionella* species have been implicated include sinusitis (Lowry and Tompkins 1993), cellulitis (Waldor et al. 1993, Kilborn et al. 1992), pancreatitis (Kesavan et al. 1993, Eitrem et al. 1987), peritonitis (Lowry and Tompkins 1993), brain abscess (Andersen and Sogaard 1987), perirectal abscess (Lowry and Tompkins 1993), acalculous cholecystitis (Earle and Hoffbrand 1990), transient aplastic anemia (Martinez et al. 1991), myositis (Warner et al. 1991), and various wound infections (Lowry and Tompkins 1993). Stout and Yu (1997) stated that the heart is the most common extrapulmonary site. This assertion is supported by numerous reports of myocarditis (De Lassence et al. 1994, Armengol et al. 1992, Devriendt et al. 1990), pericarditis (Lowry and Tompkins, 1993, Domingo et al. 1989), and endocarditis (Berbari et al. 1997, Chen et al. 1996).

Table V-1. Frequency of Symptoms of Legionnaires' Disease

Symptoms	Frequency (% of Patients)	
	A [1]	B [2]
Fever	--	99
> 38.2°C	--	71

> 39°C	70-95	--
> 39.4°C	--	79 (65)
Cough	75-95	89
Chills	59-73	78
Headache	32-75	50
Myalgias	38-75	--
Dyspnea	--	48
Neurological/Confusion	25-50	37 [3]
Diarrhea/Nausea	13-54	45 [4]
Chest Pain	30-42 [5]	45

1 The source of information is Davis and Winn 1987. The authors did not provide any indication regarding the number of patients evaluated, but noted that the frequency was a "composite estimate from published series."
2 The source of information is Ampel and Wing 1990. The authors indicated the frequency was based on 231 patients. Numbers indicated in parentheses are exceptions.
3 Symptom was listed as "neurologic abnormalities."
4 Symptom was listed simply as "diarrhea."
5 Symptom was listed as "pleuritic pain."

The kidney is also a common extrapulmonary site. In the Philadelphia epidemic of 1976, 14 of the 123 cases of legionnaires' disease developed acute renal failure (Shah et al. 1992). Since 1976, at least 53 additional cases of legionnaires' disease complicated with acute renal failure have been reported (Lin et al. 1995). Based on the limited histopathology that has been conducted, the acute renal failure appears to be a result of either acute tubulointerstitial nephritis (Shah et al. 1992, Haines and Calhoon 1987) or acute tubular nephritis (Shah et al. 1992, Fenves 1985), although acute pyelonephritis (Shah et al. 1992) and glomerulonephritis (Pai et al. 1996, Wegmüller et al. 1985) have been reported.

Typically, extrapulmonary infections occur concurrently with pneumonia and are believed to result from bacteremia (Stout and Yu 1997, Edelstein 1993). Where extrapulmonary infections develop prior to the onset of pneumonia, identifying the primary site of infection may be difficult. Several cases of infections attributed to *Legionella* species in the absence of pneumonia have been reported (Edelstein 1993). These infections may be the result of direct inoculation of a site with water contaminated with

Legionella bacteria. Table V-2 summarizes those extrapulmonary sites in which *Legionella* infections have been implicated in the presence and absence of pneumonia. As a final note, extrapulmonary infections tend to occur with greater frequency in immunocompromised patients or in patients with severe cases of legionnaires' disease (Edelstein 1993).

Table V-2. Extrapulmonary Sites of *Legionella* Infection (Source: Edelstein 1993)

Presence of Pneumonia		Absence of Pneumonia
Blood	Pericardium	Blood
Brain	Bone marrow	Surgical wounds
Bowel	Skin and fascia	Bowel
Kidney	Rectum	Respiratory sinus
Liver	Myocardium	Endocardium
Spleen	Thyroid	Peritoneum
Hemodialysis shunt	Pancreas	Pericardium
Peritoneum	Testes	Skin and fascia
Prostate	Muscle	
Peripheral lymph nodes		

B. Clinical Laboratory Findings

Many abnormalities in standard clinical laboratory tests have been noted in patients with legionnaires' disease. Some of the more common findings are summarized in Table V-3. The clinical laboratory findings that are most frequently associated with legionnaires' disease are hyponatremia (Stout and Yu 1997, Roig et al. 1994, EPA 1985) and elevated levels of serum transaminase or transpeptidase enzymes (Edelstein 1993, EPA 1985). Edelstein (1993), however, reported that in only one of four prospective studies, patients with legionnaires' disease showed an increased incidence of hyponatremia compared to patients with pneumonia caused by some other agent. Furthermore, hyponatremia was observed in only about 20 percent of the patients with legionnaires' disease in these studies. Edelstein also reported that only one of four prospective studies showed an increased incidence of elevated serum enzyme levels in patients with legionnaires' disease compared to patients with

pneumonia caused by some other agent. One study indicated the converse, and two indicated no difference. Edelstein noted that "the most reasonable conclusion is that nonspecific test results cannot be used to clearly distinguish between those with or without legionnaires' disease."

Table V-3. Common Clinical Laboratory Findings in Patients with Legionnaires' Disease [1]

Leukocytosis	Serum glutamic oxaloacetic transaminase
Hyponatremia	Serum glutamic pyruvic transaminase
Hypophosphatemia	Serum lactic dehydrogenase
Proteinuria	Alkaline phosphatase
Hematuria	Creatine phosphokinase
Liver function abnormalities	

1 Information was compiled from the following sources: Ampel and Wing 1990, Muder et al. 1989, Strampfer and Tu 1988, Ching and Meyer 1987.

Although investigators appear to agree that no one clinical feature or laboratory finding distinguishes legionnaires' disease, some have recently reported that a diagnosis of legionnaires' disease may be made using a multifactorial clinical model (Breiman and Butler 1998, Cunha 1998). Cunha (1998) noted that one problem is that the "literature does not address the diagnostic significance of characteristic signs and symptoms in concert." He recently reported a weighted point evaluation system to aid physicians in the diagnosis of legionnaires' disease.

The majority of patients with legionnaires' disease exhibit abnormalities in the chest radiograph (Muder et al. 1989). Although "all types of roentgenographic patterns are seen in cases of legionnaires' disease" (Edelstein 1993), unilateral alveolar infiltrates, which may be segmental, lobar, or diffuse, are typically observed in the early stages of the disease (Muder et al. 1989). These infiltrates may enlarge and consolidate as the disease progresses (Ampel and Wing 1990). Pleural effusion is typically observed in one-third of patients with legionnaires' disease (Ampel and Wing 1990, Stout and Yu 1997). The frequency, however, ranges from 6 to 63 percent and, therefore, is not a distinguishing feature (Edelstein 1993). Nodular opacities and cavitation are uncommon, except in immunocompromised patients (Strampfer and Tu 1988, Muder et al. 1989, Stout and Yu 1997). Radiographic progression can occur even with appropriate antibiotic therapy, and resolution is typically slow (i.e., may require one to four

months) (Muder et al. 1989, Stout and Yu 1997). Similar to clinical laboratory findings, clinicians have concluded that "no characteristic radiographic pattern helps to distinguish any one type of pneumonia" (Coletta and Fein 1998).

C. Mechanism of Action

The typical progression of a *Legionella* infection can be characterized by the following steps (Cianciotto et al. 1989). First, *Legionella* is inhaled or instilled into the lower airways of the lungs. The mechanism for evasion of the body's non-specific defenses has not been established. Second, alveolar macrophages phagocytize the bacteria by either a conventional or coiling mechanism. The resulting phagosome becomes studded with ribosomes within four to six hours. Intracellular survival of the bacteria may be attributed to one or more of the following factors: reduced oxidative burst, failure of phagosome to acidify, failure of phagosome to fuse with lysosome, and/or bacterial resistance to lysosomal contents. Third, the bacteria undergo rapid intracellular growth. In fact, the bacterial growth within infected macrophages has been estimated at 100- to 1000-fold within 48 to 72 hours of infection, which is considered remarkable compared to that of other intracellular opportunistic bacteria (e.g., *Salmonella*, *Mycobacterium*, *Listeria*) (Friedman et al. 1998). Finally, the host cell dies and releases the bacteria. Intracellular infection and bacterial growth is then escalated. At this stage, tissue damage and induction of an inflammatory response may occur as a result of exposure to bacterial cellular components and/or extracellular products from the bacteria.

Significant effort has been invested into elucidating the factors responsible for the pathogenesis of *Legionella*. The 1985 *Legionella* Criteria Document described three "toxic" bacterial components: a lipopolysaccharide (LPS) located in the outer membrane of *Legionella* bacteria, an extracellular acid-soluble toxin isolated from several *Legionella* species, and an extracellular cytotoxin isolated from *L. pneumophila*. A variety of proteolytic enzymes were also recognized as potentially important factors in the pathogenesis of *Legionella*. Since completion of the 1985 *Legionella* Criteria Document, a variety of cellular components and extracellular products have been identified (Rechnitzer 1994). Their involvement in the pathogenesis of *Legionella*, however, has not been established.

