Legionella[1]

Description

Species of the genus *Legionella* are Gram-negative, non-spore-forming, rod-shaped, aerobic bacteria. They contain branched-chain fatty acids, have a non-fermentative metabolism, and require L-cysteine and iron salts for growth. They have been placed in the family Legionellaceae, which contains the single genus *Legionella*; there are at least 42 species, which are listed in Table 5 (Drozanski, 1991; Adeleke et al., 1996; Hookey et al., 1996; Fry & Harrison, 1998; Riffard et al., 1998). The type species is *L. pneumophila*. Two other genera have been proposed but have not received general recognition (Garrity, Brown & Vickers, 1980): *Fluoribacter* for the blue-white fluorescing species such as *L. bozemanii* and *L. dumoffii*, and *Tatlockia* for the species *L. micdadei*. Some species of *Legionella* can be further differentiated into serotypes, of which there are at least 15 for *L. pneumophila* but so far no more than two for any other species.

Free-living legionellae are rod-shaped, 0.3–0.9μm wide and approximately 1.3μm long. They will grow to 2–6μm in vitro, but can form filaments 20μm or more in length. Although they are Gram-negative, legionellae actually stain poorly in the Gram procedure and by other similar staining methods, particularly in infected tissues. This has been attributed to the presence of the branched-chain fatty acids that are a major component of the cell walls. Other staining methods have been described, such as the silver impregnation method of Dieterle (Dieterle, 1927); the most effective methods include antibody-coupled fluorescent dyes and immunoperoxidase staining.

The legionellae are usually motile by means of one or more polar or subpolar flagellae. The cell wall consists of a cytoplasmic membrane on the inner surface, a thin peptidoglycan layer, and an outer membrane that contains the heat-stable lipopolysaccharides (LPS) with species- and serogroup-specific O antigens. There is no definitive evidence of a capsule.

The optimal temperature for in-vitro growth is 36°C (limits 15–43°C), with a generation time of 99 minutes under optimal conditions (Brenner, Fealey & Weaver, 1984; Brenner, 1986; Fallon, 1990; States et al., 1993). In the natural habitat—fresh water and soil—growth requires the presence of other bacteria or of protozoa, which are considered to be the natural hosts of legionellae (Rowbotham, 1980; Tison et al., 1980; Wadowsky & Lee, 1985; Fields et al., 1993).

Pathogenicity for humans

Legionellae were first detected in 1976 after a particularly notable outbreak of pneumonia in a hotel on the occasion of a United States army veterans' meeting (Fraser et al., 1977). Since that time, it has been established that these organ-isms are an important cause of pneumonia, both community-acquired (1–15%) (Lieberman et al., 1996; Butler & Breiman, 1998) and hospital-acquired (up to 50%) (Butler & Breiman, 1998). To date, disease due to *Legionella* has been detected almost exclusively in humans, but some animals (e.g. guinea-pigs, rats, mice, marmosets, and monkeys) are susceptible to experimental infection. One case of *Legionella* pneumonia has also been reported in a calf (Fabbi et al., 1998). Evidence of past infection can also be found in other animal species, including wild animals, but no animal reservoir of the bacteria or transmission between animals has been demonstrated (Collins, Cho & Reif, 1982; Boldur et al., 1987).

Two kinds of disease are observed in humans. Legionnaires disease is a severe pneumonia (incubation time 2–10 days); mortality is about 15% and *Legionella* may be detected in sputum and tissues. Pontiac fever is a febrile illness of 2–6 days' duration, with an incubation time of 1–7

(normally 3) days; it is non-pneumonic (cough is observed in about 50% of cases) and self-limiting, and accompanied by headache and myalgia. Bacteria are not detectable in body fluids or tissues nor are bacterial antigens found in urine, but blood antibodies are elevated (Glick et al., 1978; Fallon et al., 1993).

Legionnaires disease is commonly accompanied by extrapulmonary manifestations, such as renal failure, encephalopathy, and pericarditis (Oredugba et al., 1980; Posner et al., 1980; Riggs et al., 1982; Mayock, Skale & Kohler, 1983; Johnson, Raff & Van-Arsdall, 1984; Nelson et al., 1984). Lung abscesses, other local infections, and wound infections involving *L. pneumophila* and *L. dumoffi* have also been reported (Arnow, Boyko & Friedman, 1983; Bauling, Weil & Schroter, 1985; Lowry et al., 1991).

L. pneumophila serogroup 1 is most commonly isolated from patients (58% of isolates in England and Wales, 71.5% in the USA) (Joseph et al., 1994; Marston, Lipman & Breiman, 1994), followed by *L. pneumophila* serogroup 6 (Tang & Krishnan, 1993). Other serogroups of *L. pneumophila* and another 19 species of *Legionella* are associated to a varying degree with human disease. In the USA, *L. micdadei* is the second most frequent cause of Legionnaires disease and has also been repeatedly identified as the causative agent of Pontiac fever (Goldberg et al., 1989; Luttichau et al., 1998). In Australia, *L. longbeachae* seems to be an important cause of Legionnaires disease (Steele, Lanser & Sangster, 1990). Between 9.3% and 29.0% of infections are caused by species other than *L. pneumophila* (Tang & Krishnan, 1993; Joseph et al., 1994; Marston, Lipman & Breiman, 1994), but for most of these there are neither properly validated serological tests nor optimized isolation media (Edelstein, 1993).

