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Ecology of *Legionella:* From Data to Knowledge with a Little Wisdom

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Abstract. The respiratory diseases produced by the *Legionella* genus of bacteria are collectively called Legionellosis. Presently more than 34 species of *Legionella* have been identified, 20 of which have been isolated from both environmental and clinical sources. The diseases produced by *Legionella* include the pneumonic form, Legionnaires' disease, and the flu-like form, Pontiac fever. Because the vast majority of Legionellosis is caused by the *L. pneumophila* species, this bacterium is the thrust of the discussion.

Legionella is a global bacterium. The relationship of the bacterium to its environment has told us many things about infectious diseases. Not until Legionellosis and the discovery of its etiologic agent, *Legionella,* has such a successful modern-day marriage been consummated between the agent and its environment. Nearly two decades have passed since the term Legionellosis found its way into the vocabulary of the scientific journals, the popular press, and courtroom proceedings. Too often the scientific development, engineering implementation, and societal acceptance are disconnected. The focus of scientific research sometimes does not reflect engineering or societal needs and thus contributes little to the solution of immediate and important problems. At other times, scientific knowledge that could contribute to solutions is overlooked because of poor communication between the problem holders, the scientific community, regulatory agencies, the problem makers, and the public. The scope of this paper provides insights on the ecological niche of *Legionella,* describes the organism's ecological relationships in the natural world, and provides wisdom for effective control of the bacterium for the industrial and user communities.

Introduction

A large amount of information has been obtained about the bacterium *Legionella pneumophila* since the first recognized outbreak of Legionnaires' disease occurred. In July of 1976 during a bicentennial celebration of the founding of the United States of America, an outbreak of acute respiratory illness occurred at an American Legion convention in Philadelphia, Pennsylvania. Of the 4,400 attendees, along

with other individuals not directly associated with the convention, 221 became ill and 34 died [70]. The cause of the epidemic was unknown until the winter of 1976 when investigators at the Centers for Disease Control (CDC) isolated the responsible bacterium, subsequently named *Legionella pneumophila* (lung-loving) [102, 104]. Although *Legionella pneumophila* was undoubtedly the cause of previous pneumonia outbreaks, the failure to discover this organism prior to the Philadelphia incident, was due in part to the requirement by the bacterium for a specialized culture medium and slow growth on such medium.

The Bacterium

> *Legionellosis is caused by a group of rod-shaped bacteria.* Legionella *are described in Bergey's Manual of Systematic Bacteriology as follows: Rods 0.3–0.9μm in width and 2–20μm or more in length. Do not form endospores or microcysts. Not encapsulated. Not acid-fast. Gram-negative. Motile by one, two or more straight or curved polar or lateral flagella; nonmotile strains are occasionally seen. Aerobic. L-cysteine-HCl and iron salts are required for growth. The oxidase test is negative or weakly positive. Nitrates are not reduced. Urease-negative. Gelatin is liquefied. Branch chain fatty acids predominate in the cell wall. Chemoorganotrophic, using amino acids as carbon source. Carbohydrates are neither fermented nor oxidized. Isolated from surface water, mud, and from thermally polluted lakes and streams. There is no known soil or animal source. Pathogenic for man. The mol% G+C of the DNA is 39–43% [24].*

Legionella require special media supplemented with L-cysteine, soluble iron, and a pH adjusted to 6.9 [51, 52]. When cultured on charcoal yeast-extract agar, the colonies are circular, gray to white, and present a characteristic cut glass appearance. Biochemical characteristics, molecular weight of the genome, guanine-cytosine content, and DNA homology have demonstrated these bacteria are distinct from other known families of bacteria [130].

Selective media have been developed that consist of a buffered charcoal yeast-extract base supplemented with iron, cysteine, and a variety of bacterial inhibitors [20, 49, 155]. Isolation of legionellae has also been improved by the development of pretreatment techniques. Exposure of water samples to acid, pH 2.2, for 5 min [20], heating to 50°C for 30 min [67], or both prior to plating on selective media have further enhanced recovery. A polycarbonate membrane technique has also been used to filter large volumes of water in order to provide a more sensitive method for isolation of low numbers of legionellae [58, Wolford *et al.* (1988) Abstract. Annu. Meet. Am. Soc. Microbiol. Q26]. While all of these methods are somewhat effective, studies on the sensitivity of *Legionella* spp. to selective isolation procedures indicate a need for substantial care when interpreting quantitative culture results from heavily contaminated environmental samples [31, 123]. Even at the present time only a few cultural and biochemical tests are used for the routine identification of *Legionella.* Most bacteriological media and buffered-charcoal-yeast extract (BCYE) (the media of choice with a variety of modifications) do not selectively support the growth of *Legionella.*

All of the carbon and energy needs of *Legionella* spp. can be met with nine amino acids [146]. *L. pneumophila* can apparently synthesize all other necessary constituents *de novo* and has no apparent vitamin requirements [122]. In aquatic environments, *Legionella* must obtain these nutrients either from other living organisms that produce them in excess, or from the decomposition of organic matter, or both. Since legionellae typically exist in association with other microorganisms, this suggests that growth of legionellae may be supported by these microorganisms. Although the importance of associations between *Legionella* and other microorganisms for survival, growth, and pathogenicity is still not completely understood, it has been the subject of a number of studies. Evidence indicates that some serogroups and strains of *Legionella* are more virulent than others. *L. pneumophila* accounts for 90% of all the Legionellosis cases reported to CDC [22]. Serogroup 1 is the most frequently identified form of the bacterium having been isolated from both the environment and Legionellosis patients. Serogroups 3 and 6 are the next most frequently identified forms of *L. pneumophila* linked with disease. Serogroups 1, 3, 4, and 6 make up nearly 90% of all Legionnaires' disease cases.

Symptomology

Legionnaires' disease is pneumonic with an incubation period from 2 to 10 days and an attack rate from 0.1–4.0% [141]. The disease begins with a mild cough and low fever and advances through rapidly progressive pneumonia and coma. Early symptoms of the disease include malaise, muscle aches, and a slight headache, while later symptoms include a high fever (105°F) followed by an unproductive dry cough and shortness of breath. Gastrointestinal symptoms, including vomiting, diarrhea, nausea, and abdominal pain are commonly reported with the disease. The disease is effectively treated with either erythromycin or a combination of erythromycin and rifampin.

