Ann. Ist. Super. Sanità
Vol. 19, N. 2-3 (1983), pp. 215-240

ILL WINDS AND TROUBLED WATERS: SELECTED WHIFFS OF LEGIONELLOSIS

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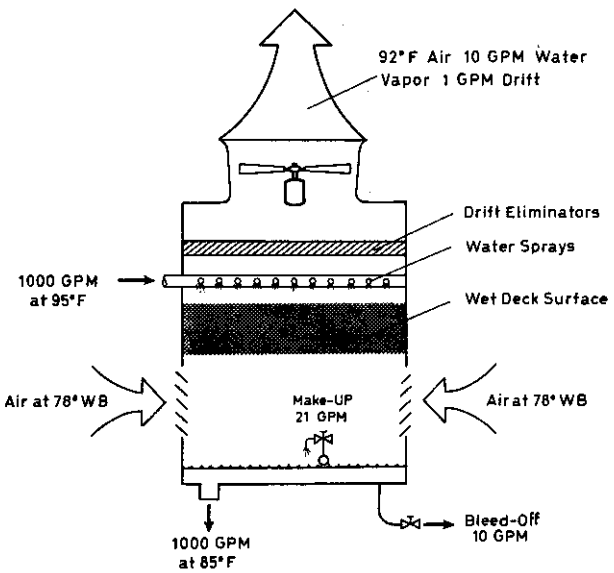

It is ill arguing with the master of thirty legions.

Favorinus (c. A.D. 130), yielding to the Emperor Hadrian in an argument and paraphrasing the proverb cum principe non pugnandum *(avoid contesting with the powerful).*

But there went up a mist from the earth, and watered the whole face of the ground.

Genesis i i .6

Summary. – Legionella pneumophila *is a recently recognized obligately aerobic gram–negative bacterium found in natural and artificial fresh–water habitats. It is the type species of a family of phenotypically similar but genetically distinct bacteria designated the* Legionellaceae, *several members of which, like* L. pneumophila, *are pathogenic for man. Selected aspects of the history, microbiology, epidemiology, clinical aspects, pathology, pathogenesis, host–defense mechanisms, diagnosis, therapy, and control measures relevant to* Legionella *and legionellosis are briefly reviewed.*

Riassunto (Venti malsani e acque torbide: una rassegna sintetica della Legionellosi). – Legionella pneumophila *è un batterio aerobico obbligato, di recente riconoscimento, che si trova in habitat di acqua dolce. E' la specie tipo di famiglia di batteri, fenotipicamente simili ma geneticamente diversi, che si designa col nome di* Legionellaceae, *a cui appartengono anche patogeni umani, come la stessa* L. pneumophila. *Questa rassegna sintetica sottolinea particolari aspetti riguardanti la storia, la microbiologia e l'epidemiologia, la clinica e la patologia, i meccanismi patogenetici ed immunologici, la diagnosi, la terapia e la prevenzione della* Legionella *e della legionellosi.*

Prologue

St. Elizabeths Hospital is the United States Government's institutional establishment for the reclamation and treatment of men and women afflicted with loathsome or incurable diseases and insanity in all its phases and of criminal degenerates.

The Book of Washington (1927)

In March 1947 Elizabeth Jackson and her colleagues at the Army Medical Service Graduate School isolated a novel microorganism from a guinea pig that had been inoculated with blood from a colleague, the serologist in the laboratory, who had a mild febrile respiratory illness. At a meeting of the Society of American Bacteriologists five years later, her group reported that the isolate was pathogenic for guinea pigs and embryonated eggs, that it was not susceptible to penicillin, and that it did not grow on any of a variety of bacteriologic media. Since they could detect no complement–fixing antibody in convalescent serum from the patient, they presumed

that the strain, designed *Olda*, was a clinically irrelevant "rickettsia–like agent" that had coincidentally infected one of the guinea pigs in their laboratory [1-3].

Austin, the seat of Mower County in southeastern Minnesota, is an agricultural community that once boasted the largest meat–packing plant in the United States. The city is the home of the Hormel Institute, which conducts research on dietary factors in cardiovascular disease. In the summer of 1957 residents of Austin experienced an outbreak of pneumonia with 78 cases and two deaths. Investigation of the outbreak failed to identify the etiologic agent, its source, or its mode of trasmission [4, 5].

The holdings of the colonial Maryland landowner Thomas Cornwallis included the 2000 acres of Saint Elizabeth's Manor between Oxon Creek and the Anacostia River. The Government Hospital for the Insane was opened in 1855 on a portion of the old Cornwallis property in what had become the District of Columbia. In 1916 the name of the institution was changed to that of the 17th–Century estate. St. Elizabeths Hospital has played host to such illustrious Americans as Ezra Pound and John W. Hinckley, Jr. In the summer of 1965, 81 cases of pneumonia with 14 deaths occurred in an outbreak among patients there. An epidemiologic investigation provided evidence for airborne transmission of an etiologic agent associated with excavations for a sprinkler system on the grounds, but the agent was not identified [6, 7].

Named for the Ottawa Indian chief, Pontiac, the seat of Oakland County, Michigan, is an automobile manufacturing center. In July and early August 1968 at least 144 persons who had entered the County Health Department were involved in an outbreak of febrile illness characterized by headache and myalgia without pneumonia. The epidemic was linked to exposure to a defective air–conditioning system, and the etiologic agent appeared to be present in the basin of the evaporative condenser used to cool air distributed throughout the building, but the agent was not identified [8 - 10].

Benidorm, on the Spanish Riviera, is a popular destination for British holiday–makers taking inexpensive package vacations. In the summer of 1983, 89 British tourists from one Benidorm hotel developed respiratory illness, and three died with pneumonia. Investigation of the outbreak by Scottish and Spanish authorities failed to identify the etiologic agent, its source, or its mode of transmission [11 - 13].

The James River flows 340 miles from Botetourt County, Virginia, to its estuary at Newport News. In the same year as the Benidorm outbreak, ten men who had cleaned a steam turbine condenser on the James developed a febrile illness characterized by headache and myalgia without pneumonia. The etiologic agent was not identified [14, 15].

The Independent Order of Odd Fellows is the American branch of a fraternal benefit society with origins in 18th–Century England. In September 1974, 20 members of the Odd Fellows developed pneumonia after a convention at Philadelphia's historic Bellevue Stratford Hotel. Two died. The outbreak was not brought to the attention of health authorities at the time [16].

In July and August 1976 an epidemic of 221 cases of pneumonia with 34 deaths struck recent delegates to an American Legion convention and other persons who had entered the Bellevue Stratford or walked by it. An epidemiologic investigation suggested that the etiologic agent had been transmitted by the airborne route on the sidewalk in front of the hotel and in the hotel lobby, but initial efforts to identify the agent were unsuccessful [17].

In January 1977 Morris Dumoff, a bacteriologist at McLaren General Hospital in Flint, Michigan, isolated a fastidious gram–negative bacillus from the lung of a patient who had died on New Year's eve after hospitalization for pneumonia. Growth was obtained on an enriched chocolate agar but not on blood agar. Attempts to identify the organism in his laboratory and at the Michigan Department of Health were unsuccessful, and the isolate was forwarded to the Center (now Centers) for Disease Control (CDC) in Atlanta for further evaluation [18].

Among the dozens of researchers at CDC who had sought to determine the etiology of Legionnaires' disease in the summer and autumn of 1976 had been Joseph McDade, a rickettsiologist. McDade had been asked to look for *Coxiella burnettii* in autopsy material in an effort to exclude Q fever as a diagnostic possibility. He had inoculated lung tissue from fatal cases of Legionnaires' disease into guinea pigs before passage in embryonated eggs. These guinea pigs had rapidly become febrile after inoculation, suggesting the presence of bacteria in the inocula, so McDade had pretreated his eggs with penicillin and streptomycin to inhibit bacterial contaminants. No rickettsiae had been recovered. Microscopical examination of tissues from the febrile guinea pigs had revealed only occasional cocci in the lungs and extremely rare bacilli in the liver and spleen. Bacteriologic culture of the lungs had confirmed the presence of contaminating gram–positive cocci. The bacilli seen in the liver and spleen had not grown [19].

Annoyed by a snide remark about CDC at a Christmas party late in December 1976, McDade resolved to conclude his unfinished business by isolating the apparent contaminants from guinea pig viscera. Reviewing a section from one spleen, however, McDade for the first time found an apparently intracellular cluster of the bacilli. Intrigued by the thought that these might represent not contaminants but rather a new rickettsial species of etiologic significance, McDade inoculated spleen tissue into embryonated eggs without antibiotics and recovered an unfamiliar organism. 111 persons from the Legionnaires' disease epidemic were found to have developed an antibody response to this agent [20].

