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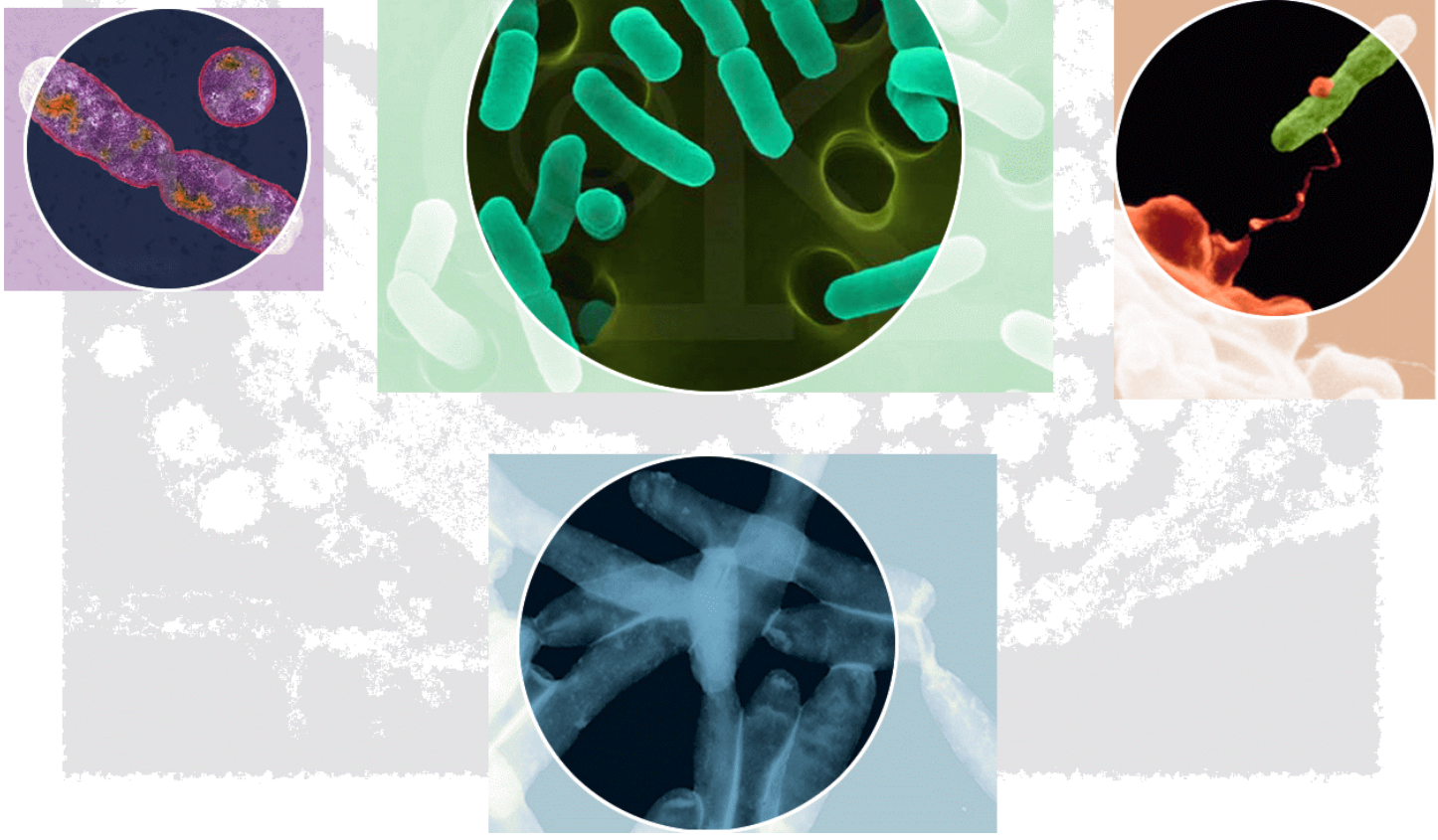
Legionella: Drinking Water Health Advisory



I. INTRODUCTION

The Health Advisory Program, sponsored by the Office of Water (OW), provides information on the health effects, analytical methodology and treatment technology that would be useful in dealing with the contamination of drinking water. Most of the Health Advisories (HAs) prepared by the Office of Water are for chemical substances. This Health Advisory however addresses contamination of drinking water by a microbial pathogen, examines pathogen control, and addresses risk factors for exposure and infection.