Pathogenicity of *Legionella* in humans is largely dependent on host susceptibility. Children and young people are rarely affected, while immunocom-promised individuals—especially transplant recipients—are at very high risk of disease. However, since any population may exhibit both extremes of susceptibility, even people considered to be "fit and well" may become ill (World Health Organization, 1990). Lieberman et al. (1996) observed that 39 out of 56 patients with community-acquired *Legionella* pneumonia had no chronic comorbidity, although coinfection with another microorganism was frequent. Smoking and alcoholism are commonly acknowledged to be predisposing factors, and infection is more common in males than females and in people over 40 years of age (World Health Organization, 1990; Butler & Breiman, 1998). Individual risk factors also include working more than 40 hours per week and spending nights away from home (Straus et al., 1996).

Individuals with terminal renal insufficiency or blood malignancies, people receiving steroid treatments, and severely immunocompromised individuals (including those with HIV/AIDS) are at significant risk for acquiring Legionnaires disease (Marston, Lipman & Breiman, 1994). Patients with chronic lung disease, cirrhosis of the liver, or diabetes are also at risk, though to a slightly lesser extent. An indwelling nasogastric tube is a further independent risk factor for nosocomial Legionnaires disease (Marrie et al., 1991; Blatt et al., 1993). Pontiac fever, by contrast, affects children and healthy adults just as frequently as immunocompromised individuals (Goldberg et al., 1989).

During an outbreak of disease, exposed populations frequently show elevated serum antibody levels but no symptoms of disease. The same is true of people working in high-risk areas. It has been reported that 62 out of 143 (43.4%) healthy people exposed to a contaminated environment had positive antibody titres against distinct serogroups (Paszko-Kolva et al., 1993).

Virulence factors

Legionellae are intracellular pathogens of macrophages, by which they are phagocytosed in a process involving the complement fragment C3 and the monocyte complement receptors CR1 and CR3. Both virulent and non-virulent strains are phagocytosed, remaining intact inside the phagocytes. Virulent strains can multiply inside the phagocytes and are able to inhibit the fusion of phagosomes with lysosomes; non-virulent strains do not multiply (Horwitz, 1993). Only two products of *Legionella* have so far been shown to be associated with virulence (Fields, 1996)—the 24-kDa protein, macrophage infectivity potentiator, thought to be conserved throughout the genus

(Ciancotto et al., 1989, 1990; Riffard et al., 1996), and the 113-kDa integral protein of the cytoplasmic membrane, which is the product of the *dotA* gene (defect in organelle tracking) (Berger & Isberg, 1993; Berger, Merriam & Isberg, 1994; Roy & Isberg, 1997). Helbig et al. (1995) have proposed that differences in the virulence of *Legionella* species or serogroups are associated with differences of epitopes of the LPS. Within *L. pneumophila* serogroup 1, the strains most commonly associated with disease in humans share a common epitope, as revealed by monoclonal subtyping (Watkins et al., 1985; Ehret, von Specht & Ruckdeschel, 1986; Dournon et al., 1988). Aerosol survival (Dennis & Lee, 1988), growth temperature (Mauchline et al., 1994), the possession of tissue-destructive protease (Baskerville et al., 1986), and the expression of flagellae (Bosshardt, Benson & Fields, 1997) may also be important virulence factors.

The host defence against L*egionella* relies principally on cell-mediated immune mechanisms. One protein produced by *L. pneumophila*, the major secretory protein (MSP, 39kDa), is able to induce protective cell-mediated immunity without being a virulence factor (Blander & Horowitz, 1991). Circulating antibodies are produced during infection with *L. pneumophila* in humans, but they do not seem to be protective and antibody titres rise only slowly; 30% of patients do not produce antibodies detectable by immunofluorescence-coupled antigens up to 4 weeks after infection. Rising levels of serum antibodies, however, are of great diagnostic and epidemiological value. No vaccine has so far been tested in humans.

Dose–response relationship; animal studies

Inoculation of guinea-pigs with material from the lungs of infected individuals resulted in the first isolation of *L. pneumophila* in 1977 (McDade et al., 1977). Since that time, guinea-pigs have been used repeatedly for experimental infection and have proved susceptible to infection by inhalation, although aerosol infection is in fact very difficult to achieve (Yu, personal communication). The lethal dose varies from 2400 to 100000 viable bacteria, but infection can be initiated by as few as 130 organisms. Infections have also been induced in monkeys, rats, and mice, although mice seem to be somewhat resistant, at least in terms of mortality (Baskerville et al., 1981; Collins, 1986). The susceptibility of the A/J mouse strain is due to a single recessive gene conferring permissiveness on A/J macrophages (Beckers et al., 1995). Suckling CD1 mice have been shown to be susceptible to infection and seem to provide a promising animal model for studies of *L. pneumophila* virulence (Castellani Pastoris et al., 1997).

The infective dose for humans can be assumed to be low—possible even a single organism— since *Legionella* infections have frequently been traced to contaminated aerosols generated at distances of up to 3.2km (Addiss et al., 1989). Given the frequency of *L. pneumophila* in human surroundings, the virulence of the organism, and the fact that the infective dose is so low, a much larger number of infections would be expected than is actually the case. It therefore follows that there must be other, as yet unknown, determinants of infection. Infectivity may be substantially enhanced if amoebae are inhaled or aspirated (Brieland et al., 1996). Vacuoles in infected amoebae may contain many hundreds of *Legionella* cells which, when liberated, provide a large inoculum in a restricted area of the respiratory tract (Rowbotham, 1986; O'Brien & Bhopal, 1993; Berk et al., 1998).