It is now clear that the organism identified by McDade, which has been given the name *Legionella pneumophila* [21], is the same as those isolated by Jackson and Dumoff as well as the etiologic agent of Austin pneumonia, Pontiac fever, steam–turbine–cleaner's disease, and the outbreaks associated with Saint Elizabeths, Benidorm, and the Bellevue Stratford.

Microbiology

Culture Media. – *L. pneumophila* does not grow on conventional bacteriologic media [22]. Culture *in vitro* was first accomplished at CDC [23] using a medium developed for antimicrobial sensitivity testing of *Haemophilus influenzae* [24] – Mueller–Hinton agar enriched with hemoglobin and IsoVitaleX (MHIH), a proprietary mixture of nutritional supplements for bacteria. Hemoglobin can be replaced by iron salts, and the essential constituent of IsoVitaleX is L–cysteine hydrochloride [23]. F–G agar, Mueller–Hinton agar supplemented with ferric pyrophosphate and L–cysteine hydrochloride, is superior to MHIH for primary isolation of the organism from clinical specimens [23].

A substantial improvement in the ability to recover *L. pneumophila* directly from clinical specimens is provided by charcoal yeast–extract (CYE) agar [25]. The sensitivity of this medium for primary isolation of *L. pneumophila* is further enhanced in the form of buffered charcoal–yeast extract (BCYE) medium, containing N–2–acetamido–2–aminoethanosulfonic acid (ACES) buffer [26], and by the addition of nutritional supplements including α–ketoglutarate [27] and albumin [28]. Incorporation of dyes into BCYE facilitates differentiation of *L. pneumophila* from other bacteria [29, 30]. Some success has been achieved in developing selective media for *L. pneumophila* by the addition of various antimicrobial agents to inhibit contaminating organisms [27, 31 - 33].

Microscopy. – *L. pneumophila* is a coccobacillary to filamentous gram–negative bacillus that stains only faintly with saffranin and is more readily seen when carbol fuchsin is employed as the counterstain [22, 34]. The modified Macchiavello staining technique for rickettsiae described by Giménez [35] can also be used but is not specific for *Legionella*. The organism is not acid-fast [22]. Strains that have not been extensively passed on agar media contain presumably lipid–laden sudanophilic inclusions [34]. Polar, subpolar, and lateral flagella are demonstrable on bacteria *in vitro* and *in vivo* using the silver–plating or Leifson stains for flagella [36 - 39].

Ultrastructure. – Transmission electron micrographs of *L. pneumophila* reveal the typical cell envelope of gram–negative organisms, comprising a cytoplasmic membrane, a narrow peptidoglycan layer, and an outer membrane [40 - 43]. The organisms multiply by pinching nonseptate fission [41]. The presence of diaminopimelate, an amino acid characteristic of bacterial cell walls, has been confirmed [44]. The ultrastructural characteristics of flagella and fimbriae on the organism have also been described [45, 46]. Suggestions that the organism forms spores [47, 48] have not been confirmed [40 - 43].

Antigens. – The antigenic characteristics of *L. pneumophila* have been extensively studied and include group–specific and species–specific determinants [49]. Serogroup specificity appears to be conferred by a lipid–protein–carbohydrate complex surface antigen of high molecular weight that has been partially characterized [50 - 52]. Antigenic relationships with other species of *Legionella* have been demonstrated by two–dimensional electrophoresis [53]. Evidence regarding antigenic characteristics of a capsule–like envelope [54] and of the flagella [55] have also been reported.

Cell Lipids. – Among gram–negative bacteria, cells of *L. pneumophila* are exceptionally rich in branched–chain fatty acids, and the characteristic profile of the methylated fatty acids from the organism revealed by gas–liquid chromatography is useful in confirming the species of the organism [56 - 59]. The content of phospholipids in *L. pneumophila* cells is also relatively high, and the organism is atypical in that the most prevalent class of phospholipid is phosphatidyl choline (lecithin), which is uncommon among prokaryotes [59].

Fluorophores. – Cultures of *L. pneumophila* elaborate a product that emits yellow–green fluorescence upon excitation with long–wave ultraviolet light [22]. At least a fraction of the fluorescent material is soluble [23].

Metabolism. – *L. pneumophila* is an obligate aerobe that does not ferment carbohydrates, although utilization of starch is detectable [34]. Glutamate is utilized preferentially as an energy source, whereas glucose appears to be metabolized by the pentose phosphate or Entner–Doudoroff pathways [60]. The organism is catalase–positive, gelatinase–positive, weakly oxidase–positive, and urease–negative and does not reduce nitrate [34]. Hippurate is hydrolyzed [61]. Various chemically defined media that support growth of the organism have been described [62 - 65]. The simplest of these contains only eight essential L–amino acids plus L–glutamic acid as an energy source, but this medium does not permit optimal growth [66]. The organism produces a brown pigment when furnished L–tyrosine in adequate concentrations [67]. L–phenylalanine can be substituted for L–tyrosine, but the D–stereoisomers do not support pigment production [68]. A variety of enzymatic activities have been detected in *L. pneumophila*. The organism digests egg yolk in a fashion suggesting the activity of protease, lipase, and phospholipase C (lecithinase) [69]. Aminopeptidase, esterase, alkaline and acid phosphatase, and phosphoamidase activity have been documented with specific substrates [70, 71]. Elaboration of extracellular protease, phosphatase, lipase, deoxyribonuclease, ribonuclease, and β–lactamase and starch–hydrolysing activity has been reported, but doubt has been cast on the presence of extracellular phospholipase C [72]. Particular attention has been given to *L. pneumophila* protease activity, which appears extracellularly and is active against several human serum proteins but not elastin [72 - 75].

Genetics. – *L. pneumophila* is the type species of the genus *Legionella*, the sole member of the family

Legionellaceae [21]. The DNA of the organism has a guanine–plus–cytosine content of 39%. The genome size is $2,5 \times 10^9$, comparable to that of *Escherichia coli* [76]. No relationship to any of a large number of previously described bacteria with similar characteristics has been revealed by DNA hybridization studies [21].

Some strains of *L. pneumophila* 39%. The plasmids [77-79]. One report suggested an association between an 80–megadalton plasmid and surface antigens on the bacterium; plasmid–bearing isolates were recovered from the inanimate environment, but clinical isolates were plasmid–free [80].

Ecology. – *L. pneumophila* is found as a free–living bacterium in natural and artificial collections of fresh water, including lakes [81, 82] and streams [83] and riparian soil [83, 84], cooling towers [83, 85-93] and evaporative condensers [9, 10, 84] for air conditioning systems, and potable water systems [92, 94-98]. In nature it has been found in association with cyanobacteria (formerly known as blue–green algae) [82], which can provide all the nutrients required for growth of *L. pneumophila in vitro* [99] and stabilize *L. pneumophila* in aerosols [100].

Complement–fixing antibodies to *L. pneumophila* were not detected in sera from 13 genera of small mammals [101].

Antimicrobial Susceptibility. – *L. pneumophila* produces a β–lactamase bound at the surface of the bacterial cell in the periplasmic space [102, 103]. The enzyme is primarily a cephalosporinase. It is most active against cefamandole, moderately active against cephacetrile, cephalexin, cephaloridine, cephalothin, penicillin G, and ampicillin, and slightly active against cefotaxime. Cephaloglycin, cefoxitin, cefuroxime, and cefusulodin are not inactivated. The enzyme is inhibited by the β–lactamase inhibitors CP–45,899 and clavulanic acid [103].

Antimicrobial susceptibility studies of *L. pneumophila in vitro* using the agar dilution method with MHIH reveal marked susceptibility to rifampin ($\leqslant 0.01$ μg/ml) as well as susceptibility to cefoxitin, erythromycin, aminoglycosidic aminocyclitols, minocycline, doxycycline, chloramphenicol, ampicillin, penicillin G, carbenicillin, colistin, and trimethoprim sulfamethoxazole. The organism is less susceptible to tetracycline, methicillin, cefamandole, cephalothin, and clindamycin and resistant to vancomycin [104].

Tests is supplemented Mueller–Hinton broth indicate susceptibility to cefoxitin, cefuroxime, penicillin G, ampicillin, and carbenicillin and intermediate susceptibility to cephaloridine, cephalothin, and cefamandole [103].