One biologically important extracellular bacterial component that has been isolated is a zinc metalloprotease. This enzyme has exhibited proteolytic, hemolytic, and cytotoxic activity and has received a variety of names (e.g., tissue-destructive protein (TDP), major secretory protein, cytolysin) (Rechnitzer 1994). This enzyme has been shown to elicit the same type of pulmonary lesions that develop in legionnaires' disease and has been found in lungs of guinea pigs infected with *L. pneumophila* at a level equal to the dose of the purified protease known to cause death in experimental animals (Conlan et al. 1988). This enzyme has also been shown to degrade two phosphorylated proteins generated by a phosphokinase system isolated from the pulmonary cells of rabbits (Belyi 1990). Although the significance of this specific system is unknown, phosphokinase systems generally are involved in controlling intracellular metabolic processes. Therefore, this enzyme may disrupt metabolic processes of the host cell in addition to causing necrosis.

One additional factor recently recognized that may contribute to the pathogenesis of *Legionella* is the symbiotic relationship of the bacteria with amoebae. Brieland et al. (1996) investigated the effect of intratracheal coinoculation of *L. pneumophila* and *Hartmannella vermiformis* into A/J mice. A/J mice are recognized as an animal model for human legionnaires' disease and have been used extensively to investigate many aspects of *Legionella* infections. *H. vermiformis* is "the most prevalent species of amoebae in potable water supplies in the United States and has been epidemiologically linked to outbreaks of legionnaires' disease." They found that coinoculation resulted in significantly increased intrapulmonary growth of *L. pneumophila*, an increased severity of infection, and significant mortality when compared to inoculation with only *L. pneumophila*. Furthermore, they found that coinoculation with *L. pneumophila* and *H. vermiformis* into a resistant host (i.e., BALB/c mice) resulted in an eight-fold increase in intrapulmonary bacterial growth when compared to inoculation with only *L. pneumophila*.

To confirm that the amoebae were providing a niche for bacterial replication, Brieland et al. (1997a) investigated the effect of coinoculation of A/J mice with *H. vermiformis* and mutant strains of *L. pneumophila* that had reduced virulence for *H. vermiformis*. They found that the intrapulmonary bacterial growth was not significantly increased in mice coinoculated with *H. vermiformis* and the mutant strains. The authors concluded that virulence for the amoebae is necessary for increased bacterial

growth and, therefore, "inhaled amoebae may potentiate intrapulmonary growth of *L. pneumophila* by providing a niche for bacterial replication."

As a final note, Brieland et al. (1997b) investigated the effect of inoculating A/J mice with *H. vermiformis* infected with *L. pneumophila*. They found that the infected amoebae were more pathogenic than an equal number of *L. pneumophila* or a mixture of *L. pneumophila* and uninfected amoebae. The authors concluded that amoebae infected with *L. pneumophila* may be the infectious particles in *Legionella* infections.

D. Immunity

Both humoral and cell-mediated immune responses to *Legionella* infections have been documented (EPA 1985, Friedman et al. 1998). Based on the results of serological tests, antibodies are produced in response to *Legionella* infections that interact with specific bacterial components. Furthermore, bacterial infection results in the activation of complement, which has been shown to occur through both the classical and alternative pathway (Friedman et al. 1998, Mintz et al. 1992). Opsonization of bacteria (i.e., binding of antibodies and/or complement to the bacteria) has been shown to increase phagocytosis by human peripheral blood monocytes and animal macrophages; however, the ability of the bacteria to replicate within these cells does not appear to be diminished (Friedman et al. 1988). Therefore, the protection provided by specific antibodies *in vivo* is not currently known.

Cell-mediated immunity is recognized as the primary defense to *Legionella* infection (Susa et al. 1998). Research indicates that cytokines secreted by T_H1 helper cells or macrophages play a primary role in limiting bacterial replication (Friedman et al. 1998). For example, interleukin-2, which is secreted by T_H1 helper cells, appears to activate natural killer cells to lyse cells infected with *Legionella*, thus limiting bacterial growth by killing the host cell (Friedman et al. 1998).

Interferon- is also an essential component in host resistance to *Legionella* infection. This cytokine, which is also secreted by T_H1 helper cells, appears to activate macrophages and monocytes to inhibit bacterial growth. In fact, bacterial growth has been shown to decrease 100-fold in activated macrophages compared to non-activated infected macrophages (Friedman et al. 1998). The limited

growth may be the result of down-regulation of the transferrin receptors, which results in a decreased availability of intracellular iron, an essential component in *Legionella* growth (Skerrett and Martin 1991, Susa et al. 1998).

Many studies have confirmed the important role of interferon- in the resistance to *Legionella* infection. For example, based on a comparison of aged mice to young mice, Fujio et al. (1995) proposed that the susceptibility of the elderly to *Legionella* infection may be the result of a decreased capacity to produce interferon- . Finally, Heath et al. (1996b) investigated the effect of *Legionella* infection in BALB/c mice (i.e., a resistant species) and in BALB/c mice in which the interferon- gene was disrupted. Mice were inoculated intratracheally with *L. pneumophila*. Bacterial growth was not observed in the BALB/c mice; however, the mutant BALB/c mice developed "persistent, replicative intrapulmonary *L. pneumophila* infections with extrapulmonary dissemination of the bacteria to the spleen." Intratracheal administration of interferon- to the mutant BALB/c mice increased clearance of the bacteria from the lungs. The authors concluded that these results confirm the importance of interferon- in the resistance to *Legionella* infection.

One additional factor that appears to play an important role in resistance to *Legionella* infection is tumor necrosis factor- , which is a cytokine secreted by macrophages. Blanchard et al. (1988) reported that polymorphonuclear leukocytes treated with tumor necrosis factor- exhibited increased bactericidal activity against *L. pneumophila*. Furthermore, mice treated with tumor necrosis factor- prior to infection exhibited reduced mortality, which correlated with increased clearance of bacteria from the lungs. Matsiota-Bernard et al. (1993) reported that treatment of human peripheral monocytes with tumor necrosis factor- significantly inhibited the growth of *L. pneumophila*. Inhibition was not observed when an inhibitor of tumor necrosis factor production or anti-tumor necrosis factor antibodies were added to the culture medium. The mechanism by which tumor necrosis factor- inhibits bacterial growth has not been established, although one proposal is that this cytokine may potentiate nitric oxide release (Susa et al. 1998, Skerrett and Martin 1996).

E. Chronic Conditions

As discussed in the 1985 *Legionella* Criteria Document, most patients with legionnaires' disease recover without chronic manifestations. Ching and Meyer (1987), however, reported that fatigue and weakness may persist for several months following treatment. Furthermore, as noted above, resolution of infiltrates on chest radiographs is slow and may take from one to four months (Stout and Yu 1997).

Respiratory abnormalities resulting from legionnaires' disease occasionally occur. Gea et al. (1988) reported the outcome of 11 patients with legionnaires' disease followed for 53 months. Mild to moderate ventilatory and/or gas exchange abnormalities were observed several months following discharge from the hospital. At study termination, the majority of patients (8/11) exhibited one or more of the following respiratory abnormalities: a restrictive ventilatory defect, a low transfer factor, and/or hypoxemia. Because the majority of patients were smokers, some with chronic bronchitis, the authors could not dismiss the possibility that these manifestations were the result of pre-existing conditions.

More serious respiratory abnormalities are rare. Pulmonary pathology that has been reported includes pulmonary fibrosis, bronchiolitis obliterans, chronic vasculitis, and chronic organizing pleuritis (Ching and Meyer 1987, EPA 1985).

F. Treatment

Early initiation of appropriate treatment is recognized today as crucial for a successful outcome of legionnaires' disease. Heath et al. (1996a) conducted a retrospective analysis of serologically confirmed cases of legionnaires' disease to determine factors associated with increased mortality. After multiple logistic regression analysis, the only factor associated with increased mortality was a delay in initiation of appropriate therapy.

Retrospective analyses of early epidemics of legionnaires' disease have helped define "appropriate" therapy. Early studies indicated that patients treated with erythromycin had a lower mortality rate than patients treated with aminoglycosides, -lactam antibiotics, or chloramphenicol (6% versus 30-40%) (Roig et al. 1993). The poor response to these antibiotics has been related to their inability to penetrate phagocytic cells. In fact, studies have indicated that clinically effective antibiotics

must have the following features: (1) superior *in vitro* activity against *Legionella* species, (2) "the ability to enter and concentrate within phagocytic cells," and (3) "the ability to achieve high concentrations in lung tissue and alveolar exudate" (Roig et al. 1993).

Current recommendations for antibiotic treatment of legionnaires' disease are provided in Table V-4. Erythromycin has historically been considered the first choice in treatment of legionnaires' disease (Stout and Yu 1997). Treatment with this antibiotic, however, is associated with several adverse side effects, including transient hearing loss, phlebitis, gastrointestinal intolerance, and, more rarely, ventricular arrhythmia (Roig et al. 1993). Newer macrolides, such as azithromycin and clarithromycin, are attractive because they have exhibited superior activity against *Legionella* species and greater intracellular penetration with potentially fewer adverse effects (Klein and Cunha 1998, Stout and Yu 1997, Roig et al. 1993). With development of intravenous formulations, these newer macrolides (e.g., azithromycin) may replace erythromycin as the treatment of choice (Stout and Yu 1997).