Mode of transmission

Inhalation of airborne droplets or droplet nuclei containing legionellae is generally thought to be the commonest mode of transmission. The aerosols may be generated by mechanical devices (e.g. cooling towers of air-conditioning systems) or by the use of potable water, especially from domestic hot-water installations (e.g. showers) (Breiman et al., 1990). In one cluster of infections, *L. longbeachae* was isolated from potting mixes and the soil of potted plants in the vicinity of patients (Steele et al., 1990, 1993, 1996). Three cases of Legionnaires disease due to *L. pneumophila* were reported following the flooding of the basement

of a bar; bacteria were isolated from the sump water (Kool et al., 1998).

Aerosol formation is deemed necessary to cause pneumonic disease, but aspiration following ingestion of contaminated water, ice, and food has also been implicated as the route of infection in some cases (Marrie et al., 1991; Blatt et al., 1993; Venezia et al., 1994; Graman, Quinlan & Rank, 1997). Some authors believe aspiration to be the major mode of transmission (Yu, 1993). Sporadic cases in hospitals have arisen from use of the taps in wash-basins. Even when it is possible to demonstrate that the disease strain and the strain colonizing a plumbing system are identical, the exact route of transmission sometimes remains a matter of speculation. There is no evidence of person-to-person transmission (Fraser, 1977; Yu, 1983).

Outbreaks and single cases of Legionnaires disease have been traced to the cooling towers and evaporative condensers of air-conditioning systems, decorative fountains, ultrasonic nebulizers, room humidifiers, hot whirlpool and spa baths, hot water from taps and showers, and medical devices containing water (e.g. respiratory care devices) (Butler & Breiman, 1998). Of 20 hospital outbreaks of Legionnaires disease in England and Wales between 1980 and 1992, 19 were attributed to *Legionella*-contaminated potable water systems (Joseph et al., 1994). The hot-water plumbing systems of many hospitals are contaminated and colonized by legionellae. The same strain may be identified over extended periods at particular sampling points (Chang et al., 1996), but different strains may colonize different parts of the same building (Marrie et al., 1992).

Disease occurrence: outbreaks, sporadic cases, and prospective studies

Outbreaks

Since the 1976 outbreak in Philadelphia led to the detection and description of the family Legionellaceae, many outbreaks—a number of them spectacular, but most on a smaller scale—have been reported, frequently involving hospitals. Infections have often been traced to colonized parts of air-conditioning plants (Dondero et al., 1980; Addiss et al., 1989; O'Mahoney et al., 1990; Watson et al., 1994), but most outbreaks and recurrent single cases in hospitals are associated with contaminated potable water and hot-water systems (Joseph et al., 1994). Decontamination of colonized installations has been shown to interrupt outbreaks and prevent recurrence of sporadic cases. In two prospective studies in hospitals, the frequency with which *L. pneumophila* was isolated from patients with pneumonia was reduced from 16.3% to 0.1% over a 6-year period and from immunocompromised patients from 76% to 0.8% over a 10-year period (Grosserode et al., 1993; Junge-Mathys & Mathys, 1994). Measures used to achieve this included decontamination of the plumbing systems, monitoring of *Legionella* in the water, examination of all clinical specimens for signs of *Legionella* infection, use of sterilized water for all applications in high-risk patients, and ensuring that all patients and clinical staff were adequately informed of the risks of infection, especially with respect to the use of hot water in high-risk wards.

Travel-associated Legionnaires disease

Legionnaires disease is often associated with travel and with staying in hotels—as was the case in the 1976 outbreak in Philadelphia. A study carried out in Ohio (Straus et al., 1996) on domestic acquisition of Legionnaires disease identified nights spent away from home as a risk factor. In England and Wales, 56% of the 160 cases reported in 1995 occurred in travellers (Newton et al., 1996), and in 1997 the same was true for 114 of the 226 reported cases (Joseph et al., 1998). Among 52 Finnish patients with Legionnaires disease, 76% of those who were not immunosuppressed and had no underlying disease ($n = 17$) had made recent journeys (Skogberg et al., 1994). Small clusters of cases have repeatedly been reported among tourists staying at certain hotels in holiday resorts, especially in the Mediterranean region: 55% of 119 hotels in

various European countries had legionellae in their water distribution systems and 73% had amoebae (Starlinger & Tiefenbrunner, 1996).

Outbreaks reported among passengers on cruise ships have been traced to contaminated water in whirlpool baths (Jernigan, 1996) or to drinking-water (Castellani Pastoris et al., 1999). Gerchikova et al. (1990) have found immunological evidence of increased exposure among railway conductors, subway personnel, and railroad construction workers, and have isolated two strains of *L. pneumophila* from water samples taken from railway dining cars. Water pipes and reservoirs on ships, railway carriages, and the like are often subject to warming and are not easily emptied for cleaning. Chlorine decay and bacterial growth are thus more likely in the water they contain.

The link between travel and Legionnaires disease was discussed at a WHO meeting in 1989 (World Health Organization, 1990). A surveillance scheme for travel-associated Legionnaires disease, instituted by the European Working Group on *Legionella* Infection, coordinated by the Public Health Laboratory Service in London, England, and monitored by WHO, has led to the detection of many cases and improved disease prevention.