Testing of *in vitro* susceptibility by agar dilution in CYE agar indicates greatest activity with erythromycin, rifampin, and rosaramycin. Aminoglycosidic aminocyclitols, chloramphenicol, and cefoxitin are very active, whereas other agents including moxalactam, cefoperazone, and cephalosporins show moderate to little activity [105]. Susceptibility in vitro to other macrolide antibiotics has also been reported [106].

On BCYE agar *L. pneumophila* is highly susceptible to erythromycin, rosaramycin, rifampin, and chloramphenicol, susceptible to ampicillin, carbenicillin, cefoxitin, moxalactam, and aminoglycosidic aminocyclitols, intermediate to resistant to cephalothin and cefamandole, and resistant to penicillin G, oxacillin, clindamycin, and vancomycin [107].

Both gentamicin and erythromycin but not cefamandole demonstrate bactericidal activity against *L. pneumophila*, whether grown in F–G broth or in human embryonic lung fibroblast cell culture [108].

Work with the original strain of *L. pneumophila* isolated in 1947 suggested resistance to penicillin *in ovo* [1], but McDade did not recover the organism from eggs pretreated with penicillin and streptomycin [19]. More recent tests of antimicrobial susceptibility in embryonated eggs indicate greatest prophylactic efficacy with rifampin, gentamicin, streptomycin, erythromycin, sulfadiazine, chloramphenicol, and cephalothin and, to a lesser extent, oxytetracycline. Chlortetracycline and ampicillin are ineffective. Therapeutic efficacy in infected embryos is greatest with rifampin and erythromycin and present with gentamicin, streptomycin, sulfadiazine, and chloramphenicol [109].

Erythromycin and rifampin were effective in treating guinea pigs experimentally infected with *L. pneumophila* by the intraperitoneal route, but no significant effect was observed with penicillin, tetracycline, gentamicin, or chloramphenicol, although the dose of the last antibiotic may have been inadequate [110].

Epidemiology

Seroepidemiology. – Yoncke *et al.* detected antibody to a mixture of three strains of serogroup 1 by indirect hemagglutination in 16.8% of 1200 sera submitted for premarital and preemployment syphilis serology in Michigan. Antibody prevalence was higher in sera collected in the summer than in sera collected in winter and decreased with increasing age of the donor after the fourth decade. Antibody prevalence to antigens of serogroups 2–4 ranged from 1.7–6.2% in the same specimens [111].

The prevalence of antibody in healthy persons detected by indirect immunofluorescence (IFA) appears to vary with location. None of a group of Pennsylvania health department employees had titers $\geqslant$ 64 [20]. Serum from persons in various occupational groups in Nottingham, England, had titers $\geqslant$ 64 and $\geqslant$ 128 in 0.54% and 0.10% of specimens, respectively [112]. Only 1.7% of serum specimens from middle–aged and elderly persons in four cities in the United States had titers $\geqslant$ 64 [113]. Titers $\geqslant$ 128 were found in 4% of 156 office workers near the Veterans Administration Wadsworth Medical Center in Los Angeles [114]. Control subjects in Kingsport, Tennessee, had a 5.2% prevalence of IFA titers $\geqslant$ 128 [89]. Of 110 industry employees in Burlington, Vermont, 15% had titers $\geqslant$ 128 [87]. The same prevalence was observed in hotel employees in Blooming-

ton, Indiana [83]. Of 21 employees of hotels near the one associated with the outbreak in Benidorm, 19% had titers ≥ 128 [13]. Healthy control subject in Austin, Minnesota, had a prevalence of indirect immunofluorescence antibody (IFA) titers ≥ 64 of 43% [5].

One group of asthmatic patients had a higher prevalence of antibody to *L. pneumophila* serogroup 1 than did other patients with chronic pulmonary disease or healthy adults [115].

IFA titers ≥ 128 to serogroup 1 of *L. pneumophila* were found in only 3.4% of patients from whom serum specimens had been submitted to CDC for *Pneumocystis carinii* serology and in only 1 of 48 patients undergoing bone marrow transplantation [116].

Outbreaks in the Community. – Outbreaks of legionellosis (Table 1) have been associated with specific buildings, including hotels [11–13, 16, 17, 91, 98], office buildings [9, 10, 117], a student union [83], a clubhouse [84], and a meat–packing plant [5].

Endemic Occurrence. – Over a 13–month period Macfarlane and colleagues in Nottingham, England, prospectively studied all patients aged 13–79 years admitted to the City Hospital with community–acquired primary pneumonia. Seroconversion to *L. pneumophila* serogroup 1 was observed in 16 (13%) of 127 patients, with cultural confirmation in one. Two additional cases of seroconversion to *L.micdadei* and one to *L.bozemanii* were also documented. Serologic testing for infection with serogroups 2–6 of *L. pneumophila* and with *L. dumoffii* and *L. gormanii* was unrevealing. Evidence of concurrent pneumococcal infection was present in six of the 19 patients with *Legionella* infection [120].

L. *pneumophila* was the etiologic agent in 15% of 58 cases of community–acquired pneumonia seen over 11 months at the Pittsburgh Veterans Administration Center [121].

Nosocomial Infection. – Nosocomial legionellosis has been a persistent problem in some centers. The largest series of cases of *L. pneumophila* infection from a single hospital has come from the Veterans Administration Wadsworth Medical Center in Los Angeles, where a persistent environmental focus of the organism led to 65 cases in patients and employees between May 1977 and December 15, 1978 [122 - 124]. *L. pneumophila* was the etiologic agent in 30% of 74 cases of nosocomial pneumonia seen over 11 months at the Pittsburgh Veterans Administration Medical Center [121]. Legionellosis was diagnosed from autopsy material in 3.8% of a sample of 263 persons dying of nosocomial pneumonia in 40 hospitals in 24 states [125].

Outbreaks of nosocomial legionellosis have occurred in hospitals in the United States and Europe [7, 85–87, 90, 92, 94–97, 126, 127] (Table 2).

Table 1. – *Selected outbreaks of legionellosis in the community* [88]

Year	Months	Location	Cases	Attack rate %	Case–fatality rate %	Reference
1957	June–August	Austin, MN	78	0.3	2.6	5
1968	July–August	Pontiac, MI	144	95	0.0	9
1973	July	Benidorm (SP)	89	–	3.4	13
1973	August	James River, VA	10	100	0.0	15
1974	September	Philadelphia, PA	20	2.9	10.0	16
1976	July–August	Philadelphia, PA	221	6.8	16.1	17
1977	August–September	Kingsport, TN	33	0.6	9	89
1977	May–December	Burlington, VT	69	–	25	87
1977–78	May–August	Bloomington, IN	39	0.2	10	83
1978	August–September	New York, NY	38	–	8	117
1978	August–September	Dallas, TX	18	6	0.2	118
1979	June–July	Eau Claire, WI	13	0.3	31	91
1980	March	San Francisco, CA	14	2.4	0.0	119
1980	June–September	Lido di Savio (IT)	23	8.7	7.9	98

Table 2. – *Selected outbreaks of nosocomial legionellosis* [88]

Year	Months	Location	Cases	Attack rate %	Case–fatality rate %	Reference
1965	July–August	Washington, DC	81	1.4	17.0	7
1977	July–September	Columbus, OH	15	–	7	85
1977–78	May–July	Los Angeles, CA	49	0.5	31	126
1976–79		Norwalk, CT	28	0.7	38	88
1978	August–September	Memphis, TN	39	2.8	13	90

Seasonality. – Outbreaks (Table 1) and sporadic cases [128] of legionellosis are concentrated in the summer and early autumn [88].

Legionella infection was demonstrated in nearly half of the primary community–acquired pneumonia seen at Nottingham City Hospital from July through September but was found in no more than 10% of cases admitted during the rest of the year [120].

In hospital with persistent problems with nosocomial *L. pneumophila* infection, cases have occurred throughout the year [86–88, 124].

Mode of Transmission

Fear death?.....to feel the fog in my throat,
The mist in my face.

Browning, Prospice

The most important mode of transmission of *Legionella* infection appears to be by the airborne route [88]. At Saint Elizabeths hospital illness was concentrated in patients who either slept by windows left open during the hot Washington summer or had permission to walk about the hospital grounds [7]. Even before the etiologic agent of the 1976 Philadelphia outbreak was recognized, the epidemiologic characteristics of the outbreak were judged to be most consistent with airborne transmission [17].