Health Advisories serve as informal technical guidance to assist Federal, State and local officials responsible for protecting public health when emergency spills or contamination situations occur. They are not to be construed as legally enforceable Federal standards. The HAs are subject to change as new information becomes available.

This Health Advisory is based on information presented in the Office of Water's Criteria Document (CD) for *Legionella*. Individuals desiring further information should consult the CD. This document will be available from the U.S. Environmental Protection Agency, OW Resource Center, Room M6099; Mail Code: PC-4100, 401 M Street, S.W., Washington, D.C. 20460; the telephone number is (202) 260-7786. The document can also be obtained by calling the Safe Drinking Water Hotline at 1-800-426-4791.

II. GENERAL INFORMATION

History

- *Legionella* bacteria were discovered following a pneumonia outbreak at the 1976 American Legion Convention in Philadelphia (Brenner 1987). The bacteria isolated from infected lung tissue and identified as the causative agent of this pneumonia outbreak was named *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. Pneumonia occurs in approximately 95 percent of *Legionella* infections (Nguyen et al. 1991). Less commonly, *Legionella* bacteria cause an influenza-like infection in humans called Pontiac fever (Hoge and Brieman 1991).

Taxonomy

- Although some phenotypic characteristics (i.e., gram stain, cell membrane fatty acid and ubiquinone content, morphology, and growth on specific media) can be used to recognize *Legionella* bacteria at the genus level, more specific diagnostic techniques are required to differentiate individual species (Bangsborg 1997, Fang et al. 1989, Winn 1988). Currently, the best methods to classify *Legionella* species are DNA analysis and antigenic analysis of various proteins and peptides.

- Currently, the *Legionella* genus consists of 42 species, seven of which are 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). The majority of human infections (70-90%) have been caused by *L. pneumophila*, especially serogroups 1 and 6 (Lo Presti et al. 1997).

Microbiology, Morphology, and Ecology

- *Legionella* bacteria are small gram-negative rods. They are unencapsulated and nonsporeforming, with physical dimensions from 0.3 to 0.9 m in width and from 2 to more than 20 m in length (Winn 1988). Most species 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 branched-chain cellular fatty acids and also ubiquinones with side chains of more than 10 isoprene units (Brenner et al. 1984). *Legionella* 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 from 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).

Symbiosis in Microorganisms

- *Legionella* proliferation is dependent on their symbiotic relationships with other 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 have been shown to 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).

- Currently, *Legionella* are known to infect a total of 13 species of amoebae and two species of ciliated protozoa (Fields 1996). *Legionella* also can multiply intracellularly 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 proliferate within these symbiont hosts provides *Legionella* with protection from otherwise harmful environmental conditions. Thus, *Legionella* are able to 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. Their enhanced resistance to water treatment has major implications for both disease transmittance and water treatment procedures.

- *Legionella* also grow symbiotically with the aquatic bacteria attached to the surface of biofilms (Kramer and Ford 1994). Biofilms provide the bacteria with nutrients for growth and also offer protection from adverse environmental conditions (including during water disinfection). The concentration of *Legionella* in biofilms depends upon water temperature; at higher temperatures, they can more effectively compete with other bacteria. 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 environmental monitoring for *Legionella* 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 section considers the worldwide occurrence or incidence of legionellosis and outbreaks of legionellosis as well as the occurrence of *Legionella* bacteria in water, soil, and air. Environmental factors influencing *Legionella* survival also are discussed.

Worldwide Distribution

- Cases of legionellosis have been reported 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 maintained 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 artifactual due to differences in definitions, diagnostic methods, surveillance systems, or data presentation (Bhopal 1993).

- National surveillance programs currently are conducted in the United States, 24 European countries, 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 (CDC 1994, CDC 1996, CDC 1997b). These figures represnt passive sureillance and accounts for <1000 reported cases when compared to Marston et al. data. Which suggest 10-15 times as many projected cases from active sureillance.

- In England and Wales, annual totals of reported cases declined briefly after a peak in 1988 but have been increasing since 1993 (Joseph et al. 1997).

- In 1996, cases of legionnaires' disease were reported in 24 European countries including England, Wales and Scotland (Anonymous 1997b). 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. This increase was attributed mainly to a large community outbreak in Spain in 1996.

