Bulletin of the Puerto Rico Medical Association 75:403-407 September 1983

Legionellosis and Legionella Spp. in the Waters of Puerto Rico*

Carmen Ortiz-Roque, B.S., M.S. (Student)
Terry C. Hazen, Ph.D.

Summary: Legionellosis is common as an atypical pneumonia in the United States; however, patients infected from the same source can develop non-pneumonic disease, a short review is given. Few cases (4) of legionellosis have been documented in Puerto Rico. Examination of water with fluorescent antibodies against 6 different species of *Legionella* and 6 serotypes of *L. pneumophila* reveals high densities of these bacteria in Puerto Rico. This report also demonstrates for the first time *Legionella* in near-shore coastal waters. Densities of *L. pneumophila* were at levels only slightly below what is considered pathogenic to humans. Densities of *L. pneumophila* were well correlated with organic and inorganic pollution, sampling sites in the San Juan area had the highest densities observed on the island. Strains of *L. pneumophila* isolated in Puerto Rico have proven to be pathogenic in the guinea pig model. Due to the high potential for exposure and the confusing symptomolgy of the disease it is suggested that many cases of legionellosis go unreported in Puerto Rico.

In order to give the clinician a better understanding of legionellosis we first present a short review. For a more detailed review see Meyer[1] and Jones and Herbert.[2]

History. In the summer of 1976 Legionnaires' disease affected 182 members of the American Legion attending a state convention at the Bellvue Stratford Hotel in Philadelphia. Twenty-nine of the affected legionnaires died and 39 other cases (5 fatal) were reported from people who had been near the hotel during the same period.[3] Characteristically, pneumonia and acute diffuse alveolar damage were observed.[4] This epidemic due to it's unknown etiology and high case fatality rate captured the attention of the press and produced numerous speculations. In an effort to determine the cause of Legionnaires' disease (LD), tissue samples were analyzed for abnormal concentrations of metallic elements, tissue and urine samples were examined for toxic organic substances, 14 bacteriological and mycological media and 13 virological host systems were assayed. Identity of the responsible agent was also attempted by testing seroreactivity of patients to 77 infectious agents.[5] The thirty-three Epidemic Investigative Survey (EIS) officers from the Center for Disease Control in Atlanta were dogged by reporters during the entire six months of their fruitless investigation. Only the use of rickettsioligical techniques combined with indirect immunofluorescence (IFA) resulted in the isolation of the etiological agent *Legionella pneumophila*,[5] a previously unknown fastidious, gram-negative bacteria.

Subsequent examination of patient sera, collected during the epidemic, for antibodies to *L. pneumophila* demonstrated seroconversion,[6] while the ventilation system of the hotel was shown to have high densities of *L. pneumophila*. The same techniques served for the retrospective identification of *L. pneumophila* as the causative agent of other epidemics in the District of Columbia, Washington (1965) and Pontiac, Michigan (1968). DNA hybridization and indirect fluorescence studies demonstrated that OLDA, and organism isolated from guinea pigs inoculated with blood of a patient from Jackson, Mississippi (1947), was indeed *L. pneumophila*.[7] One of the more notable cases was the epidemic which occurred in the Oakland County Public Health Building in Pontiac, Michigan in 1968. The serum collected at the time of the epidemic was positive for *L. pneumophila;* however, the symptoms were quite different and there were no fatalities. The clinical features presented in the Pontiac case were quite different from the Philadelphia case. Studies of many other epidemics and individual cases reveal that all can be grouped into two symptomatic types: Pontiac fever and Legionnaires' disease after their aforementioned discovery sites.[1]

Clinical Features of Legionella pneumophila Infection. Infection by *Legionella pneumophila* produces a spectrum of effects that goes from asymptomatic seroconversion to severe pneumonia. Among the clinical manifestations, the first and universal symptom of the infection is unremitting progressively rising fever (>40°C). The incubation period for Legionnaires' disease is 2-10 days averaging 4; however, more rapid onset is common in immunosuppressed patients, eg. nosocomial infections.[1] Malaise, lethargy, headache and profound weakness are demonstrated in more than 50% of all cases. Chills and rigors are also observed in 75% of all patients during the first three days.[1] More then 90% of all patients studied have a dry cough after three days of onset. As the pneumonia spreads this cough becomes productive. Confusion, disorientation and other neurological symptoms are frequently observed.