In many instances, outbreaks have taken place in a setting in which exposure occurred to evaporative condensers or cooling towers, which are heat–rejection devices for air–conditioning systems. These devices, which are different in structure and operation from the familiar small window–mounted air conditioners, range in size from the small units seen on the roofs of drug stores and small office buildings to the giant hyperbolic natural draft towers familiar from photographs of nuclear power plants. Evaporative condensers and cooling towers share two characteristics that are incidental to their design: they wash particulate matter from large volumes of ambient air and they expel aerosols of particulate–laden water. Thus samples of any bacteria that are wafted into the air, around one of these devices will be washed into a reservoir of warm water inside, and any such bacteria that can persist or multiply in this environment will be present in the aerosol plume forced out of the device [129].

An outbreak at an Atlanta country club was restricted to frequent golfers who were exposed to the exhaust from an evaporative condenser from which *L. pneumophila* was subsequently recovered [84]. A cluster of cases that chiefly involved persons who had used one of the meeting rooms in a hotel occurred in a setting in which exhaust from a contaminated cooling tower traveled down a chimney and through an open damper into the room [91]. The clearest evidence of a casual role for a heat–rejection device was obtained in studying an outbreak in a Memphis, Tennessee, hospital. The outbreak was temporally associated with the activation of a disused auxiliary cooling tower when the main units were put out of commission by a flood. Cases were concentrated in areas of the hospital that were exposed to the exhaust from the suspect unit, from which *L. pneumophila* was recovered [90]. A particularly striking example of the risk associated with contaminated cooling towers occurred in Burlington, Vermont, when a unit was inadvertently activated while a maintenance worker was still inside it. He promptly developed Legionnaires' disease.

Airborne transmission is also responsible for outbreaks of Pontiac fever. In the original outbreak, illness occurred only in persons exposed to the Oakland County Health Department when the air conditioning was operating. Air discharged from the evaporative condenser, later shown to be a source of *L. pneumophila*, gained access to the building air supply through cracks in the exhaust and supply ducts in the building and by recirculation on the rooftop [9]. The presence of aerosols of *L. pneumophila* in the building was documented by recovery of the organism from guinea pigs caged in the building but not from similarly placed animals provided with filtered air supplies [10]. No recurrences occurred after the air conditioning system was replaced [9].

Nosocomial transmission of legionellosis may occur through generation of aerosols by respiratory therapy equipment using contaminated potable water [96]. The potential of showers to serve as generators of infectious aerosols when the water supply of the hospital is contaminated is also a source of concern, although the importance of shower aerosols remains speculative [95].

The weight of evidence suggests that secondary transmission of infection from person to person rarely, if ever, occurs [5, 9, 17, 88].

Age. – Attack rates of clinical illness are highest in the middle–aged and elderly [5, 17, 88, 93, 128]. Occasional cases in children have been documented [128, 130, 131].

Sex. – Attack rates of clinical illness in outbreaks [5, 17, 87, 88] and sporadic cases [88, 128, 132, 133] are higher in males than females.

Smoking. – Forty per cent of the 65 cases reported from Wadsworth Medical Center were current cigarette smokers [124]. Cigarette smoking has repeatedly been shown to be a significant risk factor [17, 87, 88], even in comparisons with control subjects who had pneumonia that was not caused by *L. pneumophila* [132], a group that itself would be expected to have an excess of smokers.

Alcohol. – Heavy alcohol use has been shown to be a risk factor in sporadic Legionnaires' disease [132], although no significant correlation of illness with reported alcohol consumption was found in the study of the Legionnaires' outbreak in 1976 [17].

Underlying illness. – The importance of *L. pneumophila* as an opportunistic pathogen has been most evident in reports of nosocomially acquired infection. Thirty–six per cent of cases diagnosed in Burlington, Vermont, in 1977 were in immunosuppressed patients [87]. In the series of nosocomial legionellosis reported from the Wadsworth Medical Center, 61 (94%) of 65 cases

had underlying medical problems, including immunosuppression (42%), heart disease (35%), cancer (29%), lung disease (25%), and kidney disease (22%) [124], and the additional risk associated with immunosuppression was epidemiologically documented [126].

Underlying immunosuppressive illness may also be a risk factor in community–acquired infections [128], although it is difficult to exclude the influence of referral bias on the method of case ascertainment. That is, diagnostic evaluation of a case of pneumonia is likely to be most thorough in the immunosuppressed host, so immunosuppressed patients are probably overrepresented in collections of cases of laboratory–proven Legionnaires' disease. No association with immunosuppression was found in one study of sporadic cases that was specifically designed to minimize the influence of referral bias [130].

Travel. – The large numbers of outbreaks reported in recent hotel lodgers emphasize the frequency with which recent travel is a part of the history of persons with Legionnaires' disease [11–13, 16, 17, 83, 88, 98, 118]. Legionnaires'disease has been an unwelcome addition to the schedule of summer vacationers in southern Europe [13, 131]. Recent travel was significantly associated with Legionnaires' disease in a case–control study designed to minimize ascertainment bias arising from any tendency to obtain extensive diagnostic tests in cases of pneumonia with a history of recent travel [130]. Several hypotheses to explain the risk posed by travel have been proposed but not tested [130]: travel may take susceptible adults to endemic areas. Travel may increase exposure to excavation sites and to public buildings with contaminated cooling towers. Finally, the stress of travel may simply lower resistance to infection in a nonspecific fashion.

Construction and Excavation. – Numerous anecdotes of sporadic cases of infection after exposure to excavation or construction sites have raised the question as to whether these activities sometimes generate infectious aerosols from *Legionella* organisms in the soil [88]. The cases of legionellosis at Saint Elizabeths hospital occurred in patients housed near exvacations that were in progress for the installation of a sprinkler system [7]. The extended outbreak at Wadsworth Medical Center in Los Angeles began within two months of the opening of the new hospital in a setting of uncovered excavation sites [124, 126]. Residence near a construction or excavation site has been found to be a risk factor in a case–control study of sporadic cases of legionellosis [132].

Clinical Manifestations

Pontiac Fever. – Our concept of the clinical course of Pontiac fever is dominated by the observations made on the cases in the original outbreak in 1968. This form of legionellosis is an acute self–limited febrile illness with an incubation period of approximately 36 hours. It is characterized by malaise, myalgias, and headache lasting two to five days but often followed by more protracted asthenia in convalescence (Table 3). Respiratory symptoms are minimal to absent in most cases. There are no fatalities [9].

Table 3. – *Selected Clinical Manifestations in Pontiac Fever*

Finding	Prevalence (%)
Malaise	97
Myalgias	95
Headache	88
Fever	86
Chills	83
Dizziness	51
Cough	46
Arthralgias	42
Nausea	42
Meningismus	39
Chest pain	38
Sore eyes	30
Sore or dry throat	28

Physical examination is unremarkable except for the presence of fever, usually below 39.5°, and transient tachycardia and tachypnea. Urinalysis may reveal minimal proteinuria, hematuria, or pyuria, but these findings are of uncertain significance. There is a polymorphonuclear leukocytosis, with counts as high 17,700 WBC/μl reported. No abnormalities in blood chemistries have been recognized, and there are no electrocardiographic or radiologic abnormalities [9].

At Pontiac 95% of exposed employees and 29% of documented visitors to the building became ill [9], and all of the workmen at risk in the James River episode developed symptoms [14, 15]. Because of the mild and nonspecific character of the clinical illness, sporadic cases and even outbreaks with low attack rates are almost certain to escape recognition. It should be noted that the brief incubation period and the absence of radiologically apparent pulmonary involvement suggest that Pontiac fever is not simply mild Legionnaires' disease [10].

One possibility is that this syndrome may arise from absorption of toxic etracellular products of the organism through the respiratory tract. However, the incubation period and apparent absence of pneumonitis are inconsistent with findings observed with experimental aerosolization of endotoxin to rabbits [134]. Furthermore, patients with Pontiac fever develop antibody to surface antigens of *L. pneumophila*, suggesting that they are indeed exposed to intact bacteria [10].

There is no evidence that antimicrobial therapy has any beneficial effect in Pontiac fever [9].

Incubation Period. – Data from the 1976 outbreak of Legionnaires' disease in Philadelphia indicated that

most cases occurred after an incubation period ranging from two to ten days [17], and data from other outbreak investigations are consistent with this interval [7, 16, 83, 90].

Prodrome and Respiratory Symptoms. – Patients frequently complain of anorexia, malaise, and weakness in addition to respiratory and other systemic symptoms (Table 4) [124, 135].