- There have been 1,041 notifications of legionellosis in Australia since 1991, with similar numbers of cases reported each year (Anonymous 1997a).

Occurrence in Water

- *Legionella* are considered to be ubiquitous in the aquatic environment, including both natural water bodies and man-made waters (EPA 1985). Research has revealed that *Legionella* thrive in biofilms, and interaction with other organisms in biofilms is important for their survival and proliferation in water (Kramer and Ford 1994, Yu 1997, Lin et al. 1998a).

Natural Surface Water

- Studies clearly demonstrate the widespread occurrence of *Legionella* in freshwater (e.g., lakes and streams) and marine waters (EPA 1985, Ortiz-Roque and Hazen 1987, Palmer et al. 1993).

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*.

Man-Made Waters

- As noted previously, *Legionella* bacteria thrive 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).

- In the potable water supply, *Legionella* bacteria occupy niches suitable for their survival and growth (e.g., components of water distribution systems, cooling towers, and whirlpools), which function as amplifiers or disseminators of these bacteria (EPA 1985).

- In 1980, British investigators first demonstrated that plumbing fixtures in potable water systems contained *Legionella* (EPA 1985). Water distribution systems of hospitals, hotels, clubs, public buildings, homes, and factories continue to be a major source of *Legionella* exposure (EPA 1998, 1985).

- Studies have shown that *Legionella* are present in all segments of community water supplies, including water treatment facilities (Campo and Apraiz 1988, Colbourne and Dennis 1989, Colbourne et al. 1988, Voss et al. 1986).

- Numerous outbreaks of legionellosis have been linked to heat-exchange units (e.g., cooling towers and evaporative condensers) in hospitals, hotels, and public buildings, clearly establishing these reservoirs as habitats for *Legionella* (EPA 1998, 1985). However, as knowledge and awareness of the ecology and epidemiology of *Legionella* have increased, attention has shifted from heat-exchange units to potable water distribution systems as the most important sources of human exposure and infection (Lin et al. 1998b, Yu 1997).

- Whirlpools and spas also serve as ideal habitats for *Legionella* because they are maintained at temperatures ideal for their growth and organic nutrients suitable for bacterial growth often accumulate in these waters (Hedges and Roser 1991, Fallon and Rowbotham 1990, Hsu et al. 1986, Jernigan et al. 1996). In addition, whirlpools and spas can produce water droplets of respirable size that have the potential to transmit *Legionella* to humans (Jernigan 1996). Other related sources where *Legionella* have been identified include spring water spas and saunas (Bornstein et al. 1989a, 1989b, Den Boer et al. 1998).

- Legionella also have been detected in all phases of the sewage treatment process, including treated effluent (Palmer et al. 1993, 1995).

Occurrence in Soil

- Although water is the most documented source of *Legionella* in the environment, these bacteria have been isolated from mud, moist soil, and potting soil (EPA 1985, Steele et al. 1990). One species in particular, *L. longbeachae*, has been shown to inhabit and thrive in potting soil. *L. longbeachae* was able to persist for seven months in two potting mixes stored at room temperature (Steele et al. 1990). Soil rather than water may be the natural habitat of this species and may be a source of human exposure.

Occurrence in Air

- *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 (Bollin et al. 1985, EPA 1985, Seidel et al. 1987). Inhalation of *Legionella*-contaminated aerosols is an important source of human exposure and infection (EPA 1985).

Specific Disease Outbreaks

- Human exposure to *Legionella*-contaminated sources can result in outbreaks of legionellosis. Legionellosis outbreaks have been attributed most frequently to exposure to contaminated potable water, cooling towers, or components of water distribution systems. Outbreaks of legionellosis caused by contaminated cooling towers can be dramatic, with numerous cases occurring over a short period of time (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 be revealed only after active surveillance is introduced (Brady 1989, Colville et al. 1993, Goetz et al. 1998, Guiget et al. 1987, 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).

- Outbreaks of legionellosis are typically 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 cooling towers (EPA 1998).

- Travelers are usually exposed to *Legionella* in contaminated hotel potable water or contaminated whirlpool spas (EPA 1998).

- Community outbreaks are caused by exposure to the widest variety of sources, but potable water and cooling towers are the most common (EPA 1998).