Pontiac fever is a nonpneumonic, flu-like disease that is asociated with and may be caused by the *Legionella* bacterium. This disease has a high attack rate (>90%) and a short incubation period of 48–72 h. Complete recovery usually occurs in 2–5 days without medical intervention. The factors that cause the same bacterium to produce two different illnesses with major differences in attack rate and severity are not currently understood. Characteristics of neither the organisms nor the mode of transmission have been identified that account for the difference between Legionnaires' disease and Pontiac fever. Legionellae have never been recovered directly from Pontiac fever patients although diagnosis has been established through seroconversion following characteristic clinical symptoms [110]. Several hypotheses to explain Pontiac fever include a change in virulence factors [29], toxic or hypersensitivity reaction [89], or hypersensitivity of amoebae containing *Legionella* [126, 128]. The first documented outbreak of Pontiac fever occurred in 1968 in Pontiac, Michigan [74]. Ninety-five percent (144/152) of the employees at the Oakland County Health Department developed illness that generally lasted 2–3 days and consisted of fever, headache, and generalized muscle aching. Ultimately *Legionella pneumophila* serogroup 1 bacteria were isolated from a defective air-conditioning system that allowed water from the evaporative condenser to enter into the general air circulation ducts of the building [89]. Subsequently, several

outbreaks of Pontiac fever have occurred where *L. anisa, L. micdadei* and *L. feeleii* [81] have been the suspected agents. *L. pneumophila* has also been implicated in wound infections, pericarditis, and endocarditis without pneumonia being present [16].

It is not fully understood how the virulence of legionellae is expressed in nature and how such strains are transmitted from the natural environment to humans. The major mechanism of infection appears to be direct transmission from the environment by inhalation of the bacterium in aerosolized contaminated water. Person to person spread has not been documented.

According to CDC guidelines [33] a confirmed case of *Legionella* requires a physician's diagnosis of pneumonia which is based on a chest x-ray and a positive clinical laboratory test result. A laboratory test including elevated serology, culture of the organism from the patient, immunofluorescent staining of the bacterium in patient's sample, or a positive radioactive immunoassay test (RIA) for urine antigens is necessary for confirmation because the symptomology and x-ray patterns for Legionnaires' disease are unremarkable.

Epidemiology

Legionella is frequently characterized as an "opportunistic" pathogen, in that it most frequently attacks individuals who have an underlying illness or a weakened immune system. While Legionnaires' disease is treatable with antibiotics, the overall case-fatality ratio remains high. In the United States, Legionnaires' disease is considered to be a fairly common and serious form of pneumonia. The *Legionella* organism is one of the top three bacterial agents in the United States that causes sporadic community-acquired pneumonia. Because of the difficulty in distinguishing this disease from other forms of pneumonia, many cases go unreported.

Retrospective examination of preserved sera and bacterial specimens from earlier explosive outbreaks of pneumonia indicated that Legionellosis is not a new syndrome but has occurred undetected for decades [103]. The earliest documented case occurred in 1947 with a soldier at Fort Bragg, North Carolina who had an unidentified pneumonia. The earliest outbreak of Legionellosis appears to have occurred in a meat packing plant in 1957 [113].

Since the original Philadelphia outbreak, over 50 additional epidemics and numerous sporadic cases of Legionellosis have been reported. Because this disease can be difficult to diagnose, it is probably under reported. It is estimated that Legionellosis affects 25,000–100,000 persons annually in the United States [68]. Serologic surveys indicate that many people in the general population have antibodies to legionellae suggesting previous infection or exposure [17, 43, 44]. Because increasing age of the host can cause the antibody titer to deteriorate or require a longer time to appear, Poszka-Kolva et al. [116] have suggested that a significantly greater proportion of an apparently healthy population harbors antibodies to *Legionella* than was previously suspected. Breiman [22] has indicated that using the most restrictive criteria for Legionnaires' disease, *Legionella* species make up as much as 5% of the community-acquired penumonias in the United States.

A key retrospective study conducted by Foy et al. [68] used stored paired sera from 500 patients treated for pneumonia from 1963–1975 in Seattle, Washington

to determine the community incidence of Legionnaires' disease. Based on 1% of the patients showing a fourfold rise in antibody titer to the Legionnaires' disease antigen, the incidence of Legionnaires' disease was estimated to be 0.4–2.8 cases per 10,000 persons in the population per year. These findings led to the often-used estimate of 25,000 cases of *Legionella* pneumonia occurring annually in the United States. According to a 1991 community-based pneumonia incidence study conducted by the U.S. Public Health Service, the estimated incidence of pneumonia among adults due to *Legionella* is 6.1/100,000 or 11,000 cases a year in the United States. Although only approximately 1,000 cases are reported to CDC annually, Horowitz, et al. [85] has indicated that the estimated cases of Legionnaires' disease ranges from 10,000 to 100,000 cases annually in the United States alone. Thus, many cases go unrecognized and unreported [30] due in part to the lack of specialized techniques necessary for detection in a large number of laboratories.

Among individuals who are exposed and have healthy immune systems 7–9% still die when treated with erythromycin, while 25% die when hospitalized but not treated with proper or effective antibiotics [91, 141]. Among individuals with impaired immune systems, the mortality rate is 24% for the adequately treated and 80% for those incorrectly treated [91, 141]. The most susceptible people include those with chronic obstructive pulmonary disease (COPD), the aged, smokers, immunosuppressed individuals (e.g., organ transplant patients and those on corticosteroid therapy).

Most of the early outbreaks have been traced to aerosols contaminated with these organisms from either cooling towers or evaporative condensers [12, 30, 42–44, 47, 51, 72], while recent outbreaks have been traced to potable water services and components such as water heaters, showers, faucets, decorative fountains [83], grocery spray misters with reservoirs [104], whirlpool baths [19, 51a; Pfiffner (1991) PhD Thesis, Florida State University], and respiratory therapy equipment [92]. Although Legionellosis appears to be seasonal with most of the epidemics occurring in the summer, sporadic cases, especially in hospitals, occur throughout the year.

Legionella Ecology

Microbial ecologists recognize that the vigor of aquatic microorganisms generally follows the thermal cycle of their habitat. This means that a seasonal change is reflected in the activity and subsequent density of the microbial populations in the habitat. *Legionella* follows such a pattern. Because *Legionella* survives and multiplies in aquatic habitats, many of the Legionella studies have been concerned with the location and conditions in which the bacterium flourishes, and the health risks posed by those occurrences.