Legionnaires' disease has an attack rate of less than 5%. Untreated, the disease gradually worsens during the first week with extension of the pneumonia to other lobes in which case mechanical ventilation is frequently required. If untreated, the fatality rate runs as high as 30% contrasting with a 7% rate in the treated patients. Immunocompromised patients have mortality rates greater than 50%.[1]

Pontiac fever differs from Legionnaires' disease in that a shorter incubation period (24-72 hr.) is frequently observed. Fever, chills, headache and malaise which resemble influenza are common symptoms. In contrast to Legionnaires' disease the attack rate of Pontiac fever is 100% with all patients recovering (Dr. D.W. Fraser, personal communications).

Microbial Ecology Laboratory, Department of Biology, Faculty of Natural Sciences, University of Puerto Rico, Río Piedras, Puerto Rico 00931.

** This paper was presented in part at the annual meeting of the American Society for Microbiology in Atlanta, GA. March, 1982. This work was supported in part by Public Health Service Grant RR-8102 from the National Institutes of Health, Sea Grant (National Oceanographic and Atmospheric Administration, Department of Commerce) UPR SGO4F15844030 project EN/P-45 and by funds from the Oficina de Coordinación de Estudios Graduados e Investigaciones of the University of Puerto Rico, Río Piedras.*

Diagnosis of Legionnaires' Disease. Clinical diagnosis of Legionnaires' disease is difficult because it presents symptoms which are similar to other atypical pneumonias. Chest X-ray of early infections usually notes a single lobar infiltrate. Consolidation of the lobe and spread is observed upon later examination. Leucocytosis is found in more than 50% of all patients.[1] The use of nebulizers, corticosteroid therapy and cigarette smoking have been identified as important risk factors while the low incidence of legionellosis among the pediatric population is puzzling (Dr. D.W. Fraser, personal communication). To date, definite diagnosis of *L. pneumophila* infections is attainable by either of three methods: IFA, direct fluorescent antibody (DFA) and/or direct isolation of the bacterium from a specimen.

Isolation of *L. pneumophila* from culture media has been very difficult. No media has proved to be selective for the isolation of *L. pneumophila* from heavily contaminated samples and direct inoculation into guinea pigs is still recommended.[8 9] Charcoal yeast extract (CYE) agar is universally used as a support media (this media is commercially available through Remel, Lenexa, Kansas).

Serological evidence of Legionellosis is defined as a four-fold increase in titer to greater than 1:128 in sera from acute to convalescent phase as determined by indirect immunofluorescence.[10] Validation of IFA with epidemic sera has a sensitivity of >78% and a specificity of 99%[11] but does not consistently show a four-fold rise between acute and convalescent sera until at least three weeks after-the onset of legionellosis.[12] Kits are available for legionellosis diagnosis by IFA from Diodx, Denville, NJ and Bionetics, Charleston, SC.

The use of direct immunofluorescence, for the detection of legionellosis permits early diagnosis.[13] DFA against sputum and pleural aspirate has a 50-60% sensitivity.[14] With a high fatality rate[12] early diagnosis and appropriate treatment of Legionnaires' disease are desirable.

Treatment of Legionnaires' Disease. The treatment of choice (based on retrospective analysis) for legionnaires disease is 2-4 gm per day of Erythromycin for three weeks to prevent relapse.[1] Erythromycin inhibits intracellular multiplication of *L. pneumophila* but does not kill the bacteria which is a facultative intracellular parasite that requires host defenses for the containment of infection.[15] Tetracycline gives variable results while penicillin, cephapirin, cefazolin, carbenicillin, aminoglycosides and chloramphenicol have little effect on the disease's course.[16] In fact cases treated with ampicillin seem to have a higher fatality rate.[1] This illness is quite debilitating with effects lasting from several weeks to several months. Because of the potential for prolonged recovery, nutrition and vitamin therapy are also desirable upon diagnosis.