Table 4. – *Common Symptoms in Legionnaires' Disease*

Proportion (%) with:							
Malaise	89			55			
Cough	86	69	92	80	77	79	75
Chills	74	72	77	50	69	79	88
Night sweats					64		
Dyspnea	59		36				
Myalgias	55	46		14		79	
Headache	53	54	28	41		93	38
Chest pain	52	36	33		43		
Sputum production	50	37			36		
Vomiting	23	19					
Number of cases	123	78	61	56	27	14	8
Reference	135	5	124	86	89	119	84

Virtually all the patients reported from Wadsworth Medical Center had a cough, which was usually mild and occasional, unlike the constant hacking cough of *Mycoplasma pneumoniae* infection. Initially it was dry in most patients, but production of nonpurulent or minimally to moderately purulent secretions ensued in 59% of those with cough. Two–thirds of those with cough had minimal to gross hemoptysis. Recurring shaking chills began within the first three days of illness. Chest pain was usually pleuritic. Symptoms of upper respiratory infection were rare [124].

Emphasis has been placed on the prodrome of Legionnaires' disease, which resembles that of a viremia [136], and on the absence of antecedent upper respiratory tract symptoms [137] in this airborne infection to distinguish legionellosis from pneumonia caused by other common pathogens. Yu and colleagues have cast doubt on the concept that distinctive clinical and laboratory abnormalities exist in legionellosis to help the clinician recognize which cases of pneumonia are likely to be caused by *L. pneumophila* [121], however scrutiny of their data reveals that the chief difficulty lies in identifying nosocomial legionellosis in the midst of other cases of hospital–acquired pneumonia.

Diarrhea. – Watery diarrhea may be observed with onset either early or late in the illness and may even be a prodromal complaint [124] (Table 5).

Diarrhea was reported by 21% of the ill persons in the original outbreak of Pontiac fever [9].

Abdominal Pain. – Patients with legionellosis may complain of abdominal pain (Table 6).

Abdominal pain was reported by 24% of the ill persons in the original outbreak of Pontiac fever [9].

Table 5. – *Occurrence of Diarrhea in Legionnaires' Disease*

Number of patients	Proportion with diarrhea (%)	Reference
123	41	135
78	13	5
61	47	124
56	13	86
32	25	121
27	24	89
14	36	119
8	13	84

Table 6. – *Occurrence of Abdominal Pain in Legionnaires' Disease*

Number of patients	Proportion with abdominal pain (%)	Reference
123	20	135
61	0	124
56	7	86
15	47	138

Encephalopathy. – Depression, emotional lability, confusion, disorientation, delirium, lethargy, and obtundation are among the abnormalities in mental status seen in patients with *L. pneumophila* infection [124, 135] (Table 7).

Table 7. – *Occurrence of Neurologic Dysfunction in Legionnaires' Disease*

Number of patients	Proportion with neurologic disorders (%)	Description	Reference
113	21	depressed mental state or agitation	135
61	38	neurologic findings	124
56	0	(excepting headache)	86
32	45	neurologic signs	121
27	59	confusion, delirium, or obtundation	90
24	46	neurologic symptoms and signs	138
14	36	confusion	119
7	57	marked abnormality of mental status	139

Confusion was reported by 19% of the ill subjects in the original outbreak of Pontiac fever [9].

Physical Examination. – The typical case of *L. pneumophila* pneumonia presents as an acutely ill febrile patient [135]. High fever is common. Nineteen per cent of 123 patients from the 1976 Philadelphia outbreak had a fever over 40.0° on admission [135]. The maximum temperature in 63 patients reported from Wadsworth Medical Center was 40° or higher in 62% patients and 40.5° or higher in 19% [124]. The fever characteristically has an insidious onset and a continuous configuration and is minimally affected by antipyretics. Relative bradycardia is common (Table 8) [124, 135].

Physical examination of the chest at the onset of illness is often normal, and rales are typically the first abnormality noted, with signs of consolidation supervening [124].

An initial respiratory rate ⩾ 30 and a initial pulse ⩾ 110 were associated with relative risks of fatal outcome of 5.8 and 4.4, respectively, in the 1976 Philadelphia outbreak [135].

Urinalysis. – Pyuria, proteinuria [124], and hematuria [135] have been reported in a substantial proportion of cases (Table 9).

Hematuria is associated with an unfavorable prognosis [135].

Table 8. – *Physical Findings in Legionnaires' Disease*

Proportion (%) with:						
Fever	97	94	99	96	100	100
Tachypnea		42				
Tachycardia		30				
Relative bradycardia			60*			
Number of cases	123	78	61	56	14	8
Reference	135	5	124	86	119	84

*of 48 patients.

Table 9. – *Findings on Urinalysis in Legionnaires' Disease*

Proportion (%) with:			
Hematuria	48	0	20
Cylindruria	33		
Proteinuria	25	45	40
Pyuria	16		
Number of cases	102	33	5
Reference	135	119	84

Hematology. – Lymphopenia and, in patients without underlying hematologic disease, leukocytosis with supranormal numbers of band neutrophils are common in legionellosis [5, 124, 136], although rarely leukopenia may appear to be the result of *Legionella* infection itself [124] (Table 10). Miller has proposed that lymphopenia (<1000/μl) in the absence of marked neutrophilia is suggestive of pneumonia caused by *L. pneumophila* [136].

Table 10. – *Hematologic Findings in Legionnaires' Disease*

Proportion (%) with:					
Leukocytosis	53	79	78	82	70
Lymphoênia (< 1000/μl)				53*	
Number of cases	117	78	58	56	23
Reference	135	5	124	86	138

*of 40 patients with leukocytosis

In the 1976 Legionnaires' epidemic a differential count with more than 10% bands and a leukocytosis > 14,000 μl were associated with relative risks of fatal outcome of 3.3 and 2.5, respectively [135].

Hyponatremia. – Hyponatremia, usually mild and apparently the result of inappropriate antidiuretic hormone secretion, is common in *L. pneumophila* infection [124, 135] (Table 11).

Table 11. – *Occurrence of Hyponatremia in Legionnaires' Disease*

Number of patients	Proportion with hyponatremia (%)	Reference
123	31	135
55	54	124
32	45	121
23	43	138
15	67	136

A serum sodium concentration ⩽130 mEq/l on admission to the hospital was associated with a relative risk of 2.4 of fatal outcome in the 1976 outbreak [135].

Hypophosphatemia. – Hypophosphatemia may be seen early in the course of infection [124] (Table 12), although this finding may not be more common in legionellosis than in other forms of pneumonia [137].

Renal Function. – Kirby and colleagues noted no increase in serum urea nitrogen and creatinine in their

patients [124], but azotemia and even renal failure have been reported in other series (Table 13).

Table 12. – *Occurrence of Hypophosphatemia in Legionnaires' Disease*

Number of patients	Proportion with hypophosphatemia (%)	Reference
43	51	124
16	31	138

Table 13. – *Occurrence of Azotemia in Legionnaires' Disease*

Number of patients	Proportion with azotemia (%)	Reference
123	15	135
23	30	138
10	0	10

In the Philadelphia outbreak of 1976, presentation to the hospital with a serum urea nitrogen > 20 mg/dl or serum creatine > 2.0 mg/dl was associated with relative risks of fatal outcome of 4.8 and 4.5, respectively [135].

Liver Function Tests. – Abnormal lactic dehydrogenase (LDH), alanine aminotranferase (SGOT), alkaline phosphatase, and bilirubin levels are commonly seen [135-136] (Table 14).

Table 14. – *Liver Function Tests in Legionnaires' Disease*

Proportion with high:			
LDH	88%(36/41)		
SGOT	49%(21/43)		90%(9/10)
Alkaline phosphatase	49%(20/41)	45%(10/22)	
SGPT		24%(4/17)	
Bilirubin	15%(7/47)	30%(7/23)	
Reference	124	138	137

Helms and colleagues found high SGOT values to be more common in patients with legionellosis than in cases of pneumococcal or mycoplasmal pneumonia [137].

Creatine Phosphokinase. – Sharrar and associates reported high creatine phosphokinase (CPK) levels in five of six sporadic cases of Legionnaires' disease, but elevated CPK determinations have been very unusual in larger series [124, 135].

Arterial Blood Gases. – Arterial blood gas measurements reveal hypoxemia and hypocarbia commensurate with the radiographic extent of pneumonia [84, 124]. Respiratory failure is the commonest mode of death. In the Legionnaires' outbreak 15|(12%)of 123 patients had an initial $P_aO_2 < 50$ torr; nine died. Nineteen (15%) of the 123 eventually required mechanical ventilation; the case–fatality rate in this subgroup was 68%. The relative risks of fatal outcome associated with presentation with hypoxemia or eventual development of respiratory failure were 2.8 and 5.2, respectively [135].