Bacteria associated with thermally elevated habitats such as occur in Yellowstone National Park have a parameter in common with the clinical isolates of *Legionella,* i.e., they have a large number of branched-chain fatty acids [82]. Initially, this parameter was used as a diagnostic tool for the identification of bacterial isolates that were suspected as *Legionella.* Such information provided an ecological as well as an epidemiological tool for the autecological investigations of *Legionella* that followed. Ecological investigations by Fliermans et al. [57, 58] in the hot

spring environments of Yellowstone National Park indicated that *Legionella pneumophila* had indeed been part of the natural environment which had remained unchanged since the early 1900s [76]. *Legionella* has also been isolated from habitats formed by the eruption of Mt. St. Helens [58] as well as the thermal hot springs of Yellowstone National Park and other locations [58]. These thermal investigations have been followed by work of Bornstein et al. [21] with regard to French resort hot spring spas. *Legionella* is more readily isolated from warm or thermally altered habitats than from ambient ones. Although temperature is an important parameter in the distribution of *Legionella,* it is not an overriding one, since no single environmental parameter has been shown to be an effective predictor of the density of *Legionella* [58].

Surveys of lakes, ponds, streams, and soils have indicated that *Legionella* is a common inhabitant of natural waters [62, 65, 109, 119, 142, 143]. The organism has also been isolated from a variety of man-made aquatic habitats including cooling towers and evaporative condensers [38, 43, 47, 74] as well as the plumbing systems of hospitals, dental offices, hotels, misters, spas, humidifiers, gymnasiums, and homes [8, 21, 23, 43, 144, 149, 157].

Hazen and coworkers [114] demonstrated the prevalence of *Legionella* spp. in both marine and freshwater environments of Puerto Rico. Using both the direct fluorescent antibody test and guinea pig inoculations, the data demonstrated the presence of six *Legionella* species in tropical waters. The densities of *Legionella* in these tropical habitat samples were considered significantly higher than those reported in non-tropical natural habitats. *Legionellaceae* are relatively common indigenous bacteria and the elevated Legionellosis cases (15% of 88 fatal atypical pneumonia cases [107]) may have their origin in naturally high densities in Puerto Rican waters.

Work reported by Fliermans and Tyndall [61] has expanded the reported habitats to include terrestrial subsurface environments to depths of 1170 m. The data are important since subsurface environments that harbor *Legionella* may be brought to the surface through water-well drilling activities and have the potential to contact susceptible hosts through spray field irrigation. The data suggest that it may be important to ensure irrigation wells are not located in geological formations that harbor high concentrations of *Legionella.*

Interactions of Legionella

Defining the ecological niche of *Legionella* provides information for the first line of control against the dissemination of the organism and possible infection. There are basically seven events that are necessary in the contraction of Legionellosis (adapted from other investigators [14, 69]). These are described in Fig. 1 and represent a flow of the organism from its natural habitat to infection and a diagnosis of Legionnaires' disease. *Legionella* ubiquity in nature asseverates its ability to survive in nature. Its survival is enhanced by a variety of parameters including but not limited to warm temperatures, particular algal and protozoa associations, and symbiotic relationships with certain aquatic plants [58].

Several studies have shown that *Legionella* exists in habitats having a wide range of temperatures, and are particularly adapted to warmer conditions. Fliermans

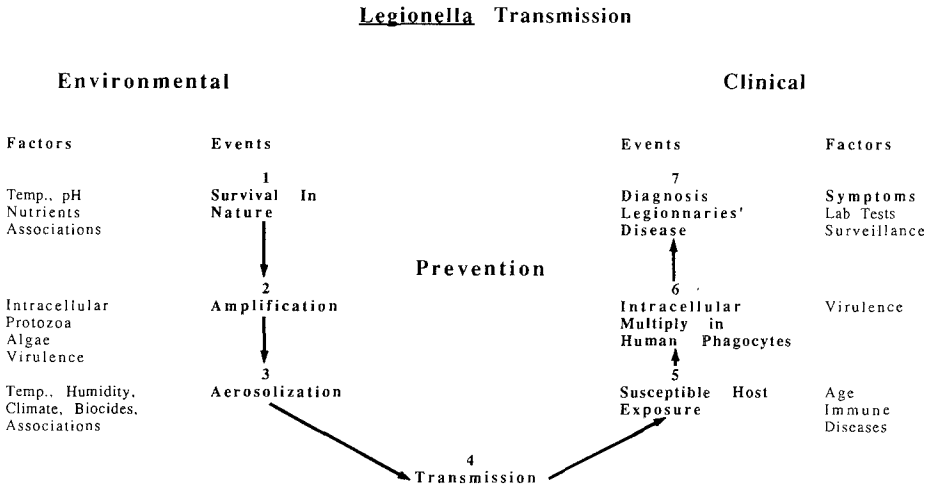

Fig. 1. Parameters in the Transmission of *Legionella.*

et al. [65] sampled 67 rivers and lakes in the United States and, using guinea pig inoculation, recovered *Legionella* from waters with temperatures ranging from 5.7 to 63°C. While these data did not reveal whether *Legionella* multiplies throughout this range, they demonstrated that the bacterium can survive and remain viable at such temperatures. The authors proposed that the relationship between *L. pneumophila* and thermal environments is also indicated by its cellular fatty acid composition which is similar to that of known thermophilic bacteria [82, 111]. Dennis et al. [46, 67] compared the effects of high temperature on several types of bacteria and found that *L. pneumophila* exhibited little loss in viability at 50°C relative to a *Pseudomonas* spp., a *Micrococcus* spp., and coliforms. Wadowksy et al. [157] investigated the effects of temperature on multiplication of naturally occurring legionellae seeded into membrane-filtered tap water. They observed that *L. pneumophila* multiplied between 25 and 37°C, with maximum increases occurring at 32 and 35°C.

Survival and growth of *Legionella* has also been shown to be substantially affected by pH. Feeley et al. [52] reported a narrow pH range of 6.5 to 6.9 for growth of *L. pneumophila* cultures maintained on artificial agar media. However, laboratory and field studies indicated that naturally occurring *L. pneumophila* multiplies at pH values ranging from 5.5 to 9.2 [65, 158]. The growth of *L. pneumophila* over such a wide pH range is believed to be a reflection of its natural habitat being the outdoor aquatic environment [65]. Work with cooling tower water suggested that elevated pH values are inhibitory to *Legionella* growth [139]. Additionally, samples being cultured for *Legionella* spp. are often pretreated with a hydrochloric acid–potassium chloride solution, pH 2.2, to eliminate contaminating organisms [20]. Such treatment has had variable results and stringent quality control measures are required for effective and reliable data.