Quinolones have shown greater activity against *Legionella* species and higher intracellular penetration than the macrolides (Klein and Cunha 1998, Stout and Yu 1997, Edelstein et al. 1996). These antibiotics have been recommended for transplant recipients with legionnaires' disease because, unlike the macrolides and rifampicin, they do not interfere with metabolism of immunosuppressive medications (Stout and Yu 1997). Although successful outcomes have been reported using these antibiotics, Baty et al. (1997) reported a case of pneumonia in an immunocompetent patient resulting from *L. jordanis* that was unresponsive to ciprofloxacin and ofloxacin. These antibiotics, however, were administered at dose levels lower than those suggested in Table V-4.

Other antibiotics that have shown variable success in treatment of legionnaires' disease include the tetracyclines (e.g., doxycycline, minocycline, and tetracycline) and the combination of trimethoprim-sulfamethoxazole (Stout and Yu 1997, Roig et al. 1993). Rifampicin is an antibiotic

Table V-4. Recommendations for Antibiotic Treatment of Legionnaires' Disease[1]

Antibiotic	Dose	Route	Frequency
Macrolides			
Azithromycin	500 mg[2]	oral or intravenous	every 24 hours

Antibiotic	Dose	Route	Frequency
Clarithromycin	500 mg	oral or intravenous[3]	every 12 hours
Erythromycin	1000 mg 500 mg	intravenous oral	every 6 hours every 6 hours
Quinolones			
Levofloxacin	500 mg[2]	oral or intravenous	every 24 hours
Ciprofloxacin	400 mg 750 mg	intravenous oral	every 8 hours every 12 hours
Ofloxacin	400 mg	oral or intravenous	every 12 hours
Tetracyclines			
Doxycycline	100 mg[2]	oral or intravenous	every 12 hours
Minocycline	100 mg[2]	oral or intravenous	every 12 hours
Tetracycline	500 mg	oral or intravenous	every 6 hours
Trimethoprim-sulfamethoxazole	160 mg/800 mg 160 mg/800 mg	intravenous oral	every 8 hours every 12 hours

1 Source of information is Stout and Yu 1997. Recommendations are based on clinical experience rather than controlled trials. Specific recommendations may vary slightly depending on the source of information.

2 Doubling of the first dose was recommended by Stout and Yu (1997). Edelstein (1998), however, does not recommend this suggested practice for azithromycin and levofloxacin.

3 Intravenous route is under investigation in United States.

that is used in combination therapy for severely ill patients and is typically administered either orally or intravenously at dose levels of 300-600 mg every 12 hours (Stout and Yu 1997). Although rifampicin has shown excellent *in vitro* and *in vivo* activity against *Legionella* species, it is not administered as a monotherapy due to the potential of developing rifampicin-resistant strains of *Legionella* (Roig et al. 1993). Rifampin has been combined with many antibiotics, but some uncertainty exists regarding the clinical efficacy of rifampicin and quinolone combinations (Roig et al. 1993, Edelstein et al. 1993). Furthermore, the clinical efficacy of the conventional combination of rifampicin and erythromycin has been questioned. Hubbard et al. (1993) conducted a retrospective analysis of patients with legionnaires' disease requiring intermittent positive pressure ventilation. Those patients receiving rifampicin in combination with erythromycin had a significantly increased incidence of jaundice, had significantly higher levels of bilirubin, and did not have decreased mortality compared to those patients that did not receive rifampicin.

For the treatment of legionnaires' disease, the preferred route of administration of any antibiotic therapy is intravenous (Stout and Yu 1997). This route ensures the greatest potential concentration of

antibiotic in the lung tissue. Intravenous treatment should continue until the patient becomes afebrile. At this point, intravenous treatment can be replaced by oral therapy. The total duration of therapy depends on the patient history. For a patient with a mild illness exhibiting significant improvement, therapy should continue for a period of approximately two weeks. For the severely ill or immunocompromised patient, therapy should continue for three weeks. Newer macrolides (e.g., azithromycin) may allow for a shorter course of treatment.

G. Summary

Since publication of the 1985 *Legionella* Criteria Document, much has been learned regarding legionnaires' disease in humans. Although its progression has been more precisely defined, no one symptom has been recognized that can distinguish legionnaires' disease from other bacterial pneumonias. Similarly, abnormalities in standard clinical laboratory tests and chest radiographs cannot be used to distinguish legionnaires' disease from other pneumonias. Some investigators, however, have recently reported that a multifactorial clinical approach may be helpful in the diagnosis of legionnaires' disease.

Although extrapulmonary diseases resulting from legionnaires' disease are still relatively rare, they have been reported with increasing frequency since publication of the 1985 *Legionella* Criteria Document. The kidney is a common site of extrapulmonary infection; however, the heart is now recognized as the most common site of extrapulmonary infection. These extrapulmonary infections can occur in the absence of pneumonia. No significant new information has been located on chronic conditions resulting from legionnaires' disease.

Since 1985, the mechanism of bacterial replication has been more precisely defined. Briefly, bacteria are inhaled or instilled in the lower airways of the lung and are phagocytized by alveolar macrophages. Bacteria undergo rapid intracellular growth within the phagosome. The host cell lyses and releases the bacteria, which escalates the bacterial infection.

Significant effort has been invested into the elucidation of factors responsible for the pathogenesis of *Legionella*. One important discovery was the isolation of a zinc metalloprotease, an

enzyme that elicits pulmonary lesions similar to those that develop in legionnaires' disease. Although not a bacterial component or product, one factor that may affect the pathogenesis of *Legionella* is their ability to infect amoebae. Recent research suggests that *Hartmannella vermiformis* may provide a niche for bacterial replication in the lungs. In fact, one study suggests that amoebae infected with *L. pneumophila* may be responsible for bacterial infection.

Recent research has continued to document that both humoral and cell-mediated immune responses to *Legionella* infection occur. Although specific antibodies are produced, the protection that these antibodies provide *in vivo* is still unknown. Cell-mediated immunity is currently recognized as the primary defense against *Legionella* infection. Research also has emphasized the importance of specific cytokines (e.g., interferon- , tumor necrosis factor-) in host resistance to *Legionella* infection. Much more research is needed to understand the host's mechanisms of resistance to these bacteria.

Since publication of the 1985 *Legionella* Criteria Document, many advancements in the treatment of legionnaires' disease have been made. Although erythromycin has historically been considered the first choice for the treatment of legionnaires' disease, newer macrolides (e.g., azithromycin) are available that exhibit superior activity to *Legionella* and greater intracellular penetration with potentially fewer adverse effects. Furthermore, quinolones show promising activity against *Legionella* infections and are recommended for patients on immunosuppressive medication. Early initiation of appropriate therapy is crucial for a successful outcome to legionnaires' disease.

VI. Risk Assessment

Risk assessment is a tool for the synthesis of available scientific information, in both a qualitative and quantitative manner, in order to characterize the probability of potential public health hazards resulting from exposure to a toxic or infectious agent. The results of such an assessment can then be employed in making informed risk management decisions. Over the past 25 years, scientists have developed methodologies to assess risks to human health from exposure to chemicals in the environment, foods, or drugs. The application of this methodology to the assessment of risks from microbial pathogens is a much newer field, however. This chapter presents the relevant information, where available, for a risk assessment of *Legionella* in water supplies.

A. Hazard Identification

As discussed in the preceding chapter as well as in the 1985 *Legionella* Criteria Document, *Legionella* are opportunistic pathogens that cause a pneumonic condition known as legionnaires' disease in some individuals. Outbreaks and sporadic cases have been reported following exposure in the general community and among hospitalized persons (i.e., nosocomial cases). *Legionella* are considered opportunistic pathogens because, although they are highly prevalent in the environment, relatively few people develop a clinical infection. Yu and colleagues (1993) characterized the attack rate for *Legionella* as "strikingly low."

Knowledge gained from advances in laboratory identification techniques and more rigorous epidemiological studies suggests that *Legionella* are responsible for a growing percentage of both community- and hospital-acquired pneumonias. These advances have allowed a better understanding of the relative impact of *Legionella*-caused pneumonia in the U.S. From a review of pneumonia patients in Ohio, Marston and colleagues (1997) estimated that between 8,000 and 18,000 (2-4 %) of the total 485,000 community-acquired cases of pneumonia requiring hospitalization annually in the U.S. are due to *Legionella*. This estimate is associated with significant uncertainty, however, because the causative organism is identified in only 50 percent of pneumonia cases (Reynolds 1996, Marrie et al. 1996).

Legionnaires' disease is the most serious illness caused by *Legionella* organisms. The clinical course of this disease, which was described in detail in Chapter V, is quite similar for community- or hospital-acquired infections (Petro-Botet et al. 1995). Other infections caused by *Legionella* are self-limiting (e.g., Pontiac fever) or are much more rare (e.g., infection of surgical incisions or other wounds) (Lowry and Tompkins 1993). Therefore, risk assessment of this organism is focused on legionnaires' disease as the endpoint of concern.