Cold Agglutinins and Other Serologic Tests. – Kirby and associates reported reciprocal cold agglutinin titers of 16 in 2 of 16 patients and high *M. pneumoniae* complement fixation titers in 2 of 16 [124]. No cold agglutinins were found in sera from the Austin pneumonia outbreak [5].

Examination of Respiratory Secretions. – Sputum is characteristically thin and watery or mucoid at the onset of pneumonia. Polymorphonuclear leukocytes are less common than erythrocytes and macrophages, and acellular material is most common. Polymorphonuclear cells become more frequent several days after institution of antibiotic therapy [140]. Giménez stains [35] of specimens are less sensitive than direct immunofluorescence (DFA), and Gram's stain is neither sensitive nor specific [140]. The combination of hemoptysis and pleuritic chest pain is not infrequent, and differentiation from pulmonary embolism with infarction may be impossible at the bedside [124, 135].

Kirby *et al.* reported the results of microscopical examination of 29 transtracheal aspirates; there were few polymorphonuclear leukocytes in 10, moderate to many polymorphs in 17, and mononuclear cells in 2 [124].

Pleural Fluid. – Kirby *et al.* reported results on 13 patients who underwent thoracocentesis. Total protein and LDH levels suggested an exudate in eight of ten specimens. The mean leukocyte count was 6,600/μl, with a range of 554-32,500/μl; polymorphonuclear leukocytes usually predominated [124].

Bronchoscopy. – Thin, watery secretions without purulence were found in each of 11 patients undergoing bronchoscopy; mucosal hyperemia and inflammation were noted in four [124].

Cerebrospinal Fluid. – Cerebrospinal fluid is almost invariably normal [124, 135].

Radiography. – Characteristically, a unilobar patchy alveolar infiltrate extends locally with or without subsequent involvement of other lobes. Progression of the infiltrate may continue for as many as five days after institution of erythromycin therapy, even in the face of other evidence of clinical improvement. Significant improvement in the radiograph is usually observed with-

in two weeks, although several months may be required for full radiographic resolution. Small pleural effusions are common and occasionally precede the appearance of an infiltrate. Mediastinal adenopathy is not seen [124, 135, 141].

Although uncommon, cavitary lung disease can occur in *L. pneumophila* pneumonia, particularly in immunocompromised patients [142-144].

Among those hospitalized in the Austin pneumonia outbreak, radiographic abnormalities were most common in those aged 40 years or older [5].

Extrathoracic Infection. – Histopathologic evidence of extrathoracic infection is uncommon in patients dying of Legionnaires' disease [124], but cases of pyelonephritis [145], peritonitis [146], perirectal abscess [147], hemodialysis fistula infections [148], and disseminated infection [149] with *L. pneumophila* have all been reported.

Concomitant Infection. – The isolation of other potential pathogens from clinical specimens does not exclude simultaneous infection with *L. pneumophila* [124, 140]. Concomitant infections with both *L. pneumophila* and *Mycobacterium tuberculosis* [150], *L. micdadei* [144], or cytomegalovirus [124] have been documented.

Other Syndromes. – Despite the association of *L. pneumophila* with cooling towers and evaporative condensers, serologic data do not support an association with hypersensitivity pneumonitis [151].

Pathology

Post-mortem examination reveals bronchopneumonia with consolidation and hepatization. The carinal and hilar nodes may be enlarged. There is an intra–alveolar exudate of polymorphonuclear leukocytes, macrophages, red blood cells, and fibrin, *L. pneumophila* is visible extracellularly as well as within macrophages. Blood vessel walls and large bronchi are spared. Adrenal lipid depletion and adrenal hemorrhage are sometimes observed [124, 152, 155].

L. pneumophila does not stain with conventional tissue Gram's stains but is readily but nonspecifically visualized with modification of the Dieterle silver impregnation stain [154, 155].

Pathogenesis

Experimental infections. – Davis *et al.* have developed an experimental model of Legionnaires' disease in which guinea pigs and rats are exposed to aerosols of *L. pneumophila* in a specially constructed chamber. Nebulization of approximately 7 x 10^9 colony–forming units reproducibly yields histologically detectable pneumonia in animals of both species, although illness is more severe and fatal infection more common in guinea pigs. *L. pneumophila* begins exponential growth in the lungs of both species without an appreciable lag phase. Infiltration of polymorphonuclear leukocytes and then mononuclear cells into involved areas begins after 24 to 48 hours, but growth of the organism continues for three to six days despite an increase in the inflammatory response [156].

Guinea pigs but not mice, rabbits, and rhesus monkeys developed pneumonia on exposure to the Oakland County Health Department at the time of the investigation of the original Pontiac fever outbreak. Naturally and artificially exposed guinea pigs had a nodular bronchopneumonia that underwent central necrosis and eventual fibrous encapsulation [10].

Endotoxin. – *L. pneumophila* cells and cell–free products are potent activators of *Limulus* amoebocyte lysate gelation, a sensitive but not specific test for bacterial lipopolysaccharide [157]. However, the febrile response of rabbits injected intravenously with these bacterial products is slight [157], and *L. pneumophila* shows little potency in other bioassays of endotoxin activity [158]. Nonetheless, Fumarola and his associates have demonstrated generation of procoagulant activity by human monocytes exposed to *L. pneumophila* and related organisms [159], as occurs when monocytes are stimulated by *E. coli* lipopolysaccharide.

Cytotoxin. – A low–molecular–weight extracellular product that is toxic for Chinese hamster ovary cells has been identified [160]. This substance impairs hexose monophosphate shunt activity and oxygen consumption by human polymorphonuclear leukocytes during phagocytosis and reduces iodination and killing of test bacteria *in vitro* [161].

Hemolysin. – Cultures of *L. pneumophila* show hemolytic activity [69], and sterile filtrates of plasma and urine from experimentally infected rabbits lyse guinea pig red cells [162]. Hemolytic activity is present in the macromolecular fraction of concentrated supernates of *L. pneumophila* cultures [163].

Dopa. – *L. pneumophila* appears to possess the enzyme tyrosine–3– monoxygenase, which catalyzes the conversion of tyrosine to dihydroxyphenylalanine (dopa) [67, 68, 164]. Thus one component of pulmonary infection with this organism may be liberation of dopa into the systemic circulation, with possible effects on the central and autonomic nervous systems.

Host Defense Mechanisms

Antibody. – Infection with *L. pneumophila* stimulates an antibody response. The immunoglobulin classes involved vary between individuals [165].

L. pneumophila maintained by passage *in ovo* is

resistant to the bactericidal effect of human serum even in presence of specific antibody, which is required to fix complement to the surface of these bacteria [166]. However, mice and guinea pigs passively immunized with specific IgG raised by immunization with the antigen characterized by Wong *et al.* were protected against experimental challenge with the organism [50].

Phagocytosis. – Optimal phagocytosis of *L. pneumophila* by human polymorphonuclear leukocytes requires both complement and specific antiboby. These phagocytes kill *L. pneumophila* only in the presence of complement and specific antibody, but bacterial killing is incomplete; however polymorphonuclear cells do not support the growth of *L. pneumophila* [166].

In contrast, *L. pneumophila* multiplies within membrane-bound cytoplasmic vacuoles of human monocytes *in vitro* under conditions that do not permit extracellular multiplication [167]. Specific antibody promotes the binding of *L. pneumophila* to monocytes. Monocytes require both specific antibody and complement to kill *L.pneumophila*, but as in the case of polymorphonuclear leukocytes, killing is incomplete. In contrast to polymorphs, monocytes permit the growth of *L. pneumophila*, even in the presence of antibody and complement, under conditions that do not permit extracellular multiplication [167, 168].

Cell–Mediated Immunity. – Human monocytes in cell culture inhibit multiplication of *L. pneumophila* if incubated with both human lymphocytes and concanavalin A or with cell–free supernates of concanavalin A–sensitized mononuclear cells. The activated monocytes inhibit multiplication by ingesting fewer bacteria than do nonactivated monocytes and slowing the rate of intracellular replication of *L. pneumophila* [169].

Laboratory Diagnosis

Primary Isolation. – Primary isolation of *L. pneumophila* from contaminated environmental and clinical specimens was initially performed after passage in guinea pigs and embryonated eggs [10, 20].