Algae. Algae were initially considered for their influence on legionellae. Tison et al. [148] isolated *L. pneumophila* from an algal-bacterial mat community growing in man-made thermal effluents. Bacteria morphologically similar to *L. pneumophila*

that reacted with specific serogroup conjugates were observed in the naturally occurring algal-bacterial mat communities in thermal effluent ranging in temperature from ambient to 55°C. This algal mat community was composed of cyanobacteria of the genera *Fisherella, Phormidium,* and *Oscillatoria.* Cyanobacteria were isolated in unialgal culture and bacteria associated with the algae were cultured and identified. Initially, only *Fisherella* cultures contained a bacterium morphologically, physiologically, and antigenically similar to *L. pneumophila* serogroup 1. Subsequently, other cyanobacteria were shown to associate with *L. pneumophila* serogroup 1. These bacteria were isolated and confirmed as *Legionella pneumophila* serogroup 1 as determined by fatty acid composition, antigenicity homology, and DNA homology as described by Cherry et al. [37], Fliermans et al. [58, 62], and Tyndall et al. [151].

Experiments initiated with *Legionella* growing in the presence of the cyanobacterium *Fisherella* indicated that at temperatures of 45°C, *Legionella pneumophila* serogroup 1 had a doubling time of 2.7 h [58, 120] which is twice as fast as that reported for the growth of *Legionella* in complex or defined media. The data further indicated that *Legionella* uses the complex organic material produced by the *Fisherella* culture as a sole carbon and energy source. Thus, it was apparent that the relationship between certain photosynthetic organisms and *Legionella* were critical in fostering and maintaining the viability of *Legionella.* The relatively rapid growth rate of *L. pneumophila* when associated with cyanobacteria is an important aspect of it's ecology, since it demonstrates the ability of *L. pneumophila* to survive and grow under natural environmental conditions. Fliermans [58] has observed the enhanced virulence for guinea pigs when *Legionella* were grown in the presence of *Fisherella* as compared to agar-cultured *Legionella.* This same virulence trigger may play a role in nature and provide conditions that enhance the virulence of the organism as well as its viability and population density. Furthermore, it may help to explain the widespread distribution of the organism in both man-made and natural habitats.

In addition to supporting growth, algae may promote the aerosol transmission of legionellae. Berendt [18] demonstrated that survival of *L. pneumophila* in aerosols was improved when the bacterium was associated with *Fisherella* spp. The enhancement may result from physical protection from desiccation provided by the mucilaginous matrix of the alga.

Protozoan. Legionella-amoebae relationships may be a cardinal factor in the ecology of *Legionella* and the epidemiology of Legionellosis. The interaction of *Legionella* and free-living amoebae was first reported by Rowbotham [125, 127] and described the deleterious effect of *Legionella* on the amoebae. The interaction of protozoa with bacteria in general is well documented [28, 53, 88, 96, 111, 121]. Sequestering of *Legionella* within the amoebae suggested growth of *Legionella* within the amoebae. Tyndall and Dominque [150] subsequently demonstrated that the interaction of *Legionella* with *Naegleria* and *Acanthamoebae* resulted in a relationship in which the amoebae did indeed support the growth of *Legionella* at concentrations as high as 10^{10} cells ml^{-1} and that *Legionella* did not replicate in the absence of the amoebae. The fact that amoebae and other protozoa act as natural hosts and amplifiers for *Legionella* in the environment has been confirmed

and expanded by a variety of investigations [3, 14, 15, 50, 54, 56, 77, 84, 117, 124, 128, 134; (Pfiffner (1991) PhD Thesis, Florida State University)].

It has been suggested that the host relationship affects the virulence of *Legionella* spp. [50, 51; Pfiffner (1991) PhD Thesis, Florida State University]. The interaction in the environment parallels the infection in man since *Legionella* multiplies within host macrophages. This ability may have importance in the pathogenic mechanism of *Legionella* [46]. It appears that *Legionella* are phagocytized by trophozoites, multiply within vesicles, and are either released when the vesicles and amoebae rupture or, under certain conditions, remain encapsulated when the amoebae encyst [119]. Interaction with amoebae could explain some of the previous observations made on the behavior of *Legionella* in the laboratory. Of particular importance is the potential protection, granted not only by algae but by amoebic hosts to *Legionella,* from the effects of disinfectants, low pH, and heat [17, 18, 45].

Protozoa of particular interest relative to the amplification of *Legionella* are the free-living amoebae *Naegleria, Acanthamoebae, Tetrahymena,* and *Hartmannella* [78, 150]. While free-living amoebae are abundant in soil and water, thermal conditions are the best-documented environmental factor enhancing the emergence of pathogenic amoebae. The ability of pathogenic *Acanthamoebae* and *Naegleria* to grow at temperatures higher than many nonpathogenic species has been demonstrated in laboratory and field studies [63, 75]. This key parameter has been useful in isolating pathogenic free-living amoebae from environmental samples.

Laboratory studies on the interactions of *Legionella* and amoebae are germane to the ecology of the bacterium. An analysis of a municipal water supply showed that areas of the reservoir and treatment plant filters supportive of *Legionella* production were also rich in amoebae populations [138, 140]. Similarly, Barbaree et al. [14, 15] demonstrated that the presence of protozoa were involved in the amplification of *Legionella* in water samples associated with a Legionellosis outbreak. During hospital-acquired Legionnaires' disease, a significant correlation was observed between *Legionella* amplification and the presence of protozoa [40].

Since free-living amoebae can be isolated from potable water, and amoebae containing *Legionella* can be found in environmental waters, CDC investigators have attempted to correlate the importance of amoebae in maintaining *Legionella* populations in hot water heaters. Fields et al. [56] studied the ability of *Legionella* to multiply in potable water samples obtained from hospitals having a history of hospital-acquired Legionellosis. These studies implicated free-living amoebae and in particular *Hartmannella* with the growth and continuing presence of *Legionella* in hot water tanks from which it could be aerosolized through faucets or shower heads.

In related studies, Breiman et al. [23] investigated the associations of protozoa and *Legionella* in hot water systems of hospitals with a history of nosocomial Legionellosis compared to hospitals without ongoing hospital-acquired Legionellosis. While *Hartmannella* was detected in 71% of water samples from hospitals with ongoing Legionellosis, amoebae were detected in only 15% of water samples from hospitals without ongoing Legionellosis. Amoebae were isolated from 80% of the samples that also yielded *L. pneumophila* serogroup 1, and the correlation between the presence of both *L. pneumophila* and *Hartmannella* was highly significant ($P < 0.001$). They concluded that the control of *Legionella* populations and their aerosolization may depend on control of the amoebae population in water systems.