- *L. pneumophila* has most frequently been implicated as the causative agent for all three types of outbreaks (EPA 1998).

- The majority of cases of legionnaires' disease are community-acquired and sporadic (i.e., non-outbreak related) (Stout et al. 1992a).

Environmental Factors Affecting *Legionella* Survival

Symbiotic Microorganisms

- The growth and survival of *Legionella* in the environment is enhanced by their ability to form symbiotic relationships with other larger microorganisms. *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).

- Because *Legionella* replicate rapidly intracellularly within protozoan hosts for prolonged periods of time, amoebic vesicles can contain hundreds of *Legionella* cells (Berk et al. 1998, Lee and West 1991). In addition, replication within protozoa may contribute to enhanced virulence of *Legionella* (Kramer and Ford 1994).

- The ability of *Legionella* to thrive within protozoa also allows them to survive over a wider range of environmental conditions and to resist the effects of chlorine, biocides, and other disinfectants (Fields 1996, Kramer and Ford 1994, Paszko-Kolva et al. 1993, States et al. 1989).

- Relationships with certain algae and bacteria in biofilms also foster the growth of *Legionella*, presumably due to the increased availability of nutrients and protection from disinfection (Kramer and Ford 1994).

Water Temperature

- *Legionella* exhibit the ability to survive in 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. Henke and Seidel (1986) claimed *Legionella* to be a "thermoresistant" organism that exhibits survival in natural warm waters of up to 60°C and artificially heated waters of 66.3°C.

- 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 and hot springs (Henke and Seidel 1986, Lee and West 1991, Verissimo et al. 1991).

- Colbourne and Dennis (1989) stated 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).

Other Factors

- Other factors influencing the survival of *Legionella* in the environment include sediment accumulation and metal content (Kusnetsov, 1993, States et al. 1987, Stout et al. 1992b, Stout et al. 1985, Vickers et al. 1987). These factors are usually amplified by ideal water temperature or coexisting environmental factors.

- "Blind Loops", caused by the positioning of heating elements in hot water tanks, and washers, grommets, etc. of various chemical composition can foster the growth of *Legionella* in plumbing systems (Hodge et al. 1991).

IV. HEALTH EFFECTS IN ANIMALS

- 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 (EPA 1985). 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 and dogs (EPA 1985).

- Experimental animals have been used primarily as hosts for the isolation of *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 (EPA 1985). Guinea pigs have been studied extensively due to similarities between the natural legionnaires' disease in humans and the experimental disease in guinea pigs (EPA 1985).

- There are varying degrees of susceptibility to *Legionella* infection among animal species In comparison to guinea pigs, other species such as rats, monkeys, marmosets and mice are more resistant to infection by *Legionella* aerosols (EPA 1985). Gerbils are highly susceptible to *Legionella* infection by the intraperitoneal route.

- The disease process in the lungs of susceptible guinea pigs is characterized by multiplication of the *Legionella* in alveolar macrophages with eventual destruction of the macrophages, release of toxic cellular products, and the accumulation of bacterial and cellular debris in the alveoli that may eventually results in impaired respiratory function and hypoxia (Davis et al. 1983). Clinical features include weight loss, fever and seroconversion (Berendt et al. 1980).

- The LD_{50} for guinea pigs exposed to *L. pneumophila* by the aerosol route is somewhat less than 10^5 cells (Baskerville 1984, Huebner et al. 1984).

- The long-term effects of *Legionella*-induced pneumonia are pulmonary fibrosis and functional impairment of the lung (Baskerville et al. 1983, Parenti et al. 1989).

IV HEALTH EFFECTS IN HUMANS

Symptoms and Clinical Manifestations

- 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 (EPA 1985).

- 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.

- The course of legionnaires' disease has been fairly precisely defined (Davis and Winn 1987, Ampel and Wing 1990; Nguyen et al. 1991, Stout and Yu 1997, WHO 1990). The incubation period is two to ten days, although incubation periods exceeding ten days have been reported. Malaise, myalgia, anorexia, headache, and fever typically occur within 48 hours. A dry cough is typically present in the early stages of the illness. 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, dyspnea, and respiratory distress may occur.

- No single symptom has been recognized that can distinguish legionnaires' disease from other bacterial pneumonias (Edelstein 1993, Roig et al. 1994, Stout and Yu 1997).