B. Dose-Response Information

The 1985 *Legionella* Criteria Document noted that quantitative data on the infectivity of *Legionella* in humans had not been reported. Unfortunately, sufficient information is still not available to support a quantitative characterization of the threshold infective dose (i.e., the dose required to produce infection) of *Legionella*. Animal models show a great interspecies variation in susceptibility to infection with *Legionella*, as described in Chapter IV. Due to the potentially serious health effects, experiments to identify the infective dose in humans are not possible. *Legionella* are opportunistic pathogens that replicate within host cells, reach target tissue via several routes (primarily inhalation or aspiration), and exhibit a very low attack rate or virulence in the general population; therefore, it is not surprising that definitive dose-response information continues to be elusive.

C. Potential for Human Exposure to *Legionella*

1. Prevalence of *Legionella* in the Environment

As discussed in Chapter III and in the 1985 *Legionella* Criteria Document, there is clear consensus that *Legionella* bacteria are widely distributed in the environment, especially in treated or potable water supplies. Important niches or reservoirs for *Legionella* can occur within treated water supply systems due to their ability to form symbiotic relationships with other microorganisms (including biofilm formation) and their subsequent resistance to standard disinfection techniques (e.g., chlorination). Since 1985, there have been numerous studies documenting the presence of *Legionella* in potable water and in water distribution systems of all types of large buildings including hospitals, office buildings and hotels, and also smaller buildings and family residences (see Chapter III). In some cases, *Legionella* occur in the absence of any reported cases of legionnaires' disease (Oppenheim et al. 1987, Stout et al. 1992b). Through the combination of environmental sampling studies with laboratory and epidemiological findings, a better understanding has been gained for the relative importance of various reservoirs for transmission of *Legionella* to humans.

2. Mode of Transmission to Humans

Given the widespread prevalence of *Legionella* in the environment and their niches within reservoirs of water supply systems, it is important to have an understanding of the circumstances under which *Legionella* bacteria can reach the lower respiratory tract of humans and potentially cause serious disease. Based on such knowledge of the important reservoirs and routes of transmission of *Legionella* to humans, the most appropriate preventive measures can be selected.

Legionella are transmitted directly from the environment to humans. There is very little, if any, evidence of human-to-human transmission, and there is no evidence of any animal reservoirs with public health relevance for this organism. In the past decade, considerable interest and controversy have been focused on the mechanisms by which *Legionella* bacteria reach the lower respiratory tract, where they are engulfed by alveolar macrophages and commence the pathological process of infection. One route is the inhalation of an aerosol containing respirable droplets of water (or other liquids)

contaminated with *Legionella*. Alternatively, *Legionella* may be deposited in upper airways and subsequently aspirated into the deeper portions of the lung. As mentioned in Chapter IV and in the 1985 *Legionella* Criteria Document, infection in animals following ingestion of *Legionella* has also been demonstrated experimentally.

At the time of the 1985 *Legionella* Criteria Document, scientists believed transmission of *Legionella* in the community or hospital setting occurred primarily via inhalation of infectious aerosols; however, this assumption was based, for the most part, on circumstantial evidence. Now, with advances in laboratory identification techniques and the availability of more rigorous epidemiological and experimental data, there is increasing emphasis on the role of aspiration as a route of transmission. Thus, it follows that there is an increased focus on potable water as a primary source of infection. Environmental sampling from outbreaks (in communities or in hospitals) most frequently has implicated potable water as the source (Stout and Yu 1997).

The 1985 *Legionella* Criteria Document reported that the most common reservoirs of transmission for community-acquired *Legionella* infection are aerosols from: heat-rejection equipment (cooling towers, evaporative condensers, steam turbine cleaning), components of plumbing systems (showers, faucets, hot water tanks), nebulizers, humidifiers, whirlpool spas, or public fountains. Less common sources include: ingestion of potable water, immersion in raw water, inhalation of contaminated oil/water mixtures, and excavations (dust or soil) (EPA 1985). Additional types of aerosol generators (e.g., grocery store mist machines) have been linked to outbreaks of legionnaires' disease (Mahoney et al. 1992). No additional categories of sources have been identified during the period since the 1985 EPA report, but the relative importance of contributions from some of these sources has shifted. Although cooling towers are still a source of some community-acquired cases (e.g., Castellani Pastoris et al. 1997, Bhopal and Fallon 1988), potable water (with subsequent inhalation or aspiration of aerosols) is acknowledged as a much more important source (Stout and Yu 1997, Neill et al. 1985). There are still insufficient data to support quantification of the relative contributions from these various sources (Bhopal 1995).

One of the most interesting and important advances made recently in the study of *Legionella* transmission concerns the role of amoebae and other larger protozoa in enabling or enhancing the

transmission of *Legionella*. Working with *Acanthamoeba* in culture with *L. pneumophila* (isolated from a cooling tower), Berk et al. (1998) examined the *Legionella*-filled vesicles formed and expelled by the amoebae. The vesicles were 2.1-6.4 m in diameter (i.e., respirable size), and the study authors calculated that, based on volumes, each vesicle could contain as many as 200 bacteria. Other investigators have estimated even higher numbers of bacteria per vesicle (Rowbotham et al. 1986 as cited in Berk et al. 1998). Berk and colleagues also demonstrated that vesicles free in the medium were resistant to biocide and that the biocide treatment facilitated the release of large numbers of vesicles as it induced encystment of the amoebae. Such infectious vesicles may represent a very important vehicle of transmission for *Legionella*, by protecting the bacteria from dessication while in the atmosphere and delivering a possibly infective dose to the respiratory tract. Thus, these preliminary findings contribute to the complexity of modeling exposure and dose-response relationships for *Legionella* infections in humans.

For nosocomial cases of legionnaires' disease, there is a growing body of evidence from case observation and experimental data that points to aerosolization of potable water (tap water) as the most important source of transmission of *Legionella*. Blatt et al. (1993) analyzed 14 nosocomial cases that occurred in a military hospital and compared them with controls. Environmental sampling for *Legionella* showed colonization of 15% of potable water sites, one hot water tank, and the groundwater supply to the hospital, while no *Legionella* were isolated from the hospital cooling towers, building air intakes or other hospital air and oxygen supplies. This case-control comparison showed a negative association between showering and acquiring legionnaires' disease, although earlier studies have sometimes reported a positive association with showering (EPA 1985, Breiman et al. 1990, Hanrahan et al. 1987 as cited in Blatt et al. 1993).

Potable water is now consistently identified as the most common source of *Legionella* in hospitals (Yu 1993, Blatt et al. 1993, Woo et al. 1992). Observation of hospital cases indicates a high risk of infection for patients who have received ventilation support or have been exposed to respiratory equipment (e.g., nebulizers), suggesting a major role for aspiration as a route of transmission for hospital-acquired legionnaires' disease. The relative significance of aspiration is also supported by the very low infection rates (or antibody titers) among hospital personnel where nosocomial *Legionella* outbreaks have occurred (Marrie et al. 1986).

The sources of *Legionella* that play an important role in transmitting the bacteria to humans have been fairly well characterized by now. Knowledge gaps exist, however, regarding the relationship between environmental concentrations of *Legionella* and the ultimate risk of infection in exposed individuals. It is, therefore, useful to review the factors that may place an individual at increased risk for developing legionnaires' disease.

D. Risk Factors

For opportunistic pathogens such as *Legionella* bacteria, identification of risk factors in susceptible individuals is an essential element for the selection of the most appropriate control and prevention measures. Based on the very low attack rates associated with this organism, it is clear that the general U.S. population is quite resistant to infection by *Legionella*.

Certain patient populations are clearly at increased risk for contracting nosocomial legionnaires' disease. These populations include patients who require intubation, patients who have received ventilation assistance (including patients who have undergone surgery), and patients receiving respiratory therapy with potentially contaminated medical equipment or whose care includes the use of aerosol generators such as humidifiers or nebulizers (England et al. 1981, Marston et al. 1994, Stout and Yu 1997).

Certain demographic factors are associated with an increased susceptibility to legionnaires' disease following exposure. Subpopulations at increased risk include men over the age of 50, heavy smokers, and heavy drinkers (Bhopal 1995, Marston et al. 1994, England et al. 1981). These findings are based on analyses of very large series of legionnaires' disease cases. For example, Marston and colleagues (1994) reviewed data for 3,254 patients whose cases were reported to the CDC between 1980 and 1989. The findings reported by England et al. (1981) represent the first 1,000 confirmed cases of legionnaires' disease reported to the CDC (through September 1979).

People with certain underlying health conditions also have a significantly increased risk of contracting legionnaires' disease. Such medical conditions include: chronic obstructive pulmonary disease, diabetes, head or neck cancer, other malignancy, or end-stage renal disease. In addition, any

disease state (e.g., AIDS) or medical treatment (e.g., drugs such as corticosteroids or cancer chemotherapy, or procedures such as hemodialysis) that suppresses or depletes a patient's immune system can lead to an increased susceptibility to opportunistic infections such as legionnaires' disease. Several patient populations (e.g., renal transplant patients, especially those requiring hemodialysis) are at an extremely high risk for legionnaires' disease, as they have both an increased risk of exposure (via their surgery and other ventilation needs), and an increased susceptibility (due to corticosteroid therapy and dialysis) (Woo et al. 1986, LeSaux et al. 1989).