Several in vitro amoebae studies have shown that *Naegleria* spp. tolerate a relatively wide pH range of 4.6 to 9.5 [32, 95, 124]. Sykora et al. [145] observed 100% survival of *Naegleria fowleri* cysts in vitro at pH levels as low as 2.1, but reduced survival at pH 8.7, and no survival at pH 10.0. These findings indicate that the susceptibility of *L. pneumophila* to high pH values in laboratory cultures, natural waters, and cooling tower waters may be related to the susceptibility of amoebae to elevated pH levels. The data also suggest that the resistance of amoebae to low pH and higher temperatures may be factors in the effectiveness of such sample preparations in isolating environmental strains of *Legionella.*

Since *Legionella* and other bacteria can be incorporated into amoebic cysts, the eradication of such bacteria becomes more difficult. King et al. [90] have shown that bacteria sequestered in amoebic trophozoites and cysts are resistant to chlorine concentrations that are bactericidal for free-living microorganisms. Since amoebae can be pathogens per se as well as hosts for bacterial pathogens, control of protozoa is desirable. However, the concentration of free chlorine needed to kill amoebic cysts ranges from 1.5 to 4 ppm with exposure times of 0.5 to >3 h [34, 44]. In some situations these concentrations may negate the use of chlorine.

While there are still many questions concerning the extent of the association between algae, amoebae, and *Legionella,* the *Legionella*-amoeba model is consistent with many of the earlier findings. This suggests that the best way to understand the ecology of environmental *Legionella* spp. may be through an improved understanding of the ecology of amoebae. Even with these advances, complete recovery of *Legionella* from environmental samples remains difficult. Legionellae often comprise only a few percent of the total bacterial population in environmental specimens, and exhibit a lag period of several days for growth. As a result, *Legionellaceae* may be inhibited or masked by other bacteria during culturing.

From Data to Information

Amplifiers and Disseminators

Any natural or man-made system that provides suitable conditions for the growth of *Legionella* is considered an amplifier. Examples of man-made amplifiers are the following:

- Cooling towers, swamp coolers, direct evaporative coolers, evaporative condensers, and fluid coolers in which the evaporative process is used to reject heat
- Domestic hot water systems that have water heaters operating below 55–60°C and deliver water below 50°C
- Spas and whirlpools
- Humidifiers and decorative fountains that create a water spray with water maintained at temperatures that promote *Legionella* growth
- Respiratory therapy equipment
- Reservior misters used for vegetables in grocery stores
- Metal-working fluid aerosols
- Other water sources may include places where stagnated water is present, i.e., fire sprinkler systems, water in recreational vehicles, water for eye-wash and safety showers

Conditions that promote the collection of warm, stagnant water and growth of microorganisms such as algae and/or flavobacteria and/or protozoa have been documented to be excellent amplifiers of *Legionella* [54, 148, 156, 162]. Since *Legionella* can multiply in both protozoa and alveolar macrophages, some investigators now believe that protozoa are the natural host of *Legionella* in the environment and that humans are accidental secondary hosts. It is proposed that the association of *Legionella* with protozoa may enable them to overwhelm alveolar macrophages in the human lung [15, 54, 55].

Airborne dissemination is generally accepted as the primary means by which legionellae are transmitted to humans. In order for this to occur, aerosols of legionellae must be generated, and the ambient air conditions, i.e., temperature, moisture, and solar radiation, must not be too extreme. Survival of legionellae appears best under humid conditions ($\geq$65% relative humidity) [5]. Sources of legionellae have usually been found to be less than 300 m from the site of the outbreak. However, in an outbreak in Wisconsin, cases occurred up to 2 miles away from the suspected source [2]. Other data indicate *Legionella* survival is enhanced by associations with algae [18], and that travel can extend great distances based on the meteorological conditions and the settling velocity of the bacterium [60, 152]. One report indicated that Legionellosis may have been contracted after the bacterium had been transported several kilometers [1].

Reservoirs

Water is the reservoir for *Legionella* in the natural environment. Many lakes and streams, especially those that are thermally enhanced, have been found to harbor legionellae [48, 62, 64, 148]. *Legionella pneumophila* tends to grow as part of the biofilms or "slimes" in such environments. It is hypothesized that *Legionella* survive the routine water treatment used to produce potable water, and are carried in the treated drinking water to buildings where they enter and colonize the plumbing fixtures, especially of hot water service systems. Low levels of the organism can colonize a system and grow to high concentrations under the right environmental conditions. Environmental conditions that promote the growth of the organism include:

- Water temperatures between 20 and 50°C. The optimal growth range is 35–40°C. Cold water systems stored below 20°C are generally not a source for amplified *Legionella* levels.
- Stagnant water
- pH range of 2.0 to 8.5
- Sediments that tend to promote growth of supporting microbiota
- Microorganisms including algae, protozoa, flavobacteria, *Pseudomonas,* and select aquatic plants that supply essential nutrients for growth of *Legionella* as well as harboring the bacterium

When *Legionella* maintains its viability in domestic water systems, it is capable of rapid multiplication under proper growth conditions. Cooling towers and other wet type heat rejection (WTHR) systems may become colonized if contaminated water is used as the source of make-up water. This is probably the most frequent

way WTHR equipment becomes contaminated, even though such systems are excellent air scrubbers.

Transmission and Virulence Factors

Although the mechanism of Legionellosis transmission is through direct inhalation of aerosols, other routes of transmission may exist, but are as yet undefined. Legionellosis has been associated with domestic hot water systems primarily in hospitals, yet in many instances it has been difficult to identify a consistent aerosolized source.

It is stressed by public health officials that the mere presence of legionellae, either in waer or in an amplifier will not in itself cause people present in the area to develop the disease. In exposed populations most healthy individuals generally do not become ill with Legionellosis. For the disease to occur, the following conditions must exist simultaneously (Figure 1;[12]):

- Legionellae must have sufficient virulence factors to cause disease. Currently, these factors are not completely delineated or understood.
- Virulent legionellae must be present in sufficient densities to cause an infection.
- Legionellae must be transported to the host without encountering much injury or loss of virulence.
- The potential host must inhale air contaminated with legionellae containing particles that are less than 5 μm in size so that the legionellae reach the deepest parts of the lungs.
- The host's defense system must be unable to stop the infection.