Sporadic community-acquired infections

In a prospective study in two counties in Ohio, USA, Marston et al. (1997) showed that most cases of pneumonia caused by *Legionella* are community-acquired and sporadic. The annual incidence (with definite diagnosis) was calculated to be 7.0/100000 adults—approximately 10 times the number of cases reported to health authorities. Community-acquired infections may be caused partly by cooling towers and other aerosol-producing devices, but certain features of domestic plumbing and potable-water supply and water-heating systems have also been shown to be associated with Legionnaires disease and must therefore also be considered as a sources of legionellae (Aldea et al., 1992; Straus et al., 1996).

Plumbing systems in residential premises—particularly one-family houses (Tiefenbrunner et al., 1993)—are less frequently colonized than those in hospitals. However, investigations in different cities in Finland, Germany, and Spain have shown that apartment blocks may be as heavily contaminated as hospitals (Aldea et al., 1992; Lück et al., 1993; Zacheus & Martikainen, 1994). The observed differences may be due to the size of water heaters, the extent of the hot-water installations, and other details of the heating (central versus point-of-use, electric versus gas or oil) and distribution systems (Alary & Joly, 1991).

Monitoring and assessment

Examination of clinical specimens

No specific clinical symptoms of *Legionella* infections distinguish them from pneumonia or localized infections of other origins, and many community-acquired infections will be treated without diagnosis. Definitive diagnosis of a *Legionella* infection relies on the following features:
   — increasing serum concentrations of antibodies;
   — detection of antigens in the urine;
   — detection of bacteria in lung tissue, or in sputum or other secretions, by direct immunofluorescence microscopy;
   — culture of *Legionella* from respiratory secretions, bronchoalveolar lavage fluid, pleural fluid;
   — detection of *Legionella* nucleic acid by DNA probes or by polymerase chain reaction (PCR).

Up to 4 weeks after infection, 30% of patients do not develop antibodies detectable by indirect immunofluorescence assay (IFA). An acute-phase antibody titre of 1:256 did not discriminate between cases of *Legionella* infection and non-cases, while a positive urine antigen assay was found in 55.9% of cases compared with <1% of non-cases (Plouffe et al., 1995). A fourfold increase in IgG and IgM titres is considered to be a reliable sign of infection, and detection of

Legionella antigen is a fairly sensitive (70%) and highly specific (>99%) method for diagnosis of *L. pneumophila* serogroup 1 infection (Plouffe et al., 1995). Urinary antigen test results will remain positive for several weeks after the onset of infection (Stout & Yu, 1997). However, infections with non-serogroup 1 *L. pneumophila* will be missed unless test kits containing antibodies against other *Legionella* serogroups and species are available. Urinary tests to detect infection with other serogroups and species are being developed.

Detection of bacteria in lung tissue and sputum by direct immuno-fluorescence as well as by DNA hybridization and PCR is no more successful than the examination of serum for antibodies or of urine for antigen—70% of cases, at best, are detected. Clearly, a positive bacterial culture is the most convincing evidence of infection, but only 9% of 160 cases reported in England and Wales in 1995 were diagnosed by culture (Newton et al., 1996).

To summarize, there is no single laboratory test currently available that will detect all infections caused by *L. pneumophila* or other *Legionella* species (Edelstein, 1993).

Analytical methods for environmental samples

A standard procedure for the isolation, culture, and identification of *Legionella* has been prepared by the International Organization for Standardization (1998). High-yield solid and liquid culture media are commercially available; these are generally optimized for *L. pneumophila*. Recovery rates using these media, and using sample preparation procedures, have yet to be fully evaluated for other *Legionella* species. In dealing with *Legionella* species other than *L. pneumophila*, therefore, the recovery rate should be determined. Steinert et al. (1997) have shown that legionellae may enter a viable but non-culturable state, but become culturable again by cocultivation with axenic *Acanthamoeba castellani*. Swab specimens from a faucet have been shown to yield 10 times as many legionellae as a 250-ml water sample taken from the same faucet (Ta et al., 1995), reflecting the prevalence of the organisms in biofilms.

Environmental samples frequently need to be concentrated or diluted to give optimal results on solid media. Moreover, background bacteria must be eliminated before, or suppressed during, primary culture. Legionellae and background bacteria can be concentrated by centrifugation (e.g. 6000g for 10 minutes at about 20°C) or by membrane filtration. Numbers of other bacterial species present in the sample can be reduced by heat treatment (50 $\pm$ 1°C for 30 $\pm$ 2 minutes, or 55°C for 15 minutes) or by acid treatment (3 minutes at pH 2.2). The material is then streaked, or the filter is transferred, onto buffered charcoal–yeast extract (BCYE) agar (Edelstein, 1981), with or without selective supplement. Various improved media for different purposes have been proposed more recently, as has incubation under 2–5% carbon dioxide. A medium containing dyes (bromocresol blue and bromocresol purple), vancomycin, and polymyxin B (DGVP) gave optimal results in a comparative study (Ta et al., 1995).