- Extrapulmonary diseases resulting from *Legionella* infection are relatively rare but can occur. The heart is the most common site of extrapulmonary infection (Armengol et al. 1992, Berbari et al. 1997, Chen et

al. 1996, De Lassence et al. 1994, Devriendt et al. 1990, Domingo et al. 1989, Lowry and Tompkins 1993, Stout and Yu 1997). The kidney is another site of extrapulmonary infection (Fenves 1985, Haines and Calhoon 1987, Lin et al. 1995, Pai et al. 1996, Shah et al. 1992, Wegmüller et al. 1985). These extrapulmonary infections can occur in the absence of pneumonia.

Clinical Laboratory Findings

- Many abnormalities in standard clinical laboratory tests have been noted in patients with legionnaires' disease. 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). However, abnormalities in standard clinical laboratory tests cannot be used to distinguish legionnaires' disease from other bacterial pneumonias (Edelstein 1993).

- Similarly, clinicians have concluded that no characteristic radiographic pattern helps to distinguish legionnaires' disease from other bacterial pneumonias (Coletta and Fein 1998).

Mechanism of Action

- The typical progression of a *Legionella* infection can be characterized by the following steps (Cianciotto et al. 1989). 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 phagosomes. The host cells lyse 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 (Conlan et al. 1988).

- Although not a bacterial component or product, another 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 (Brieland et al. 1996, 1997a, 1997b). One study suggests that amoebae infected with *L. pneumophila* may be responsible for bacterial infection.

Immunity

- Both humoral and cell-mediated immune responses to *Legionella* infection have been documented (EPA 1985, Friedman et al. 1998).

- Although specific antibodies are produced, the protection that these antibodies provide *in vivo* is still unknown (Friedman et al. 1998).

- Cell-mediated immunity is currently recognized as the primary defense against *Legionella* infection (Susa et al. 1998). Research also has emphasized the importance of specific cytokines (e.g., interferon- , tumor necrosis factor-) in host resistance to *Legionella* infection (Blanchard et al. 1988, Friedman et al. 1998, Skerrett and Martin 1996, Skerrett and Martin 1991, Susa et al. 1998).

Chronic Conditions

- Fatigue and weakness are two chronic conditions that may persist for several months following treatment (Ching and Meyer 1987). Most patients with legionnaires' disease recover without any chronic manifestations (EPA 1985).

- Mild respiratory abnormalities (e.g., restrictive ventilatory defect and/or hypoxemia) resulting from legionnaires' disease occasionally occur (Gea et al. 1988). More serious respiratory abnormalities are rare. Pulmonary pathology that has been reported includes pulmonary fibrosis and chronic vasculitis (Ching and Meyer 1987, EPA 1985).

Treatment

- Early initiation of appropriate therapy is considered crucial for a successful outcome to legionnaires' disease (Heath et al. 1996).

- Erythromycin (a macrolide antibiotic) has historically been considered the first choice for the treatment of legionnaires' disease (Stout and Yu 1997). However, newer macrolides (e.g., azithromycin) are available that exhibit superior activity to *Legionella* and greater intracellular penetration with potentially fewer adverse effects compared to erythromycin (Klein and Cunha 1998, Stout and Yu 1997, Roig et al. 1993). With development of intravenous formulations, these newer macrolides 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, they do not interfere with metabolism of immunosuppressive medications (Stout and Yu 1997).

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

- For the treatment of legionnaires' disease, the preferred route of administration of any antibiotic therapy is intravenous (Stout and Yu 1997). Intravenous treatment should continue until the patient's fever subsides. At this point, intravenous treatment can be replaced by oral therapy. The total duration of therapy depends on the individual patient's history.

IV RISK ASSESSMENT

Hazard Identification

- 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.

Dose-Response Information

- Sufficient information is not available to support a quantitative characterization of the threshold infective dose (i.e., the dose required to produce infection) of *Legionella*.

Potential for Human Exposure to *Legionella*

- *Legionella* are opportunistic pathogens with widespread distribution in the environment but a very low rate of infection in the general population.

- *Legionella* are transmitted directly from the environment to humans (EPA 1985). 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.

- 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 (Blatt et al. 1994, Stout and Yu 1997, Woo et al. 1992, Yu 1993).