Although clinical investigations have not been conducted to determine the dose response for humans, guinea pig studies demonstrated that highly virulent organisms required lower densities for infection than less virulent organisms [11, 58, 118]. Nevertheless, the greater the density of *Legionella* to which a person is exposed, the more likely it is that the disease will occur. Although, the infectious dose for humans has not been clearly defined, several authors have suggested "trigger" levels [60, 131] for control of *Legionella.* These levels may vary according to the susceptibility of the potential host and the virulence of the bacterium.

Outbreak investigations frequently involve establishing a dose-response relationship between exposure and disease outcome. There are three indirect measures for determining dose exposure: distance of cases from the source, length of time of exposure to the contaminated source, and concentration and virulence of *Legionella* in the suspected source. Numerous investigations have focused on establishing a dose-response relationship using either time of exposure [60, 66, 160] or distance from the contaminated sources [2, 108; Miller and Kenepp (1991) ASHRAE Annu. Meet., p 76.] Although little information exists on the densities of *Legionella* in sources associated with outbreaks, one study showed that cooling towers associated with outbreaks were more likely to have high counts of *Legionella* than cooling towers that were not known to be associated with outbreaks [108, 131, 132; Morris and Feeley (1990) Abstr. ASHRAE Annual Met, p 76].

Miller and Kenepp [108] showed that of the 342 cooling towers sampled, 20% of the towers had population densities considered in a high risk category. These

investigations further demonstrated that of the 1,100 cooling towers sampled, 70% of the towers were amplifiers of *Legionella.* Fliermans and coworkers [61] have demonstrated from field investigations and a decade of monitoring cooling towers in the southeastern United States results similar to those of Miller and Kenepp [108]. Such data were based on a modified direct fluorescent antibody technique, which accounts for viable nonculturable *Legionella* [60, 66, 86, 129]. These data provided further evidence that the majority of the towers in the high-risk category actually demonstrated a loss of a large segment of the competing microbiota. Thus, the use of total bacterial count data has no basis as a predictor of *Legionella* densities and should be discarded as an indicator of how clean the sysem is with regard to *Legionella.*

Control and Safety

Although sparsely documented in the literature, several workers who have either serviced or cleaned WTHR equipment and other aerosol-producing devices have been infected with either Legionnaires' Disease or Pontiac fever [71; personal communication]. Thus, commissioning, maintaining, cleaning, disinfecting, dismantling, and other procedures associated with WTHR systems should be designed to minimize risk to personnel. Procedures that create substantial aerosol sprays should be avoided whenever possible. In cases where this is unavoidable, suitable respiratory protection should be worn to minimize the risk of inhaling water mist containing *Legionella.* Consequently, the wearing of a half-face respirator mask equipped with a cartridge filter that has a HEPA-filter or "Type H" high efficiency rating is recommended [153]. Filters capable of removing aerosols, mists, particulates, radionuclides, and asbestos should provide adequate protection against respirable *Legionella.*

Maintaining a clean system is of critical importance in reducing the risk of Legionellosis [4, 5, 7, 26, 35, 93, 105, 106, 133, 136]. It is the goal of a maintenance program to provide efficient operation of the system while minimizing the risk of Legionellosis through preventing conditions that allow the amplification of *Legionella.* Well-maintained towers with proper water treatment have generally not been associated with outbreaks of Legionellosis [97, 105]. Most water treatment programs are designed to prevent corrosion, scale, and biofouling. Many water treatment companies use algacides and selected biocides with the incorrect belief that all bacteria are being controlled and, in particular, legionellae. In some areas, tower maintenance procedures are mandated by local laws, health codes [161], or as required by EPA. Any control measures must include microbiological monitoring for *Legionella* as part of the quality assurance/quality control program to insure effectiveness.

The most effective control for most diseases, including Legionellosis, is prevention of transmission at as many points as possible in the disease's chain of transmission (Fig. 1). Such rationale is from the vantage point that if one treatment fails, other treatments will be present and act as backup mechanisms. No attempt is made to compile a complete list of procedures that have been suggested to control Legionellosis [4, 9, 26, 28, 31, 154]. Concepts are presented so that readers may

develop an understanding of the types of conditions that enable the amplification of *Legionella.* This should provide recognition and correction of such conditions.

Theoretically, the ultimate method for preventing human infections of *Legionella* would be to completely eliminate the bacterium from the environment. However, this is an impossible task because of the ubiquity of legionellae. Consequently, other approaches are required. One way is point source control whereby water systems are treated as the water enters a building or potential amplifiers. The method practiced most frequently in preventing the transmission of Legionellosis is at the man-made amplifiers. If legionellae are prevented from growing and increasing in or on a device, the probability of having an infective dose of legionellae is greatly reduced. Consequently, the risk of infection should be substantially reduced if conditions are eliminated that promote the collection of warm, stagnant water.

Since cooling towers and evaporative condensers are a group of systems that have been widely implicated in the amplification of *Legionella,* routine maintenance service, including visual inspections, and mechanical and physical cleaning programs designed to maintain year-round system cleanliness, are an important part of an effective water treatment program. Clean systems generally respond to water treatment more effectively than fouled ones, thus reducing chemical requirements. *Legionella* outbreaks, unexpected shutdowns, and equipment damage are generally avoided with clean systems as well as reducing associated costs which may include litigation.

General Inspection and Routine Maintenance Work. The efficient operation and thermal performance of a cooling tower depend on its cleanliness as well as mechanical maintanence [98, 105]. Proper cleaning procedures should address the entire tower system including the distribution assemblage, strainers, drift eliminators, casing, fan and fan cylinders, louvers, and the cold water basin. Initial field research has shown that fill packs provide a poor environmental niche for the amplification of *Legionella* [28]. Thus, it is suggested that removal of the fill for routine cleanings should not be conducted or required. Towers should not be allowed to become obviously fouled, but cleaned often enough so that sedimentation and visible slime are easily controlled by water treatment protocol. The cooling tower is the only component in the condenser loop that can be viewed easily without system shutdown, and thus should be considered as an indicator of total system cleanliness.

Towers are excellent air washers and the water quality in a given location quickly reflects that of the ambient air [23, 81, 87]. Proximity to highways, construction sites, level of air pollution, operating hours, and cooling tower designs that increase thermal performance are all factors in tower soil loading [98]. Thus, recommendations by manufacturers regarding cleaning schedules should be viewed as guidelines. Such treatments are designed to enhance water quality and increase the time interval between cleanings. Conversely, one should also not expect regular cleanings to replace water treatment [98].