Many of these risk factors contribute not only to increased incidence of legionnaires' disease among these groups, but also increased severity of the disease and increased mortality. Marston and colleagues (1994) found that, among 3,254 legionnaires' disease cases reported to the CDC *Legionella* surveillance system between 1980 and 1989, the following factors were significantly associated with increased mortality attributed to legionnaires' disease: the use of steroids or other immunosuppressive drugs; the presence of cancer, diabetes, or renal disease requiring dialysis; hospital-acquired infection; older age; male gender; isolation of *L. pneumophila* subgroup 6 (Lp6); or isolation of more than one *Legionella* species or *L. pneumophila* serogroup. More severe legionnaires' disease has also been documented in smaller series of cases among bone marrow transplant patients (Harrington et al. 1996) and patients receiving immunosuppressive drugs (with or without chronic disease) (Pedro-Botet et al. 1998).

People immunocompromised due to HIV infection are also at risk of developing more severe legionnaires' disease, but *Legionella* infections (in the absence of other pneumonia-causing pathogens) in this population are relatively rare. This may be due, in part, to exposure to other more common (and more virulent) pathogens in the environment and, in part, to increased infection control vigilance (including concern for waterborne pathogens) when patients with AIDS are hospitalized. Marston and colleagues at the CDC (1994) reported an increased prevalence of legionnaires' disease among AIDS patients compared to the general U.S. population (8 people with AIDS among 2,575 legionnaires' disease cases; 0.19 expected). Bangsborg et al. (1990) reported that among 180 AIDS patients with pneumonia, only six had *Legionella* infection, but four of these six patients were also infected with the fungus *Pneumocystis carinii*. A high rate of coexistent pulmonary infection (again, with *P. carinii*) was also reported by Blatt et al. (1994): among seven HIV-infected individuals who had legionnaires' disease, six

were also infected with *P. carinii*. Of bacterial pneumonias reported in persons with AIDS, other species that are more pathogenic and hardier than *Legionella* are reported most frequently, including *Haemophilus influenzae*, various *Streptococci*, and *Branhamella catarrhalis* (Chaisson 1998).

Another population that may be at increased risk of contracting *Legionella* infection is neonates, due to their underdeveloped immune systems, intensive ventilation procedures, and corticosteroid therapy. Nosocomial cases of legionnaires' disease have been reported, albeit infrequently, in this population (Holmberg et al. 1993, Horie et al. 1992). Older infants and children who have the risk factors identified for adult populations (e.g., are receiving corticosteroid therapy or are undergoing mechanical ventilation) are also at increased risk of contracting legionnaires' disease (Carlson et al. 1990). But even though pneumonia (all types/sources) is common in the general pediatric population, reports of legionnaires' disease in otherwise healthy children is extremely rare (Abernathy-Carver et al. 1994, Carlson et al. 1990, Famiglietti et al. 1997).

E. Quantification of Potential Health Effects

The 1985 *Legionella* Criteria Document reported that our understanding of the mechanisms of transmission of and infection by *Legionella* was inadequate for quantification of the potential health effects or the development of specific recommendations for control (EPA 1985). Although improvements in laboratory isolation and identification techniques for *Legionella*, along with important experimental, epidemiological, and ecological study results, have greatly expanded our understanding of *Legionella* infections in humans, the current state of the science still does not allow estimation of the probability of the potential adverse health effects caused by *Legionella*. Estimation of the infective dose (i.e., the dose required to produce infection) is necessary for completing a risk assessment of a microbial pathogen. *Legionella* are opportunistic pathogens with widespread environmental occurrence and a very low attack rate in the general population. *Legionella* survive and thrive inside vesicles after being ingested by amoebae in water reservoirs, but much more information is needed on the implications of this potential vehicle for enhanced transmission and infectivity. More complete information is also needed concerning the conditions under which a population is likely to be exposed to the infective dose, with models that accommodate aspiration and inhalation routes, as well as the variability introduced by the bacteria's potential replication within host cells.

Despite deficiencies in understanding several of the factors necessary to determine the risk of infection by *Legionella*, the current state of knowledge is sufficient to support specific recommendations to control and prevent legionnaires' disease.

F. Minimizing Risk

In the 22-year period since the sentinel outbreak in Philadelphia of what is now known as legionnaires' disease, a great deal of knowledge has been gained on the behavior and occurrence of *Legionella*. Based on this knowledge, efforts to minimize the risks of *Legionella* infection have been instituted, especially for the protection of susceptible or high-risk individuals.

Because there is little if any person-to-person transmission of *Legionella* and no vaccine is available to prevent infection, risk minimization efforts are focused on breaking the chain of transmission between environmental sources of *Legionella* and human hosts. Approaches used for controlling the growth of *Legionella* in treated water, frequently used in combination, include heat, chlorination, ultraviolet light, copper-silver ionization, and ozone treatment. These various treatment options are detailed in Chapter VII of this report. For hospitals and other health care settings, regular environmental surveys of both hot water systems and distal sites should be conducted; some health departments have issued mandates for such testing (Allegheny County Health Department 1997). In health care institutions, these environmental surveys can also serve to raise awareness and the index of suspicion of health practitioners for consideration of *Legionella* as the causative agent in nosocomial pneumonia cases (Yu 1997).

Active surveillance for *Legionella* infection, especially among hospital patients at highest risk of acquiring nosocomial infection (i.e., transplant patients, immunocompromised patients, or patients with certain chronic underlying health conditions) is also an important tool for minimizing risk of legionnaires' disease because it allows for prompt remedial actions and rapid diagnosis and treatment of confirmed cases. As discussed in Chapter V of this document, earlier treatment is associated with reduced severity of disease and reduced mortality. Both the control measures and active surveillance for cases can be expensive, however, and ultimately require cost-benefit decisions. Several recent publications have outlined some of the important considerations in making such cost-benefit decisions.

Shelton and colleagues (1993) developed a system for determining when *Legionella* detection may justify preventive or remedial actions. Analysis of samples from buildings with reported legionnaires' disease outbreaks and with no reported cases (a total of 900 samples) revealed a strong association between amplified *Legionella* levels and legionnaires' disease outbreaks. Their system matches action levels (scale of 1 to 5) with specific environmental concentrations of viable *Legionella* detected in three types of sources: cooling towers and evaporative condensers; potable water; and humidifiers/foggers. The concentrations corresponding to the action levels vary depending on the environmental source. For example, the highest level of concern (Hazard Level 5) corresponds to *Legionella* concentrations above 1,000 organisms/mL in cooling towers, above 100/mL in potable water, or above 10/mL in a humidifier/fogger. For Hazard Level 5, the authors recommend immediate disinfection of equipment. Hazard Level 3 represents "a low but increased level of concern" and corresponds to *Legionella* concentrations above 10/mL in cooling towers and above 1/mL in potable water or a humidifier/fogger. The study authors noted, however, that among the 900 samples, some of the samples rated Hazard Level 5 were obtained in buildings without any reported legionnaires' disease cases. Such findings illustrate the lack of a direct relation between detected environmental levels of *Legionella* and risk of disease. Nevertheless, preventive or remedial actions may be warranted when *Legionella* concentrations exceed certain limits.

In 1997, the Centers for Disease Control (CDC) for the first time included information on *Legionella* infections in their revised "Guidelines for Nosocomial Pneumonia" (CDC 1997a). For hospitals without any identified cases, the CDC outlined two primary prevention measures: (a) routine culturing of the potable water system, with initiation of active surveillance (i.e., increasing the use and availability of diagnostic laboratory tests for *Legionella*) when 30 percent or more of environmental samples are positive for *Legionella*; or (b) utilizing diagnostic laboratory tests for high risk patients with nosocomial pneumonia, with routine maintenance of potable water supplies (i.e., with sufficient heat and chlorination), and initiation of an environmental investigation once one definite or two possible cases of legionnaires' disease have been identified.

The CDC Guidelines also outline secondary prevention measures for hospitals where nosocomial legionnaires' disease cases have been identified, with a caveat that full-scale environmental investigations and decontamination measures may not always be indicated in all hospitals. The decision

to initiate such measures depends on the level of risk for infection and mortality from *Legionella* in the given patient population. Nonetheless, the CDC also cautions that a low threshold for initiating an environmental investigation may be appropriate because nosocomial cases have typically been underdiagnosed, and additional recent or ongoing nosocomial cases typically are identified once several cases have been confirmed. The Guidelines (CDC 1997) describe five important steps in conducting such an environmental investigation: (1) review of medical records; (2) active surveillance to identify recent or ongoing nosocomial cases; (3) identification of risk factors for infection and comparison of cases and controls, through the collection of information on environmental exposures (e.g., showering or the use of respiratory therapy equipment); (4) collection of water samples from the implicated sources and other potential aerosol sources; and (5) subtype matching of patient isolates and the environmental samples. Decontamination or replacement of the identified environmental sources must also take place. Clearly, these secondary prevention measures can require extensive resources.

G. Summary

Given that legionnaires' disease is the most serious infection caused by *Legionella*, risk assessment of these organisms should be focused on legionnaires' disease as the endpoint of concern. *Legionella* are opportunistic pathogens with widespread distribution in the environment but a very low rate of infection in the general population. The sources of transmission of *Legionella* to humans have been well characterized, and almost all of these sources (with the exception of contaminated medical equipment) involve the aerosolization of water contaminated with *Legionella* and subsequent inhalation or aspiration. Potable water, especially in hospitals and other buildings with complex hot water systems, is considered to be the most important source of *Legionella* transmission.