Risk Factors

- The very low attack rates associated with this organism suggest 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).

- 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 an increased incidence of legionnaires' disease among these groups, but also increased severity of the disease and increased mortality (Harrington et al. 1996, Marston et al. 1994, 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 (Bangsborg et al. 1990, Marston et al. 1994).

- 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). Even though pneumonia (of all types/sources) is common in the general pediatric population, reports of legionnaires' disease in otherwise healthy children are extremely rare (Abernathy-Carver et al. 1994, Carlson et al. 1990, Famiglietti et al. 1997).

Quantification of Potential Health Effects

- 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.

Minimizing Risk

- 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 (Lin et al. 1998a, 1998b).

- For hospitals and other health care settings, where the lethal dose has been established 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.

- Both the control measures and the 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 (CDC 1997a, Shelton et al. 1993).

IV ANALYSIS AND TREATMENT

Analysis of Samples

Collection of Legionella

- The examination of water for the presence of *Legionella* is best done by taking swab samples of the medium over which the water flows (EPA 1985, Ta et al. 1995).

- The specimen should then be concentrated by filtration and treated with an acid buffer to enhance *Legionella* recovery (Bopp et al. 1981, EPA 1985, Nguyen et al. 1991, Ta et al. 1995). Acid wash treatment is used to isolate *Legionella* because unlike most bacteria, *Legionella* strains are acid resistant (Nguyen et al. 1991).

- 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 (EPA 1985). Selective buffered charcoal yeast extract (BCYE) medium is most commonly used to culture *Legionella* (Edelstein 1987).

Detection of Legionella in Environmental and Biological Samples

- The most common and rapid test for *Legionella* is the Direct Immunofluorescence Assay (DFA). Sputum, lung specimens, and bronchial and tracheal secretions are excellent samples to test by the DFA method; however, it may not be useful in the detection of environmental specimens (Grimont 1986). More recently, monoclonal antibody test have been developed and have been found to eliminate false positive results due to cross reactivity with non-*Legionella* organisms (Stout and YU 1997). Monoclonal antibody test are effective due to their high specificity for a single antigenic determinant.

- *Legionella* bacteria, and antibodies in patient sera, can be detected using the Indirect Immunofluorescence Assay (IFA). Because seroconversion only occurs after a rather long time period in humans, the IFA test is often used in conjunction with other tests (Kohler 1986). 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 1986, Ehret et al. 1986, Kashuba and Ballow 1996).

- Enzyme-linked immunosorbent assays (ELISA), radioimmunoassays (RIA), and agglutination assays have also been used to detect *Legionella* antibodies (EPA 1985). 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.

- The Polymerase Chain Reaction (PCR) test uses two disparate primers: one that is specific for *Legionella* species and one for *L. pneumophila* only (Fricker and Fricker 1995). PCR is a relatively new method not yet available commercially designed to rapidly multiply DNA target genes in a laboratory setting to yield detectable quantities for testing.

Disinfection as a Water Treatment Practice

- 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. Because 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.

- 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). In cases of outbreaks, 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).

- Hyperchlorination of water distribution systems requires the installation of a chlorinator. Shock hyperchlorination involves the addition of chlorine to a water system, raising chlorine levels throughout the system for one to two hours (Lin et al. 1998a). Continuous hyperchlorination entails the addition of chlorinated salts to the water (Stout and Yu 1997, Muraca et al. 1990). 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). Hyperchlorination may cause human health problems. Levels of trihalomethanes tend to increase in the hot water system when chlorine levels exceed 4mg/L (Helms et al. 1988, Muraca et al. 1990).

- Copper-silver ionization distorts the permeability of the *Legionella* cell, denatures proteins, and leads to lysis and cell death (Nguyen et al. 1991, Miuetzner et al. 1997, Muraca et al. 1990). A commercial system can be easily installed to perform this ionization. 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. 1998a).

- 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. 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).

- Ozone, which can be created using ozonators, can be used to kill *L. pneumophila.* 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).

- 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). In addition, any *Legionella* that may have colonized the system downstream of the heater will be unaffected.

- 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 and 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 NEEDS

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. In addition, *Legionella* are widely distributed in the environment, including treated water supplies. 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.

- 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 posed by *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*.

- Development or rapid diagnostic test are needed to detect infections caused by *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.

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