The commissioning and startup stages of a cooling tower are vitally important. There is often pressure to achieve early completion and hand-over of facilities. Several Legionellosis outbreaks have been associated with the start-up and restart of cooling tower systems [2, 48]. Proper commissioning includes taking precautions

to control risk at start-up as well as ensuring the system operates correctly and within design parameters. Precautions necessary to control risk involve inspection, cleaning, and disinfection procedures at start-up similar to those used for ongoing cooling tower maintenance.

Water Treatment. Although good maintenance may reduce the likelihood of *Legionella* amplification, it will not prevent colonization. Limited information exists regarding effectiveness of many commercial biocides in preventing *Legionella* growth under field conditions. Although cleaning of a cooling tower amy be required to enhance heat transfer efficiency, there is very little data to indicate that cleaning alone is effective in controlling *Legionella* [25]. Therefore, chemical biocidal treatment is required. Conventional water treatment should not be expected to reach inaccessible surfaces or organisms in thick biofilms. Biocides are not likely to be effective unless administered in conjunction with a clean tower, since organic sediments require a greater biocide demand to keep microbial populations under control [28, 99]. Clean towers also decrease the niches and nutrients available to the microbial consortia [6, 26, 104, 106].

Several studies have been conducted on the effectiveness of various biocides under field conditions [10, 27, 28, 59, 94, 100, 101, 103, 162, 163]. Traditional oxidizing agents such as chlorine and bromine, provided at appropriate levels, have proven effective in controlling *Legionella* levels in cooling towers. Continuous chlorinating at low free residual levels can be effective in controlling *Legionella* growth [60, 61; Fliermans and Tyndall (1992) Am. Soc. Microbiol., New Orleans, LA, N-17]. While continuous chlorination at 0.2–0.3 ppm is effective against a wide range of bacteria [92], such levels are generally not effective in removing *Legionella* from a highly contaminated cooling tower system [60, 66, 108]. Early field investigations [62, 66, 67; Fliermans and Tyndall (1992) Am. Soc. Microbiol., New Orleans, LA, N-17] demonstrated the effectiveness of 1.5 ppm free residual chlorine for a short duration in reducing the levels of *Legionella* in large industrial cooling towers.

Levels of free residual chlorine above 1 ppm may be corrosive to the metallurgy of a system and cause delignification of wood surfaces if these levels of chlorine are maintained for an extensive period of time [159]. Additionally, high levels of chlorine may also form toxic by-products with organic substances present in water and may be of environmental and public health concern. Frequent monitoring and control of pH is essential for maintaining adequate levels of free residual chlorine, since pH values above neutral reduce the chlorine effectiveness. With proper control of pH, chlorine concentrations, and contact time, the effectiveness of chlorination against *Legionella* can be maintained.

Australian studies [27, 28] indicated that slug doses of fentichlor (not registered in USA) used weekly for 4 h at 200 ppm, or a slow-release bromo-chloro-dimethyl-hydantoin (BCD) at 300 ppm were effective in controlling the growth of *Legionella.* This study also indicated that quaternary ammonium compounds which were frequently used for biofouling control in cooling towers were not effective in controlling *Legionella.* McCoy [100] found BCD to be an effective biocide at the high concentrations reported. Investigations on BCD are equivocal since Fliermans and Harvey [59] reported the lack of effectiveness of continuous bromocide treatment at 2.0 ppm free residual levels against *Legionella.* These data were derived from

sophisticated techniques combining electron transport and the modified direct fluorescent antibody (DFA) assays.

Monitoring. The analysis of water samples collected from a source suspected of amplifying *Legionella* is a valuable means of identifying potential sources of the disease. A qualified microbiological laboratory experienced in *Legionella* detection can determine the number of organisms present by the modified DFA technique, polymerase chain reaction (PCR) assay, or colony forming units per volume of water [22, 115; Bowman and Tyndall [1993] Am. Soc. Microbiol., Atlanta, GA, Q9]. Each technique has its own advantages and disadvantages. While plate counts are widely used, great caution is required in the interpretation of the data. Although each technique varies, it must be stressed that appropriate and periodic microbiological monitoring for *Legionella* be conducted to insure the proper quality control program for the selected maintenance practice.

Impaction of an air sample onto specialized culture plates using an Andersen-type sampler, or impinging of air samples into a liquid media with high volume (1,000liters/min) Litton samplers are often used tto demonstrate the presence of the organism in the air [38, 39]. Air sampling for *Legionella* is neither an efficient nor an effective means of defining the presence of the bacterium and is generally not recommended as a means of measuring potential exposure because of the high likelihood of obtaining false negatives.

The routine monitoring of cooling towers for Legionella is, at times, a hotly debated issue. On one hand it is viewed as a logical conclusion and validation of quality assurance and quality control procedures that are in place for the water treatment at the wet heat rejection facilities. Monitoring is viewed as a good business practice in order to prevent litigation. The prevention of litigation is viewed by industry as a cost-effective endeavor. In contrast, routine monitoring is not recommended by the Centers for Disease Control. CDC does recommend monitoring after an outbreak has occurred.

Emergency Treatment of Cooling Towers

Recommendations are available for emergency treatment of cooling towers, evaporative condensers, and swamp coolers, together with the associated open circulating water systems including circulation pumps, refrigerant condensers, and interconnecting piping where rapid reduction of the levels of *Legionella* are necessary [41]. Although the procedures are based on the collective experience and wisdom of many water treatment specialists, the ubiquity of *Legionella* prevents its complete elimination from cooling systems. Yet the population densities of *Legionella* can be monitored and managed in cooling systems in a reliable manner. These recommendations should leave a system with populations of *Legionella* at or below the level of *Legionella* in the make-up water so that regular chemical treatment of the circulating water can be resumed.

Procedures for Cleaning. Close off the blowdown of the system. If the system has a conductivity controller, it should be electrically removed from the system. Immediately shut off the heat source (refrigeration machines) and the fans on the

cooling tower, evaporative condenser, or evaporative cooler. The electrical supply to the fans should be locked out so that they cannot be activated until work has been completed. Continue to operate the recirculating water pumps for the condenser water system so that water is kept circulating through the cooling tower or evaporative condenser, maintaining only sufficient make-up water to compensate for evaporation needs.

Discontinue the regular chemical treatment program (corrosion inhibitor, scale inhibitor, etc.). Add a dispersant such as Cascade, Calgonite, or equivalent dishwasher compound (silicate-based nonfoaming detergent) at a dosage of 10–25 pounds per thousand gallons of water in the system. The dispersant is best added in a turbulent zone of the water system, such as the cooling tower basin near the pump suction.