Plates are incubated at 36 $\pm$ 1°C for up to 10 days and examined every 2 or 3 days. Presumptive *Legionella* colonies are examined for their L-cysteine requirement by streaking them onto cysteine-free BCYE agar or other appropriate media, e.g. sheep blood agar, with subsequent incubation. Confirmation of *Legionella*, and species and serotype identification are done using commercially available antisera, preferably by direct immunofluorescence. Commercially available latex agglutination kits may also be used. A more rapid procedure has been proposed to replace examination for L-cysteine requirements, namely a colony blot assay using a genus-specific monoclonal antibody coupled with a chromogenic reagent (Obst, 1996). PCR procedures have also been developed. The DNA-sequence information of the ribosomal 23S–5S spacer region was used to develop a genus- and species-specific detection and identification system for all legionellae, using PCR and reverse dot-blotting (Robinson et al., 1996).

Both environmental strains and clinical isolates can be successfully subtyped by molecular techniques such as ribotyping, macrorestriction analysis by pulsed-field gel electrophoresis, or PCR-based methods (Schoonmaker & Kondracki, 1993; Pruckler et al., 1995; Van Belkum et al., 1996). These yield valuable information on the sources and epidemiology of infections. However,

the results of subtyping alone, in the absence of epidemiological data, cannot reliably implicate a source because the distribution of the various subtypes in the environment is unknown.

Control

Occurrence, transport, and survival in the environment and in source waters

The legionellae have been found in natural freshwater systems, including thermal waters, all over the world and are considered to be part of the natural fresh-water microbial ecosystem (Fliermans et al., 1981; Verissimo et al., 1991). The organisms have also been found in sewage-contaminated coastal waters of Puerto Rico (Ortiz-Roque & Hazen, 1987), in well material down to a depth of 1170 metres (Fliermans, 1996), and in low concentrations in groundwater (Frahm & Obst, 1994; Lye et al., 1997). Some outbreaks have been associated with soil and excavation activities. *L. longbeachae*, *L. bozemanii*, and *L. dumoffi* have all been isolated from potting mixes made from composted wood wastes (Hughes & Steele, 1994; Steele & McLennan, 1996).

A characteristic feature of legionellae is their ability to multiply inside protozoa (Rowbotham, 1980). Protozoa that support the growth of legionellae include species of *Acanthamoeba*, *Hartmanella*, *Naegleria*, *Echinamoeba*, *Vahlkampfia*, and *Tetrahymena* (Fields, 1993). Indeed, it has been suggested that environmental growth of legionellae in the absence of protozoa has not been demonstrated, and that protozoa are the natural reservoir for these organisms in the environment (Fields, 1993). However, association with cyanobacteria of the genera *Fischerella*, *Phormidium*, and *Oscillatoria* also promotes relatively rapid growth of *L. pneumophila* (Tison et al., 1980), and cocultivation with some bacteria has been demonstrated in vitro (Wadowsky & Yee, 1985).

Legionellae will not grow in sterilized samples of the water from which they have been isolated. It follows from this that they are part of a microbial ecosystem in which they are both nourished and protected from physical removal by the water current and from antimicrobial agents. They are detected in significant numbers only after other microorganisms have colonized sediments, soil, or biofilms. Growth of other *Legionella*-like organisms in amoebae has been described repeatedly, and—on the basis of 16S rRNA similarity—it has been proposed that these organisms are indeed members of the genus *Legionella* (Rowbotham, 1993; Adeleke et al., 1996).

With the exception of thermal waters and water in tropical regions, legionellae are found in only low concentrations in natural environments (≤1cfu/ml in groundwater); this is to be expected from the low replication rates at temperatures below 25°C. The organisms will be introduced from surface water, soil, and subsoil into water used as the source for preparation of drinking-water and other purposes.

Effects of drinking-water treatment

Storage of raw water in reservoirs will not necessarily reduce numbers of *Legionella*; at elevated temperatures there may even be growth of the organisms. However, as for other bacteria, *Legionella* concentrations can be reduced by coagulation, flocculation, and sedimentation. Growth of *Legionella* may well occur inside filters used for drinking-water preparation (such as granular activated carbon filters) if there is microbial colonization that includes amoebae, but this will be controlled by low temperatures. Significant concentrations will develop only in situations where temperatures rise above 20°C for prolonged periods.

Growth and/or recontamination in distribution systems

At temperatures between 20°C and 50°C, legionellae frequently colonize water distribution systems. The main sites of colonization, bacterial growth, and contamination are the pipework in buildings, boilers (especially if they contain sediment), membrane expansion

vessels and reservoirs inside buildings, as well as the fittings, outlets, and accessory devices connected to water-supply systems. Special mention should be made of medical and dental equipment containing or supplied with water, because it is likely to be used on, or in the vicinity of, susceptible individuals. Colonization is enhanced at temperatures above 25°C, by stagnation, and by formation of biofilms that include protozoa and have an elevated iron content. All these features are common in the warm-water distribution systems of large buildings, including hospitals and other clinical establishments.

Measurable inactivation of legionellae begins at a temperature of 50°C: for *L. pneumophila*, decimal reduction times of 80–111 minutes at 50°C, 27 minutes at 54°C, 19 minutes at 55°C, 6 minutes at 57.5°C, and 2 minutes at 60°C have been recorded (Dennis, Green & Jones, 1984; Schulze-Röbbecke, Rödder & Exner, 1987).

Prolonged stagnation (of several months, for instance during building construction or over holiday periods) resulting in a heavy microbial load has been reported on several occasions when water has been identified as the source of infection (Dondero et al., 1980; Kramer et al., 1992; Breiman, 1993; Mermel et al., 1995; Straus et al., 1996). Pressure-compensation vessels (shock absorbers) also provide the conditions for stagnation (Memish et al., 1992) and should be positioned on the cold-water (i.e. intake) side of hot-water installations.