Edelstein *et al.* reported positive cultures on CYE agar from endotracheal and transtracheal aspirates, lung tissue, and blood but not from pleural fluid. Cultures from respiratory tract secretions required three to seven days to became positive [140]. *L. pneumophila* may still be isolated from respiratory secretions or lung days after initiation of erythromycin therapy [140, 170].

Recovery of *L. pneumophila* from lung tissue may sometimes be enhanced by dilution of the specimen before culture on agar media [170].

Antigen Detection. – Antigenuria has been detected in patients with *L. pneumophila* infection by radioimmunoassay [171], enzyme-linked immunosorbent assay [172, 173], and latex agglutination [174].

Direct Immunofluorescence. – DFA (Direct Fluorescent Antibody) may be used to detect the organism in environmental and clinical specimens, including respiratory secretions and tissue from biopsies and autopsies [175, 176]. By definition, DFA staining is serogroup–specific. Organisms belonging to serogroups not encompassed by the reagents used by a particular laboratory will stain poorly or not at all. Seven serogroups of *L. pneumophila* have been described [177], and recognition of additional serogroups can be expected in the future. Staining of the specimen with fluoresceinated pre–immune serum from the same animal as that from which the DFA antibody was obtained is required as a control to minimize the risk of falsely positive examinations. Unrelated bacteria that stain specifically with DFA reagents for *L. pneumophila* are infrequently encountered [175, 176].

Edelstein *et al.* reported that sputum specimens gave a higher yield than did transtracheal aspirates. Two bronchial washings and one transbronchial biopsy were even less satisfactory. In some instances the morphology of the DFA–staining bacteria became atypical after the patients began receiving antibiotics [140]. Prior erythromycin therapy apparently reduces the sensitivity of DFA in *post mortem* diagnosis [124].

Serology

Indirect Immunofluorescence. – The most widely used assay of antibody in convalescent serum specimens is the IFA (Indirect Fluorescent Antibody) test [20, 178, 179].

Fluoresceinated anti–human immunoglobulin is used as the conjugate, so the test detects IgG, IgM, and IgA [180]. Since the antigen used in the test is whole heat–killed *L. pneumophila* cells, which presumably expose multiple determinants to the test serum, there is opportunity for cross–reaction with antibody to heterologous serogroups of *L. pneumophila*, other species of *Legionella*, and even completely unrelated bacteria.

Thirty–one (84%) of 37 Pontiac fever patients demonstrated seroconversion to *L. pneumophila* serogroup 1 [10] as did 90.9% of 111 patients from the 1976 Legionnaires' outbreak [20].

Nine (41%) of 22 patients with seroconversion by IFA reported by Edelstein et al. did not show a rise in titer to serogroup 1 antigen [140].

Most patients in whom seroconversion to *L. pneumophila* occurs have diagnostic titer elevations within three weeks of onset of illness. The optimal time to draw the convalescent–phase serum specimen is 14 to 60 days after onset (Table 15). Although a single IFA titer of 256 is sometimes taken as sufficient to establish that a recent illness represents infection with *L. pneumophila* [20], serodiagnosis from standing titers is hazardous, particularly if data are not available on the background prevalence of antibody in the referral population from which the patient comes.

Administration of pharmacologic doses of glucocorti-

Table 15. – *Time Required for Seroconversion in Serologically Proven Cases*

Proportion (%) exhibiting IFA titer of at least 128		
Week of illness:		
1	12	13
2	48	55
3	82	84
4	90	94
5	94	98
6–10	89	100
Number of patients	101	31
Reference	20	124

coids does not appear to retard the antibody response in those who show seroconversion [124].

Microagglutination. – A microagglutination test has been described [181]. It chiefly detects IgM antibody [182].

Complement Fixation. – *L. pneumophila* appears to fix complement poorly [166], a phenomenon that may have contributed to the failure to appreciate the significance of Jackson's original isolate [1].

Other Serologic Tests. – Immune adherence hemagglutination, indirect hemagglutination [183], ELISA [183], and immunodiffusion [184] for antibody have all been described but are not widely used at present. Some serogroup cross–reactivity is seen with the indirect hemagglutination test. Differentiation between species and group–specific reactivity can be achieved by adsorption of sera with group–specific antigen [185].

Combined Modalities. – The prolonged outbreak of nosocomial Legionnaires' disease at the Veterans Administration Wadsworth Medical Center in Los Angeles permitted an evaluation of available methods for the diagnosis of legionellosis in a uniform setting. The diagnosis was confirmed in 32 patients studied from October 1978 to May 1979. Direct immunofluorescence examination of respiratory tract secretions and indirect immunofluorescence assay of serum antibody detected 62% and 75%, respectively, of 13 culture–positive cases. When a positive result with any of the three techniques was taken as the definition of a case, IFA serologic tests (serogroups 1 - 4) were 81% sensitive (22/27), culture on charcoal yeast–extract agar was 62% sensitive (13/21), and direct immunofluorescence of respiratory tract secretions was 47% sensitive (15/32). The use of all three techniques in conjunction was recommended for optimal results [140].

Therapy

No controlled trials of therapy in humans have been reported. The available data come from series of patients treated with various combinations of antimicrobials at the discretion of the responsible physicians.

In the 1976 Legionnaires' outbreak the case–fatality rates were worst for patients treated with cephalosporins (41%), intermediate for those receiving aminoglycosidic aminocyclitols (36%), chloramphenicol (30%), ampicillin (24%), or penicillin G (20%), and best for those receiving erythromycin (11%) or tetracycline (10%) [135].

Beaty *et al.* reported case–fatality rates of 50% for untreated patients. The case–fatality rates were 32% in those receiving gentamicin, 26% with penicillin G, 25% with a cephalosporin, and 6% with erythromycin [86].

Kirby *et al.* reported a 13% case–fatality rate in 46 patients who received erythromycin, including 7% fatalities in 29 patients without immunosuppression and 24% fatalities in 17 immunosuppressed patients. In contrast, there was a 55% case–fatality rate in 18 patients who received no erythromycin (25% of eight patients without immunosuppression and 80% of ten immunosuppressed patients). In nine of the ten fatalities in patients not receiving erythromycin, death occurred within four to 13 days (mean 6 days) of onset. Subjective improvement was noted from 12 to 96 hours after initiation of oral or intravenous erythromycin with defervescence complete in a mean of 4.2 days (range 1 - 15). Kirby *et al.* recommended a dose of 2 - 4 g/day for a total of three weeks to avoid relapse or protracted convalescence [124].

Seven patients developed Legionnaires' disease while receiving ampicillin (two patients), penicillin, cefazolin, cephalexin, cephapirin, or tetracycline. Legionnaires' disease progressed in most patients given ampicillin, carbenicillin, oxacillin, penicillin, cefazolin, cephalexin, cephalothin, cefoxitin, amikacin, gentamicin, vancomycin, or clindamycin instead of erythromycin. A possible response was seen in two of four patients receiving tetracycline and in two patients given trimethoprim–sulfamethoxazole [124].

Horwitz and Silverstein have found that growth of *L. pneumophila* in human monocytes *in vitro* is rapidly but reversibly inhibited by concentrations of erythromycin or rifampin comparable to those that inhibit multiplication of the organism in BYE broth. However neither antimicrobial in concentrations equal to or greater than attainable peak serum levels effectively kills *L. pneumophila* that are multiplying within monocytes or suspended extracellularly in medium that does not support multiplication of these organisms [186].

Control Measures

Detection of Legionella *in the Inanimate Environment.* – Environmental specimens can be screened for *L. pneumophila* using direct immunofluorescence [187]. Passage of environmental specimens through guinea pigs

and embryonated eggs has been used successfully to isolate *L. pneumophila* in the presence of large numbers of other microorganisms [188]. Enrichment steps using low pH [189] or heat [190] have also been employed as an alternative to these biologic filters. Recent improvements in selective media for *L. pneumophila* permit isolation from potable water specimens after direct plating onto agar medium [33].

Decontamination. — Decontamination of cooling towers has been attempted using chlorination at neutral pH with the goal of achieving a free chlorine residual of 100 - 250 ppm [119]. Periodic flushing of the potable water system of a building with superheated water may be the best way of dealing with contamination of storage tanks, pipes, and outlets [95].