The system should be given an initial slug dose corresponding to 10–20 ppm of free chlorine. Readily available sources of chlorine-yielding disinfectants include calcium hypochlorite (HTH) and sodium hypochlorite solution (in the form of Clorox, other household bleach, liquid swimming pool chlorine, or laundry bleach). A 10 ppm dosage of chlorine per 1,000 gallons of water in the system will require about 1/8 pound HTH, 1.5 pints of 5% sodium hypochlorite, or 3/4 pint of 10% sodium hypochlorite. The actual chlorine concentration in the water will likely be less than that calculated because some of the chlorine will be removed by reaction with organic matter in the water or lost to the air. The amount of free chlorine in the water may be monitored by use of a swimming pool test kit, such as those commonly available in hardware stores and similar sources of swimming pool supplies. Add chlorine as required to maintain 1.5–2 ppm free residual of chlorine in the system for 24–72 h following the initial slug dose.

During this operation the dispersant and disinfectant combination may dislodge sufficient solids to clog screens and filters. These should be checked at intervals and cleaned as needed. Additionally, if a system is badly fouled, the populations of *Legionella* may actually increase in the basin water during treatment because of release of biofilm from the internal plumbing. After circulating the chlorine-containing water, the blowdown valve(s) are opened and the entire system flushed and drained, until the discharge is free of turbidity. Once the system is brought back on line, it is strongly recommended that the effectiveness of the treatment be measured through *Legionella* monitoring as part of a quality control and quality assurance program.

Interpretation of Water Sample Results

The probability of infection with *L. pneumophila* is a function of the total dose obtained, the virulence of the bacterium, and the susceptibility of the host. Because total eradication of the *Legionella* organism may not be possible, an acceptable control strategy is to minimize the levels of the organism present in a water source. Ample evidence is available to indicate that *Legionella* levels are readily controllable. Surveys [60, 66, 73, 80; Fliermans and Tyndall (1992) Am. Soc. Microbiol., New Orleans, La, N-17] of *Legionella pneumophila* levels from over 1,000 cooling towers indicates approximately 60% of these systems contained levels of *Legionella pneumophila* that were below "trigger limits" when measured

by DFA analysis. In another survey of 663 cooling towers, 57% of the water sources contained nondetectable levels of *Legionella* when measured by culture [108]. Data from environmental sampling requires interpretation to determine whether remedial actions are required.

Several investigators, institutions, and agencies have provided interpretations for actions associated with the results of environmental sampling [36, 78, 79, 112, 131, 137]. There are two primary criteria to assess the risk of cooling towers for *Legionella.* The first is a function of cooling tower location with regard to its proximity to a susceptible population. Persons with compromised immune systems are most susceptible to infection from exposure to *Legionella.* the second criterion is based on the density of *Legionella* present in the cooling system. Such population densities are a function of the make-up water and the design and operation characterisitics of the cooling tower. The risk of transmission of *Legionella* to susceptible individuals is likely to depend on both these criteria.

Risk factors for determining the likelihood that a cooling tower may be associated with human illness are not well defined. However, some towers appear to be more likely to be associated with an outbreak of Legionnaires' Disease than other towers. Although currently available data are limited the following rating may be used to help assess risk [131].

Cooling Tower Rating

Rating the microbiological risk associated with a cooling tower is based on the location of the host population and the potential susceptibility of the host. The following categories reflect such a rating:

- **Category 1:** Highest risk. Cooling tower serving or in the vicinity (<200 m) of a hospital, nursing home, or other health care facility caring for persons who may be immunologically compromised.
- **Category 2:** Cooling tower serving or in the vicinity (>200 m) of a retirement community, hotel, or other buildings where a large number of people are localized.
- **Category 3:** Cooling tower in a residential or industrial neighborhood.
- **Category 4:** Lowest risk. Cooling tower isolated from residential neighborhood (>600 m from residential area).

Based on the above categories it is recommended that category 1 towers should be monitored on a monthly basis for the presence of *Legionella.* Category 2 towers should be monitored on a monthly to quarterly basis for the presence of *Legionella.* Category 3 towers should be monitored on a quarterly to yearly basis for the presence of *Legionella,* while category 4 towers should be monitored on a yearly basis during the late summer or early fall for the presence of *Legionella.* These guidelines represent the best available knowledge on risk and are to be used until better knowledge of the dose effect of *Legionella pneumophila* can be obtained.

Circumstances do exist where *Legionella* is amplified to concentrations great enough to produce infection in susceptible individuals. Such circumstances are derived either from a lack of knowledge that *Legionella* is present in such amplifiers or the lack of wisdom in treating such amplifiers adequately to control the ubiquitous organism. Both arguments suggest that closer attention to the particular habitats

are important and that effective measures of Quality Assurance and Quality Control must be implemented. These measures would inlcude selective monitoring to assure and insure that systems are not amplifying *Legionella* and that control measures are effective. The wise use of ecological information and judicial use of resources by numerous industrial companies has provided them with a level of efficacy whereby issues of *Legionella* litigation have not heretofore been a concern.

Conclusions

Based upon the scientific, medical, and engineering literature available to date the following is concluded:

1. Heating, ventilating, air-conditioning, and refrigerating (HVAC&R) systems and their components as well as potable hot water services and bathing equipment can amplify and disseminate aerosols of a wide variety of airborne contaminants including *Legionella* bacteria, the agents causing Legionellosis (Legionnaires' disease and Pontiac fever).
2. Design and good housekeeping procedures that prevent amplification and dissemination of *Legionella* should be formulated and implemented before systems are operated, and continued rigidly thereafter. Although this practice will not guarantee that a system or individual component will be free of legionellae, it should reduce the chance of it becoming heavily infected with these bacteria because of their need to receive nutrients from other organisms such as algae and protozoa.
3. Currently, the only reliable way of testing for the presence of legionellae in a system is by analyzing specifically for these organisms. No surrogate tests are available, and there is currrently no correlation between total bacterial counts and legionellae concentrations.
4. The efficacy of a specific biocide treatment in controlling legionellae can only be determined by testing specifically for the presence of legionellae in the field under actual working conditions. Laboratory trials must not be relied upon exclusively as the sole proof of the efficacy of a biocide.
5. This information is provided so that an understanding of the data generated with regard to the ecology of *Legionella* is translated into the wise use of man-made amplifiers and the control of *Legionella* in such systems.

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