The concentration of assimilable organic carbon (AOC) in water seems to have less influence on the growth of *Legionella* than on the formation of biofilms. Legionellae are not observed in the absence of other microorganisms. Biofilm formation is encouraged not only by elevated AOC levels but also by certain materials present in a plumbing system. Since legionellae are iron-dependent, it is to be expected that the use of iron piping would encourage their growth; *Legionella*-contaminated water frequently contains high levels of iron as the result of corrosion. However, in a study on the prevalence of legionellae in private homes, the organisms were found only in houses with copper pipework (Tiefenbrunner et al., 1993), and many hospitals in the United Kingdom that have experienced outbreaks of Legionnaires diseases also had copper plumbing. It therefore seems that avoidance of iron or steel pipework does not protect against colonization by *Legionella*.

Materials that promote biofilm formation by nutrients that migrate to surfaces in contact with the water should not be used in water installations, whether as coatings, fillings, or sealants for pipes, reservoirs, or containers, or for devices such as membranes of pressure-compensation vessels, tap washers, etc. (Colbourne et al., 1984; Niedeveld, Pet & Meenhorst, 1986). Biofilms will also form, however, on inert surfaces, albeit more slowly and less extensively, so that the material of which the surface is composed is actually less important than the size of the biofilm-bearing surface. The larger the surface that is available for bacterial growth in a water system, the more likely it is to become colonized by legionellae; thus small water systems in single dwellings are much less likely to become colonized than large systems in, for example, hotels or hospitals.

Control of legionellae in potable-water systems

Prevention

Entry into a potable-water system of single bacteria or bacteria-carrying amoebae from the public supply system, or during construction or repair, must always be considered as a possibility. Prevention of significant bacterial growth is best achieved by keeping water cool (preferably below 15°C) and flowing, or hot (at least 55°C) and flowing. Mains drinking-water supplies can be kept free of significant levels of *Legionella* by chlorination: a concentration of 0.2mg/litre free chlorine will keep levels below 1cfu/100ml, indicating that no active replication is occurring. The low levels of legionellae occasionally found in public water supplies have never been shown to constitute a health risk. Inside buildings, however, the residual chlorine (if any) carried over from the public

supply will not prevent growth of these organisms, and additional measures are required to prevent water stagnating at temperatures that will allow bacterial growth.

The cold water supply should be kept cool, with temperatures at outlets not exceeding 20°C. Pipework, storage tanks, and devices such as water softeners should be insulated against heat gain and should never be situated in rooms where the temperature is constantly high. Hot water must be stored and distributed at a temperature of at least 50°C throughout the system. It is recommended that the water is heated to, and stored at, 60°C, and that it attains 50°C (NHMRC, 1996; Health and Safety Executive, 2000), 55°C (DVGW, 1996), or 60°C (Gezondheidsrad, 1986) at taps after running for no more than 1 minute; temperatures inside boilers and recirculation systems should be similar. For this purpose, the design and construction of the hot-water system must meet certain requirements that are otherwise unnecessary. Water temperatures inside calorifiers and tanks must reach 60°C throughout, including at the bottom, at least once a day. The calorifier must be able to achieve this temperature consistently, even during periods of high demand. For the purpose of thermal disinfection, the calorifier must produce sufficient amounts of water to flush all outlets in the building with water at 70°C. Tanks must be accessible for cleaning and the accumulation of sludge must be avoided. Connected tanks, filters, and other appliances must be scrutinized for their potential to promote *Legionella* growth. Pipework should be as short and easy to survey as possible and should avoid "dead ends" and other zones of stagnation. Outlets should be fitted with mixer taps to reduce the risk of scalding.

Point-of-use water heaters have been proposed as a means of obviating the need for hot-water storage and distribution systems (Muraca, Yu & Goetz, 1990). Even these, however, are not totally failsafe, since growth of legionellae can occur at the outlets (Sellick & Mylotte, 1993).

Since stagnation will give rise to elevated colony counts (and also frequently to high concentrations of legionellae in the water within pipes and reservoirs), it has been proposed that water-supply systems should be drained when there is to be an extended period (e.g. weeks) during which there will be no water consumption.

Particular care should be taken to protect plumbing systems during the construction of new hospital buildings. Before a new or renovated hospital or similar building is opened, the water in the supply system should be tested for microbiological quality, including the presence of *Legionella*.

Eradication/disinfection

When hot-water systems develop problems that cannot be identified or repaired, it is often difficult to keep them permanently free of elevated concentrations of legionellae. Continuous or intermittent treatment for purposes of disinfection or permanent eradication may then be advisable. Techniques for the eradication of *Legionella* include the following:
- — thermal disinfection: heating and flushing
- — UV irradiation
- — use of chlorine, chlorine dioxide, chloramine, ozone, or iodine
- — metal ionization (copper and silver).