Despite many advances in laboratory isolation and identification techniques and the availability of findings from recent epidemiological and experimental studies, the current state of the science does not allow for quantification of the potential risks caused by *Legionella* in water supplies. Nevertheless, important preventive (or remedial) actions have been identified that can minimize the risks of *Legionella* infection, especially for the protection of high-risk or susceptible individuals.

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VII. Analysis and Treatment

A. Analysis of Samples

Legionella can survive in a wide range of conditions including variable temperatures, pH-levels, and dissolved oxygen concentrations. In addition, algae and other water bacteria can promote their growth (Nguyen et al. 1991). Detection of *Legionella* contamination in potable water and plumbing fixtures, as well as in biological samples, is a major concern, particularly of hospitals experiencing cases of legionnaire's disease. The 1985 *Legionella* Criteria Document discusses collection (disassembly, swabbing and scraping, centrifugation, filtration), isolation (culturing), and detection techniques (Direct Fluorescent Antibody (DFA), Indirect Fluorescent Antibody (IFA), monoclonal antibodies, and radioimmunoassay). However, more recent studies and additional data on the collection, isolation, and detection of *Legionella* in both water and biological samples have been published and are described below.

1. Collection of *Legionella*

Most outbreaks of legionellosis come from warm waters, as higher temperatures generally stimulate the growth of these organisms. It is difficult to culture *Legionella* in waters below 20°C (Colbourne et al. 1988). Test samples for *Legionella* typically come from anthropogenic sources such as faucets, sink outlets, taps, filters, and showerheads, which are usually sampled by disassembling, swabbing, and scraping to obtain *Legionella*-bearing debris or scale (Stout et al. 1992b, Helms et al. 1988, Stout and Yu 1997, Barbaree et al. 1987). As reported in the 1985 *Legionella* Criteria Document, the most effective manner of obtaining the sample is by insertion of sterile cotton swabs into the interior surface of the water source. Ta et al. (1995) found that swabbing recovered greater concentrations of *Legionella* organisms than two other methods (water sampling before swabbing and water sampling after swabbing), exposing an average of 30.2 CFU in each swab sample while the concentration of *Legionella* in the water samples averaged 4.7 CFU. Swab sampling is also the preferred sampling method because the swab is easier to transport and requires less processing time than straight water samples (Ta et al. 1995).

The study by Ta et al. (1995) also concluded that concentration of the water sample, either by filtration or centrifugation, greatly improved the ability to detect *Legionella* in the samples. Filtration was proven more effective than centrifugation, recovering 77 percent of the expected organism count while centrifugation recovered only approximately 34 percent (Ta et al. 1995).

The 1985 *Legionella* Criteria Document provided summaries of several studies that used filtration or centrifugation to concentrate *Legionella*, and recommended heat and acid wash treatment to isolate *Legionella* from environmental specimens. Because *Legionella* can survive at high temperatures, heating (at 60°C for 1-2 minutes) was found to reduce the strains of other bacteria contaminants (e.g., *Pseudomonas aeruginosa*) by 98 percent while leaving the *Legionella* unaffected.

Acid wash treatment is used to isolate *Legionella* because unlike most bacteria, *Legionella* strains are acid resistant (Nguyen et al. 1991). Ta et al. (1995) showed that although acid buffer treatment did not enhance the recovery of *L. pneumophila* bacteria, it was in fact required for an optimal yield of other strains. A detailed procedure for isolation of *Legionella* from environmental water samples by acid treatment was described by Bopp et al. (1981) and summarized in the 1985 *Legionella* Criteria Document. Water samples were pretreated, either concentrated by centrifugation or not concentrated, with an HCl-KCl buffer mixture at pH 2.2 for periods of 5-60 minutes. The greatest quantity of isolations were obtained by acid treatment of centrifuged samples for 5 minutes (Bopp et al. 1981). More current studies have shown that samples treated with acid for three minutes can minimize the development of competing bacteria (Ta et al. 1995).

Following the collection and pretreatment steps, the samples are plated onto appropriate media. *Legionella* do not grow on standard culture media. They have complex nutritional requirements, featuring an unusually high iron requirement. The 1985 *Legionella* Criteria Document described various media that can be used for culturing *Legionella* including a charcoal yeast extract (CYE) medium, which was improved to the buffered charcoal yeast extract (BCYE) that is presently used to successfully isolate these organisms. This medium is ACES buffered charcoal yeast extract (BCYE) agar supplemented with -ketoglutarate (BCYE), a Krebs-cycle intermediate that is readily catabolized by these bacteria (Edelstein 1987). An incubation period of two to six days ensues when *Legionella* are cultured on this medium (Grimont 1986). The buffer maintains the pH within a range that is critical for *Legionella*

(around pH 6.9) while the -ketoglutarate stimulates growth. Growth is further enhanced by the addition of L-cysteine, keto acids, and ferric ions. Antimicrobials such as glycine (inhibitor), cefamandole, polymyxin B, vancomycin (antibacterials), and anisomycin and cyclohaxamide (antifungals) are added to inhibit or prevent the overgrowth of contaminants (Nguyen et al. 1991). Selective media containing dyes, glycine, vancomysin, and polymyxin (DGVP) is used for environmental sampling (Lin et al. 1998).

2. Detection of *Legionella* in Environmental and Biological Samples

An array of serological tests have been used for detecting *Legionella* in water, sputum, blood, serum, and urine samples. Kohler (1986) reports that antigens can be detected in the urine of approximately 80 percent of patients with *L. pneumophila* serogroup 1 pneumonia, and that the specificity of these assays is greater than 99 percent. Most tests are used on lower respiratory tract secretions, specifically tissue specimens, bronchial and tracheal secretions, and sputum. Sputum specimens are pretreated with acid and cultured on selective media, similar to the pretreatment of environmental samples. Tracheal aspirate specimens on culture media can provide a yield of 90 percent sensitivity (Nguyen et al. 1991).

The two main serologic tests performed on bacteria are direct and indirect fluorescent assays, which are applicable to both environmental and clinical specimens. Fluorescent organic compounds are attached to antibody molecules that are bound to a cell or tissue's surface antigens, and then these tags are detected by a fluorescent microscope. In the direct method, the antibody against the organism is fluorescent, while the indirect method has the fluorescent antibody detected against a nonfluorescent antibody on the surface of the cell. The Direct Immunofluorescence Assay (DFA) and the Indirect Immunofluorescence Assay (IFA), which were examined in the 1985 *Legionella* Criteria Document, are described further below. Other serologic tests described in the 1985 *Legionella* Criteria Document and discussed below are enzyme-linked immunosorbent assays, monoclonal antibodies, and radioimmunoassay. Serologic tests that are currently being used for detection of *Legionella* antigens or antibodies, including Polymerase Chain Reaction, and nucleic acid and DNA probes, are also discussed below. The serologic tests differ primarily in sensitivity, specificity, predictive value, and complexity.

Direct Immunofluorescence Assay (DFA)

The most common and rapid test for *Legionella* is the DFA. According to Nguyen et al. (1991), the exhibited sensitivity of DFA tests ranges anywhere from 25 to 85 percent. Sputum, lung specimens, and bronchial and tracheal secretions are excellent samples to test by the DFA method (Grimont 1986). Kohler (1986) reports that as long as proper quality control exists, the specificity of DFA testing in respiratory specimens is greater than 90 percent. However, he cites examples of DFA accuracy results for serogroup 1 infections of 50 percent, 47 percent, 68 percent, and 33-47 percent, where sensitivity and specificity were not ideal in testing respiratory specimens. There are also DFA tests that use species-specific monoclonal antibodies that are particularly useful for lower respiratory tract samples and tissues. The monoclonal reagent is optimal for detection of *L. pneumophila* in respiratory specimens due to its ability to detect multiple serogroups of *L. pneumophila* and decreased non-specific fluorescence of the specimen and other bacteria. It may not be useful in the detection of environmental specimens (Grimont 1986).

Indirect Immunofluorescence Assay (IFA)

Legionella bacteria, and antibodies in patient sera, are detected through IFA. Heat-fixed antigens are commonly used in the United States, but formolized antigens, used primarily in Europe, are said to actually be more specific than heat-fixed antigens. Because seroconversion only occurs after a rather long time period in humans, the IFA test is often used in conjunction with other tests (Kohler 1985). A series of serological tests are typically conducted to test for antibodies, and they are most often run in conjunction with the IFA (Colbourne et al. 1988, Grimont 1986, Edelstein 1987, Kohler 1985, Ehret et al. 1986, Kashuba and Ballow 1996).