Non–pneumophila Legionellaceae

The development of CYE agar and its modifications and the search for *L. pneumophila* in environmental specimens has led to the discovery of the existence of many other gram–negative aerobic nonfermentative rods that do not grow on conventional bacteriologic media and that are characterized by a high proportion of branched–chain fatty acids. Most are known to have fresh–water aquatic habitats, and at least many are proven etiologic agents of pneumonia in man. These organisms have been classified together as members of the family *Legionellaceae* on the basis of their phenotypic similarities [191]. Since these organisms share little to no homologous sequences as determined by hybridization of intact chromosomal DNA, they represent distinct species. Some workers have chosen to emphasize this lack of genetic relatedness by proposing new genera, *Fluoribacter* and *Tatlockia*, for some of these species, but despite assertions to the contrary [192] these designations do not have priority. Since their introduction offers no real advantage while complicating the terminology of workers in microbiology, clinical infectious disease, and public health, these alternate generic terms should be avoided [193].

Legionella micdadei

In the early 1940's outbreaks of an acute febrile illness characterized by an exanthem over the anterior aspect of the legs were reported from Wrens, Georgia [194], and Fort Bragg, North Carolina [195]. The etiologic agent of Fort Bragg fever was ultimately isolated by Tatlock [196, 197] and subsequently shown to be a strain of the Autumnalis serogroup of *Leptospira interrogans* [198].

Early in the course of his investigations in North Carolina, Tatlock isolated a different microorganism from guinea pigs inoculated with blood from a case of Fort Bragg fever [199]. This agent, a poorly staining gram–negative pleomorphic bacillus, was pathogenic for guinea pigs and could be maintained by passage in yolk sacs of fertile hens' eggs. No complement–fixing or agglutinating antibody to the agent was found in convalescent sera of patients who had had Fort Bragg fever and the organism was presumed to be enzootic in the guinea pigs used in the laboratory [197, 199]. Jackson reported antigenic differences between the *Tatlock* agent and the organism that she isolated in 1947, which was ultimately shown to be *L. pneumophila* [1].

In 1959 Marilyn Bozeman and her colleagues recovered an organism, designated *Heba*, from the spleen of a guinea pig inoculated with blood from a patient with suspected pityriasis rosea. The organism was pathogenic for guinea pigs and could be propagated in yolk sacs. It did not grow on any of a variety of bacteriologic media. *Heba* was found to be antigenically related to the *Tatlock* agent. Complement–fixing antibody to these agents was not detected in sera from humans and from thirteen genera of small mammals. *Heba* was presumed to have been latent in the guinea pig from which it had been isolated [101].

In 1979 Pasculle and his colleagues described an apparently newly recognized bacterium responsible for pneumonia in patients receiving glucocorticoids or other immunosuppressive medications. A peripheral, pleurally based nodular or spherical infiltrate was felt to be radiographically typical [200]. This organism and one described in immunocompromised patients in Charlottesville [201] have been shown to represent the same species as the *Tatlock* and *Heba* organisms. These have been given the designation *Legionella micdadei* (Pittsburgh pneumonia agent, *L. pittsburgensis, Tatlockia micdadei)* [202-204]. Tatlock himself has recently emphasized that the evidence for a leptospiral etiology of Fort Bragg fever is substantial and that the agent that he isolated in 1943 was in all likelihood a contaminant in his cultures [197]. Like *L. pneumophila, L. micdadei* has an aquatic habitat and has been isolated from respiratory therapy equipment [205] and potable water systems [206].

When tested on BCYE agar, *L. micdadei* is highly susceptible to erythromycin, rosaramycin, rifampin, cloramphenicol, and the penicillins, susceptible to cephalosporins, cefoxitin, moxalactam, and aminoglycosidic aminocyclitols, and resistant to clindamycin and vancomycin. No β–lactamase is produced [107]. *L. micdadei* is also susceptible *in vitro* to other macrolide antibiotics [106].

Despite apparent differences in their efficacy in clinical infections, erythromycin, rifampin, penicillin G, cephalothin, and gentamicin are bactericidal against *L. micdadei in vitro.* Mutants resistant to rifampin can be selected by exposure to low doses of this agent [207].

Clinical experience indicates that β–lactam antibiotics are inefficacious and that erythromycin is the drug of choice in infections with *L. micdadei* [208]. Respiratory secretions may be positive by culture or DFA for days after institution of erythromycin therapy [144].

Legionella bozemanii

In 1958 a 38-year-old Navy SCUBA diver died after developing fulminant bronchopneumonia while in training [209]. In January 1959 Marilyn Bozeman and her colleagues at the Walter Reed Army Institute of Research recovered an organism, designated *Wiga*, from the brain of a guinea pig inoculated with lung tissue from the dead man. The organism was pathogenic for guinea pigs and could be propagated in yolk sacs. It did not grow on any of a variety of bacteriologic media. *Wiga* was found to share an antigenic determinant common to *Olda*, *Tatlock*, and *Heba*. It was resistant to penicillin G *in ovo*. Complement–fixing antibodies were not detected in sera from humans and 13 genera of small mammals. As with the other "rickettsia–like agent", it was considered to have been latent in the guinea pig from which it had been isolated [101].

An identical organism was isolated from the lung of a patient with chronic lymphocytic leukemia who developed a fatal case of pneumonia after a boating accident in a brackish swamp [209]. This organism has been given the designation *L. bozemanii (Fluoribacter bozemanae)* [210].

Although *L. bozemanii* does produce β–lactamase, its antimicrobial susceptibility pattern *in vitro* on BCYE agar resembles that of *L. micdadei* [107]. *L. bozemanii* is susceptible to a variety of macrolides [106].

Legionella dumoffii

During investigations of an outbreak of infection with *L. pneumophila* in the garment district of New York [117], a similar but distinct organism was isolated from a cooling tower [209]. This isolate is the type strain of the species designated *L. dumoffii* [210].

An isolate of *L. dumoffii* has been recovered from the lung of a man with small–cell carcinoma of the lung who died of pneumonia in Houston [211]. This organism has also been recognized by DFA in a specimen of lung from a woman in San Antonio who developed fatal pneumonia while receiving glucocorticoid therapy for systemic lupus erythematosus [212].

L. dumoffii has β–lactamase activity. Its antibiotic susceptibility pattern *in vitro* on BCYE agar resembles that of *L. pneumophila* [107]. *L. dumoffii* is susceptible *in vitro* to a variety of macrolide antibiotics [106].

Legionella gormanii

The fifth species of *Legionella* to be described was *L. gormanii* [213]. The type strain was recovered from soil collected from a creek bank at a country club in Atlanta that was the site of an outbreak of disease caused by *L. pneumophila* [84]. The organism has been recognized by DFA in the lung of a man in Connecticut who died of pneumonia [213].

Although *L. gormanii* does produce β–lactamase, it has an antimicrobial susceptibility pattern *in vitro* on BCYE agar similar to that of *L. micdadei* [107].

Legionella longbeachae

Four cases of pneumonia in both previously healthy and immunosuppressed patients that were associated with recovery of a new species of *Legionella* from respiratory secretions were reported in 1981. The organism has been designated *L. longbeachae*. Evidence of clinical benefit of treatment with erythromycin with or without rifampin was seen in three of the four [214]. A fatal case of pneumonia with a strain of *L. longbeachae* belonging to a distinct second serogroup of this species has been reported [215].

L. longbeachae is susceptible *in vitro* to various macrolide antibiotics [106].

Legionella jordanis

Two isolates from specimens of fresh water have been classified as belonging to a species designated *L. jordanis* [216], named after the Jordan River on the campus of Indiana University, where the first of these was recovered during the investigation of an outbreak of infection with *L. pneumophila* [83]. Serologic evidence suggests a role for this organism in cases of pneumonia in man [179, 216].

Legionella wadsworthii

L. wadsworthii is represented by an isolate recovered from the sputum of a patient with pneumonia and underlying chronic lymphocytic leukemia who was admitted to the Veterans Administration Wadsworth Medical Center in November 1981. The patient responded slowly to antimicrobial therapy that included erythromycin and rifampin [217].

Legionella oakridgensis

The most recently named species of *Legionella* is *L. oakridgensis*, which is represented by ten strains isolated from industrial cooling towers. These organisms produce illness in experimentally infected guinea pigs but have yet to be associated with human disease [218].

WO - 44

A *Legionella*–like organism represented by the isolate WO - 44 has been shown to be responsible for an outbreak of illness resembling Pontiac fever that affected 395 engine assembly plant workers at a factory in Windsor, Ontario, in August 1981. Epidemiologic studies suggested airborne transmission from the coolant system in the piston department [219].

Ricevuto il 4 aprile 1983.
Accettato il 26 aprile 1983.

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