Raising the water temperature to at least 60°C (Health and Safety Executive, 2000) is the most reliable means of eradicating legionellae from a water-supply system, although the exact temperature and the length of time necessary for heating and flushing the system, including the outlets, remain matters of some debate (Dennis, Green & Jones, 1984; Snyder et al., 1990). An $8\log_{10}$ reduction in *L. pneumophila* has been demonstrated within 25 minutes at 60°C, 10 minutes at 70°C, and 5 minutes at 80°C. The most resistant species—*L. micdadei*—is about twice as resistant to thermal disinfection as *L. pneumophila* (Stout, Best & Yu, 1986). This is consistent with the finding that *Legionella* in a hospital water system could not be eradicated by raising the temperature in the hot-water tank to 60°C; however, raising the temperature in the tank to 77°C (which produced 50–60°C in the system as a whole) successfully eradicated the organism (Best et al., 1983). At 50–60°C in a model plumbing system, a $7\log_{10}$ reduction in legionellae occurred in under 3 hours (Muraca, Stout & Yu, 1987).

From the observation of Stout, Best & Yu (1986), it follows that thermal disinfection of plumbing systems requires the water in boilers and tanks to be heated to 70°C and taps (outlets) to be kept open for 30 minutes; see also Plouffe et al. (1983). This measure should succeed in eliminating *Legionella* for some weeks. Reappearance of the organisms in the water is usually accompanied or preceded by elevated concentrations of other bacteria, resulting in an elevated heterotrophic plate count (HPC), i.e. >100 colony-forming units/ml (cfu/ml), which is more easily monitored than the concentration of legionellae. Chlorination of the water, however, may make this indicator useless, because   chlorine does not affect legionellae to the same extent as HPC (Zacheus & Martikainen, 1996).

Compared with other Gram-negative bacteria, the legionellae are highly susceptible to UV irradiation (Antopol & Ellner, 1979). In the dark, a 90% reduction has been achieved at 5W·s/m^2, 99% at 10W·s/m^2, and 99.9% at 16W·s/m^2. On exposure to photoreactivating light, however, doses at least 3 times higher are needed because of the organism's effective light-dependent DNA repair system (Knudson, 1985). In hospitals, UV irradiation units installed near "points of use", together with prefiltration systems to prevent accumulation of scale, have been successful in keeping water outlets free of legionellae (Farr et al., 1988; Liu et al., 1995). However, these units are effective only over short distances.

Chlorine is much better tolerated by *Legionella* spp. than by many other bacteria, including *Escherichia coli*. Achieving a given reduction in different species of media-grown *Legionella* required more than 40 times longer than the same reduction in *E. coli* (Kuchta et al., 1983). Tap-water-adapted strains have been reported to be 68 times as resistant as *E. coli* when computed as a product of concentration and time (CT), and experiments using iodine suggest that cultures associated with stainless-steel surfaces are even more resistant (Cargill & Pyle, 1992). The resistance of *Legionella* to chlorine is further enhanced by inclusion of the organisms in amoebae or by growth in biofilms (Kuchta et al., 1993), and it is therefore unsurprising that legionellae have repeatedly been found in chlorinated water that complies with microbiological standards for drinking-water. In reality, the calculation of CT values in laboratory experiments with cultured legionellae is an inadequate indication of resistance to chlorine and other antimicrobial agents.

A chlorine concentration of 2mg/litre will kill free legionellae (Kuchta et al., 1993) and appears to be sufficient to keep the organisms at low levels in hot water (Snyder et al., 1990; Grosserode et al., 1993); even at chlorine levels of 4mg/litre, however, amoebae containing *L. pneumophila* will liberate viable organisms (Kuchta et al., 1993). Continuous hyperchlorination (>2mg/litre) may cause corrosion of pipes and formation of trihalomethanes as by-products (Helms et al., 1988; Grosserode et al., 1993). Nevertheless, supplementary chlorination of the hot-water supply may produce satisfactory results in many situations.

There have been fewer studies of chlorine compounds than of chlorine itself. Chlorine dioxide is probably more effective than chlorine because of its superior oxidative power and effect on biofilms (Walker et al., 1995; Hamilton, Seal & Hay, 1996). Chloramines have a slower action than chlorine but greater stability; Cunliffe (1990) reported that legionellae were much more sensitive than *E. coli* to monochloramine—a compound that is used in Australia to control the growth of *Naegleria fowleri*. In a comparison of hospitals that had reported Legionnaires disease with others that had not, Kool, Carpenter & Fields (1999) revealed that outbreaks were 10 times as likely in a hospital with residual free chlorine in its water than in one where the residual disinfectant was chloramine.

Electrolytically generated copper and silver ions have been shown to be effective in reducing legionellae in vitro (Landeen, Yahya & Gerba, 1989). At 45°C, a 5$\log_{10}$ reduction in legionellae was achieved after 1 hour with silver and copper ion concentrations of 80 and 800μg/litre respectively, and after 24 hours with concentrations of 20 and 200μg/litre (Rohr, Senger & Selenka, 1996). Lin et al. (1996) have also demonstrated a synergistic effect of copper and silver ions. Copper/silver ionization has been used successfully in hot-water recirculating systems, reducing *Legionella* concentrations to 10–100cfu/litre (Colville et al., 1993; Liu et al., 1994; Selenka et al., 1995; Rohr et al., 1996), although there is still no proof of continuing efficiency with prolonged use.