Enzyme-Linked Immunoabsorbent Assays (ELISA)

Enzyme-Linked Immunosorbent Assays (ELISA), radioimmunoassay (RIA), and agglutination assays have also been used to detect *Legionella* antibodies. These methods employ enzymes and radioisotopes to detect antibody molecules. The ELISA method is used to detect *Legionella* antibodies in patient sera, but it has also been used to detect *Legionella* antigens in urine. The RIA method has also

been used for the detection of *Legionella* antigens in urine, but is no longer commercially available. The agglutination method has been used to detect antibodies in serum and antigens in urine. These tests are all extremely sensitive because radioactivity and enzyme reaction products can be measured in very small amounts. Enzyme-linked methods are preferred over radioactively tagged methods of discovery to eliminate the problem of radioactive material disposal even though a longer incubation period is required for these types of tests. With ELISA, preheating of the specimen is required to avoid false positives. Agglutination assays, which clump organisms to other particles, are simpler and faster assays that are generally easier to perform than the others (Kohler 1986).

Monoclonal Antibody

Monoclonal antibody tests, tests with antibodies formed from a single clone of cells, have been found to be more accurate than polyclonal tests due to the suppression of background fluorescence. Also, false positive results from cross-reactivity with non-*Legionella* organisms are eliminated (Stout and Yu 1997). Monoclonal antibody tests are effective due to their high specificity for a single antigenic determinant. Monoclonal antibodies can be produced to react only with a particular species, or even strain, of bacteria. According to Kohler (1986), numerous laboratories have asserted that antibodies for *L. pneumophila* have been developed to be species-specific and even serogroup-specific. These monoclonal antibodies can be aimed against subsets of a specific serogroup and then used for antigen detection by ELISA (Kohler 1986).

Polymerase Chain Reaction (PCR)

The Polymerase Chain Reaction (PCR) test uses two disparate primers. One is specific for *Legionella* species, and the other is specific for *L. pneumophila* only. The primers are specific for the gene sequences of the 5S ribosomal RNA gene. The PCR was converted into a kit called the EnviroAmp® Legionella Kit; however, the kit is no longer commercially available. PCR is a relatively new method designed to rapidly multiply DNA target genes in a laboratory setting to yield detectable quantities for testing. A study done by Fricker and Fricker (1995) compares this technique to standard culture methods for water samples. The positive results of the PCR matched those of the culture in all but 4 of 87 cases, where the PCR reaction was apparently inhibited. Generally, the PCR was found to be

a very useful screening test because it is both fast and accurate. The main problem with the PCR method is that it identified several negative cultures as positive. This issue is being investigated, but it is either due to false positive results, discovery of dead *Legionella*, or detection of viable but non-culturable *Legionella* (Fricker and Fricker 1995).

Murdoch et al. (1996) studied the ability of PCR tests to detect *Legionella* DNA in urine and serum samples of pneumonia patients. There was a 64 percent detection rate in the urine and/or serum samples, with this figure rising to 73 percent if testing was done within four days of the onset of symptoms.

Nucleic Acid and DNA Probes

Nucleic acid and DNA probes can also be used to detect *Legionella*. With these methods, probes are marked with RNA or DNA sequences that are specific to a particular species or strain of bacteria. Nucleic acid probes require that the nucleic acids of the bacteria become accessible and prepared to react with the tagged probe. Detection using this method has been reported to be anywhere from 5 to 100 percent, with *L. pneumophila* giving the highest values (Grimont 1986). According to Edelstein (1987), a probe kit generally has a sensitivity of 75 percent and a specificity of 100 percent "if certain samples are excluded from the analysis." Although it is more sensitive to *L. pneumophila* detection, it will still quickly recognize all *Legionella* species (Edelstein 1987). This new detection method, however, as reported by Nguyen et al. (1991), has yet to be clinically validated and is rather insensitive and costly.

Urinary Antigen

There is a commercially available enzyme immunoassay (EIA) test for the *Legionella* antigen in urine from the company Binax, Inc. in Portland, Maine. Nguyen et al. (1991) reports that the test exhibits 99 percent specificity and greater than 90 percent sensitivity and that it is relatively inexpensive. The main drawback to this urinary antigen test is that it only detects antigens of *L. pneumophila* serogroup 1. However, since this species accounts for upwards of 80 percent of all legionellosis infections, this weakness is rather slight (Nguyen et al. 1991).

B. Disinfection as a Water Treatment Practice

Legionella are found in natural aquatic environments, artificial aquatic environments such as heat rejection devices (cooling towers and evaporative condensers), and water distribution systems (Muraca et al. 1990). Water distribution systems in hospitals, hotels, institutional buildings, and domestic homes, as well as personal respiratory therapy equipment, freestanding room humidifiers in hospitals, industrial cutting oil/water emulsions, and communally used whirlpools and spas have been shown to be reservoirs for *Legionella* (World Health Organization 1990, Moreno 1997). *Legionella* colonization is promoted by temperatures below 50°C (122°F), scale and sediment accumulation, stagnation (which prevents disinfectant from reaching the bacteria), and design of the hot water tank (see Chapter III, Section F for further discussion of factors affecting *Legionella* survival) (Muraca et al. 1990). The 1985 *Legionella* Criteria Document indicated that *Legionella* surviving initial water treatment may colonize pipe joints, cul-de-sacs, and corroded areas or adhere to the surface or sediment of storage tanks, especially those constructed of wood (EPA 1985). New distribution systems may also be a source of *Legionella* contamination; the 1985 document cited cases in which *Legionella* outbreaks have occurred in new distribution systems (EPA 1985).

There are several control methods available for disinfection of water distribution systems. These include thermal (super heat and flush), hyperchlorination, copper-silver ionization, ultraviolet light sterilization, ozonation, and instantaneous steam heating systems. These disinfection methods are discussed below. The use of heat, chlorine, ultraviolet sterilization, and ozone were discussed in the 1985 *Legionella* Criteria Document, however, recent studies have been conducted that provide updated information. Because one methodology may not be sufficient, a combination of these techniques may be more effective in eradicating *Legionella* from the system and preventing recolonization (Yu et al. 1993).

Thermal Disinfection

Thermal disinfection is a common practice for water distribution systems in hospitals, hotels, and other institutional buildings. The hot water temperature is elevated to above 70°C (158° F), and distal sites, such as faucets and showerheads, are flushed for thirty minutes (Nguyen et al. 1991, Miuetzner et al. 1997, Stout and Yu 1997). *L. pneumophila* is killed at temperatures above 60°C (140°F). At 70°C

(158°F), it takes ten minutes to eliminate *L. pneumophila* from water, and at 60°C (140°F) *L. pneumophila* are eradicated in 25 minutes (Muraca et al. 1990). In cases of outbreak, thermal disinfection can be quickly implemented. No special equipment is needed, and it is relatively inexpensive (Stout and Yu 1997, Muraca et al. 1990, Nguyen et al. 1991). The disadvantages to this method are the potential for scalding and the fact that many personnel are required to monitor distal sites, tank water temperatures, and flushing times (Nguyen et al. 1991, Muraca et al. 1990). In addition, recolonization will occur within months because disinfection using this method is only temporary (Lin et al. 1998).

In state development centers for mentally and physically handicapped people, hot water tanks positive for *Legionella* were heated to 71°C for 72 hours followed by flushing for 15 minutes. In one center, *Legionella* reoccurred after three months. Consequently, a quarterly heating schedule was established in both centers (Beam et al. 1984).

Hyperchlorination

Hyperchlorination of water distribution systems requires the installation of a chlorinator. Shock hyperchlorination involves the addition of chlorine to a water system, raising chlorine throughout the system to a concentration of 20 to 50 mg/L. The chlorine levels of the system are returned to 0.5 to 1 mg/L after one to two hours (Lin et al. 1998). Continuous hyperchlorination entails the addition of chlorinated salts (e.g., calcium hypochlorite (solid) or sodium hypochlorite (aqueous)) to the water at concentrations ranging from 2 to 6 mg/L (ppm) (Stout and Yu 1997, Muraca et al. 1990). Domestic residual levels are typically 1 mg/L (ppm) (Muraca et al. 1990). The 1985 *Legionella* Criteria Document suggests using chlorine levels of 1-2 mg/L (ppm), however, recent studies have shown that using chlorine levels of 3-5 mg/L is more effective (Helmes et al. 1988). The chlorinator will maintain a set level of chlorine throughout the system, which should completely eliminate *Legionella.* Unfortunately, this method is relatively expensive, and it does have some drawbacks. This method leads to corrosion of the pipes of the system after five to six years of operation, and eventually parts of the system may be destroyed. Corrosion can be reduced by the use of a silicate coating on the water pipes (Nguyen et al. 1991). In addition, mechanical failure of the chlorinator, if not detected, could result in *Legionella* recolonizing the system (Nguyen et al. 1991). Human health problems are another result of

hyperchlorination. High levels of trihalomethanes develop in the hot water of the system when chlorine levels exceed 4 mg/L (Helmes et al. 1988, Muraca et al. 1990). Trihalomethanes are potentially carcinogenic, and can be reduced by maintaining the concentration of the chlorine below 4 mg/L (Muraca et al. 1990).

Ezzeddine et al. (1989) describes disinfection in a hospital where 6 ppm of free residual chlorine was used in a heating tank during a 6-hour period of time. *Legionella* was eliminated from the tank; however, chlorination of the mixer tank, where the temperature was 45°C, was not successful even when chlorine levels were raised to 6 ppm over 48 hours.

Helmes et al. (1988) combined the hyperchlorination method with an elevated water temperature at a University of Iowa hospital after a 1981 outbreak of nosocomial legionellosis. Chlorine levels were set at 3-5 mg/L, while temperatures were raised to 60-70°C. After six months of hyperchlorination, *Legionella* was no longer detected in samples.