The resistance of legionellae to ozone is comparable to that of *E. coli* and *Pseudomonas aeruginosa* (Domingue et al., 1988): at an ozone concentration of about 0.3mg/litre, a 4–5$\log_{10}$ reduction in the number of organisms was achieved within 20 minutes (Edelstein et al., 1982; Domingue et al., 1988). However, results for the use of ozone to eradicate legionellae from water systems remain ambiguous. It is probably difficult to achieve a sufficient contact time, since adequate levels of residual ozone will not persist in extended domestic water-supply systems. Additional considerations include the safety and corrosive effects of ozone, and compliance with local regulations.

Although each of the disinfection techniques described in this section has proved effective in reducing *Legionella* under controlled conditions, there are differences in their costs and in their suitability for use in large domestic water-distribution systems. In the event of a disease outbreak or other situation requiring immediate action, heat flushing—alone or combined with hyperchlorination—may be the most appropriate measure to apply. To prevent recurrence, the affected system should be checked for any peculiarities of design or operation that predispose to *Legionella* colonization, and these should be corrected, if possible, before any further action is taken.

Conclusions and recommendations

Health risk assessment

The risk of infection following exposure to *Legionella* is difficult to assess and remains a matter of some debate (O'Brien & Bhopal, 1993). Since *Legionella* is ubiquitous in both natural and man-made environments, it must be supposed that most people are frequently exposed, at least to single organisms. Generally, there is either no reaction to such exposure or asymptomatic production of antibodies. Drinking-water from natural sources and from public supplies may carry single organisms or *Legionella*-containing amoebae but, outside hospitals, there are no reports of outbreaks or recurrent cases of disease following consumption or use of drinking-water that has been kept cool and not subjected to prolonged periods of stagnation. However, the inference to be drawn from the many reported outbreaks and documented single cases is that inhalation of small numbers of bacteria, or aspiration following ingestion, will lead to disease.

Risk of infection is acknowledged to be high among transplant patients, patients receiving high-dose steroid treatment or intensive care, individuals being fed by nasogastric tube, and people with malignancies and end-stage renal disease. Special measures of protection and surveillance are essential for people in these categories. Increased susceptibility during outbreaks has also been observed among males, diabetic patients, the elderly, and people with reduced resistance to respiratory disease (e.g. smokers). Nonetheless, no unequivocal dividing line between those at risk and those not at risk has yet been established.

Risk management strategies

Most outbreaks reported to date have been associated with cooling towers, evaporative condensers of air-conditioning devices, potable water at elevated temperatures (especially in hospitals and hotels), hot whirlpool and spa baths, nebulizers, and certain potting composts. The greatest risk seems to be associated with water subjected to prolonged periods of stagnation and in systems that are frequently maintained at temperatures of 25–50°C; this range of temperatures should therefore be avoided as far as possible.

Water systems—particularly cooling towers and evaporative condensers—should be designed, constructed, and operated in such a way that microbial growth is minimized. High water temperature is the most efficient approach to both intermittent disinfection and continuous control. In hot-water distribution systems, water temperatures should exceed 60°C in boilers, reservoirs, and circulating pipes, and reach 50°C at outlets. Continuous surveillance and disinfection have

been proposed for water systems in hospitals and in public swimming pools, hot whirlpool and spa baths and the like, and for medical and dental equipment that uses water. However, opinion continues to be divided on this issue (Centers for Disease Control and Prevention, 1997), and there is no generally accepted threshold limit for the concentration of legionellae in water. Surveillance of the drinking-water and hot-water supply systems in hospitals is recommended by some (Allegheny County Health Department, 1997), and considered prudent in institutions for the elderly and, possibly, in large hotels.

Total prevention of sporadic infections is impossible, because of the widespread occurrence of *Legionella* in all environments. In hospitals, however, all clinical specimens from patients with symptoms of pneumonia should be examined for *Legionella*, *Legionella* antibodies, and *Legionella* antigen. Transplant patients should be scrupulously protected from exposure to *Legionella* during immunosuppression; their drinking-water should be sterilized, and sterilized water should be used for washing these patients.

There are insufficient data to support widespread disinfection of water-supply systems in the absence of any linkage to *Legionella* infections, but in all cases of nosocomial pneumonia every effort must be made to identify the source of infection and implement measures to interrupt transmission. Continuous monitoring of the water, however, is advocated only when antimicrobial measures have to be checked.

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Table 5. Legionella species and serogroups and their association with disease

Legionella species	Serogroups	Pathogenicity for humans

L. adelaidensis		
L. anisa		+
L. birminghamensis		+
L. bozemanii	2	+
L. brunensis		
L. cherrii		
L. cincinnatiensis		+
L. donaldsonii [a]		
L. dumoffii		+
L. erythra	2	
L. fairfieldensis		
L. feeleii	2	+
L. gesstiana		
L. gormanii		+
L. gratiana		
L. hackeliae	2	+
L. israelensis		+
L. jamestowniensis		
L. jordanis		+
L. lansingensis		
L. londiniensis	2	
L. longbeachae	2	+
L. lytica [b]		
L. maceachernii		+
L. micdadei		+
L. moravica		
L. nautarum		
L. oakridgensis		+
L. parisiensis		+
L. pneumophila	15	+
L. quateirensis		
L. quinlivanii	2	
L. rubrilucens		
L. sainthelensi	2	+
L. santicrucis		+
L. shakespearei		
L. spiritensis		
L. steigerwaltii		
L. tucsonensis		+
L. wadsworthii		+
L. waltersii [c]		
L. worsleiensis		

[a] Adeleke et al., 1996.
[b] Drozanski, 1991.
[c] Hookey et al., 1996.