Copper-Silver Ionization

Copper-Silver Ionization distorts the permeability of the *Legionella* cell, denatures proteins, and leads to lysis and cell death. A commercial system can be easily installed to perform this ionization. This system sends an electrical current to copper/silver electrodes, which generate positively charged ions. These positively charged ions electrostatically bond to the negatively hypercharged sites on the cell walls of the microorganisms (Nguyen et al. 1991, Miuetzner et al. 1997, Muraca et al. 1990). The *Legionella* are then killed, making it unlikely that recolonization will occur. Copper-silver ionization is less expensive than hyperchlorination and provides residual protection throughout the water distribution system (Nguyen et al. 1991, Muraca et al. 1990). A disadvantage of this approach is that the system's performance will suffer unless scale is removed regularly from the electrodes and the pH of the system is maintained below 8. Also, extremely high concentrations of copper and silver ions will turn the water a blackish color, which can stain porcelain (Lin et al. 1998). Another disadvantage is that over an extended period of time, human consumption of the water from this system may result in accumulation of copper and silver and toxic effects (Muraca et al. 1990). However, because copper and silver ions are typically only added to hot water recirculating lines, human exposure would be minimal since

consumption of large amounts of water is unlikely (Lin et al. 1998). In addition, the levels of ions in hot water are maintained below the EPA recommended levels for cold drinking water which are 1.3 ppm copper and 100 ppb for silver (a secondary minimum contaminant level).

Miuetzner et al. (1997) used a flow-through cell containing two sets of four copper-silver electrodes. A single cell was installed in each of three hot water circuits of a hospital. The copper-silver ionization system significantly reduced the amount of *L. pneumophila* recovered from the faucets from 72 percent to 2 percent within one month. Control of *Legionella* was maintained for at least 22 months after the ionization treatment.

Ultraviolet Light Sterilization

Ultraviolet light kills *Legionella* by disrupting cellular DNA synthesis (Muraca et al. 1990). An ultraviolet light sterilization system can be installed easily. It can be positioned to disinfect the incoming water, or it can be installed at a specific place in the pipe system that services a designated area. The UV system consists of low-pressure mercury lamps in quartz sleeves. Sterilization is most effective at UV energy wavelengths of 254 nm and temperatures of 40°C (104°F) (Muraca et al. 1990). A filter should be used to remove particulates from the water to keep UV light transmission optimal (World Health Organization 1990). No chemical by-products are produced, and the taste and odor of water from a water distribution system containing a UV sterilizer are not affected (Muraca et al. 1990). The UV sterilization system requires continuous maintenance in order to prevent scale from coating the UV lamps. The system does not provide residual protection, so distal areas must be disinfected (Nguyen et al. 1991, Muraca et al. 1990). Operational problems, such as electrical malfunction and water leaks, are possible, in which case experienced technicians are needed (Muraca et al. 1990).

Ozonation

Ozone can be used to kill *L. pneumophila*. It can be created using ozonators, which electrically excite oxygen (O_2) to ozone (O_3). Ozone instantaneously inactivates *Legionella*; however, it has a short half-life and decomposes quickly back to oxygen. A second form of disinfection may be required in the distribution system for residual protection. Also, ozonation is more expensive than hyperchlorination,

and a large amount of space is required for the air preparation equipment or oxygen tanks and contacting tank (Muraca et al. 1990). Ozonation was described in the 1985 *Legionella* Criteria Document as a possible method of eliminating *Legionella* from a water distribution system. At the time, few studies had been conducted and the results were inconclusive.

Muraca et al. (1987) recommend using a 1-2 mg/L ozone residual for treatment of domestic water. They demonstrated that a 1-2 mg/L ozone residual caused a 5 log decrease in a *L. pneumophila* population of 10^7 CFU/mL over five hours within a model plumbing system.

Instantaneous Steam Heating

Instantaneous Steam Heating systems entail flash heating water to temperatures greater than 88°C (190°F) and then blending the hot water with cold water to attain a designated water temperature (Nguyen et al. 1991, Muraca et al. 1990). These systems are often cost-effective because specialized personnel are not needed to operate them; maintenance can be performed by regular building staff. The maintenance is, however, more complex than the maintenance of a conventional hot water tank. Instantaneous Steam Heating systems work best when installed as the original system of a building rather than when the building has already been contaminated by *Legionella.* Another drawback to this system is that it can only be used to control *Legionella* in the hot water supply system. The cold water portion of the distribution system is not disinfected (Muraca et al. 1990). Any *Legionella* that may have colonized the system downstream of the heater will be unaffected. In order for disinfection to be complete, the hot water temperature at outlet sites must exceed 60°C. These heaters may not have the ability to flush large amounts of outlets with superheated water for thirty minutes (Lin et al. 1998).

C. Summary

The examination of water for the presence of *Legionella* is best done by taking swab samples of the medium over which the water flows. The specimen should then be concentrated by filtration, treated with an acid buffer to enhance *Legionella* recovery, and cultured on a BCYE agar medium. *Legionella* can be detected in environmental and biological samples by a number of tests, the most common of which are direct and indirect immunofluorescence assays.

Contamination by *Legionella* has occurred in the water distribution systems of many hospitals, hotels, and other buildings. Various means of disinfection have been established and utilized. Some methods have not always proven completely successful or have not provided permanent protection from recolonization. A combination of these methods may be the most effective way of managing water systems and preventing future outbreaks. Yu et al. (1993) defines two categories of disinfection, focal and systemic. Focal disinfection is directed at a specific portion of the system and would include ultraviolet light sterilization, instantaneous heating systems, and ozonation. Systemic methods, such as thermal, hyperchlorination, copper-silver ionization, disinfect the entire system. Selecting a combination of focal and systemic disinfection techniques would ensure eradication of present *Legionella* colonies and prevent recolonization of the water distribution system.

VIII. Research Requirements

From all of the information presented in the previous chapters, it is clear that *Legionella* bacteria are an important cause of community- and hospital-acquired pneumonia, and they can be associated with serious morbidity and mortality, especially when the infection is not rapidly diagnosed and treated. *Legionella* are widely distributed in the environment, including treated water supplies. In the past 13 years (i.e., during the time since publication of the 1985 EPA Criteria Document on *Legionella*), dramatic advances have been achieved in our understanding of the behavior and transmission of *Legionella*, including information on: special ecological niches occupied by these organisms, including their presence in biofilms and their symbiotic relationships with larger microbes such as amoebae; improved techniques for the clinical isolation (e.g., culture techniques) and characterization (e.g., PCR technology) of these organisms; improved methods for identifying patients recently or currently infected with these organisms (e.g., urinary antigen assay); factors important for understanding the epidemiology of legionellosis infection; and effective measures for eradicating these organisms from treated water supplies.

Despite the important advances in the 13 years since the previous EPA *Legionella* Criteria Document, additional information is needed to institute optimal prevention and control measures and to minimize the morbidity and mortality associated with *Legionella*. Specific information gaps include the following:

- The relative influence of the symbiotic relationship between *Legionella* organisms and larger microbes on *Legionella* survival, transmission, virulence, and susceptibility to disinfection. More information is also needed on the implications of the intracellular replication of *Legionella* inside host microbes.

- Key environmental factors promoting the growth of *Legionella* in biofilms. Additional information is needed about the structure and physiology of biofilms, and in particular, the effects of changing environmental conditions on their ecology.

- More comprehensive data on the occurrence of *Legionella* in groundwater, especially as it relates to potable water supplies.

- Further information on the relative importance of various reservoirs of the organism (and thus the allocation of expenditures for disinfection); in particular, the diminishing role of cooling towers and the increasing prominence of potable water distribution systems as reservoirs for *Legionella*.

- The nature of the dose-response relationship for this organism, including the development of models, particularly for exposures from potable water. An effort should be made to determine the predictive value of *Legionella* concentrations found in a given reservoir. Research is also needed to establish the minimal infectious dose for high-risk populations.

- A clearer definition of the important factors involved in transmission of this infectious agent from a specific source, which would be facilitated by more accurate identification of legionellosis cases, especially of sporadic cases, and the corresponding improved epidemiological and environmental analyses.

- The further characterization of risk factors for acquiring legionellosis, particularly for community-acquired, sporadic cases. Many cases of legionellosis undoubtedly still go unrecognized. Information indicating patients at greatest risk of *Legionella* infection should also be disseminated more widely to clinicians, with the hope of more accurately and rapidly identifying (and treating for) *Legionella* as the causative agent, thus reducing morbidity and mortality associated with these organisms.

- The risk for development of legionnaires' disease from *Legionella* present in residential water systems (single family or multi-family dwellings).

- Identification of the most effective (and most cost-effective) biocidal treatments for a given source of *Legionella*.

- Delineation and development of specific design and operational/physicochemical modifications for building water supply systems, in order to minimize colonization by *Legionella* and symbiont hosts, including biofilm eradication.

Given the potentially high costs of surveillance for, and eradication of, *Legionella* from treated water supplies, new information that fills some of these gaps will be of great value in identification and institution of the best strategies for prevention of legionellosis.

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