Epidemiology of Legionella spp. Since 1976, 8 serogroups of *Legionella pneumophila* and 8 other species of the genus *Legionella* have been discovered: *L. oakridgensis, L. gormanii, L. jordanis, L. longbeachae, L. Bozemanii, L. wadsworthii, L. dumoffii and L. micdadei.* Of the above, all except *L. gormanii, L. jordanis* and *L. oakridgensis* have been directly implicated in human pathogenicity. *Legionella gormanii* and *L. jordanis* have been isolated from environmental sources, but only on the basis of IFA seroconversion have been implicated in respiratory disease.[17] *Legionella oakridgensis* has not been implicated in human pathogenicity but presents a pathogenic pattern very similar to the other *Legionella* species when injected into guinea pigs.[18]

Epidemics of Legionnaires' disease have been reported in: District of Columbia, Michigan, Pennsylvania, Kansas, Virginia, Tennessee, Georgia, California, New York, Vermont, Indiana, Connecticut, Ohio, Maine, Wisconsin, Florida, Texas, and Washington. In addition Legionnaires' disease has been documented in: England, Sweden, Spain, Netherlands, Italy and France.[1]

The mode of transmission of this airborne pathogen has been traced to aerosols produced by evaporative condensers and cooling towers.[19] The isolation of *L. pneumophila* from the plumbing fixtures and showerheads of potable water systems and their potential as a source of infection has also been documented.[20] Recent studies have demonstrated that airborne spread of *L. pneumophila* from infected to healthy guinea pigs does not occur.[21]

A CDC survey in the USA found that legionellosis accounts for 3.8% of the fatal nosocomial pneumonias and 10% of the cases of atypical pneumonias,[1] and they estimate that among the flu and pneumonia infections that *Legionella* is responsible for 250,000 cases each year in the USA.[22]

Since its discovery, four cases of Legionnaires' disease have been retrospectively diagnosed in Puerto Rico by IFA (Dr. C.H. Ramírez-Ronda, Veterans Administration Medical Center, San Juan, personal communication).

Ecology of Legionella spp. Although *L. pneumophila* has been isolated from aquatic environments associated with various epidemics, it has also been isolated from many non-epidemic related habitats.[23] The ubiquitous nature of this bacterium was demonstrated by an extensive study in which 792 out of 793 samples were found to be positive for *L. pneumophila* by DFA.[9] Isolation of *L. pneumophila* from guinea pigs was attained in 15% of the injected samples confirming the identitiy of the organism. Seven physico-chemical parameters describing the habitats of the aforementioned isolates had the following ranges: temperature, 5.7 to 63°C; conductivity, 18 to 106 uohms; pH, 5.5 to 8.1; dissolved oxygen, 0.3 to 9.6 ppm; chlorophyll A, 0.7 to 24.mg m^{-3}; phoephytin, 0.2 to 18.8 mg m^{-3}; and water clarity, 1 to 4 m secchi disk readings.[9] Being part of many natural aquatic habitats, *L. pneumophila* can comprise as much as 10% of the bacterial population. A "reasonable level of acceptance" of *L. pneumophila* has not yet been defined but densities in the order of 10^5 - 10^6 viable cells per ml has been suggested as potentially pathogenic to humans.[24, 24]

With the exception of the apparent association of *L. pneumophila* with blue-green algae[25] and *L. pneumophila 's* facultative thermophilic nature, no other findings define the conditions favoring high densities of LD bacterium nor the mechanism(s) of transmission converting an ubiquitous member to the natural aquatic flora into a potential pathogen.

Considering the ubiquitous nature of *L. pneumophila* and it's apparent thermophilic character, the fact that this bacterium had not been reported for the tropics became the object of our curiosity. During the summer of 1981, concentrated water samples were sent to Dr. Carl B. Fliermans (Ecological Microbes Unlimited, Aiken, South Carolina), which upon intraperitoneal injection into guinea pigs and isolation of *L. pneumophila* from the diseased

animals, confirmed the identity and established the pathogenicity of the Puerto Rican isolates. We then started a study to define the abundance and distribution of *L. pneumophila* in tropical aquatic habitans in Puerto Rico.

Materials and Methods

The following study sites (Fig. 1) were chosen for their accessibility, differences in basic water quality and differences in polluting effluents: Mameyes River watershed (Luquillo), Boca Vieja cove (Cataño), San Juan Beach, Canas River (Ponce) and Mata de la Gata island (Parguera), for detailed descriptions of sampling sites see Biamon and Hazen;[26] A.J. López-Torres, MS thesis, University of Puerto Rico, Río Piedras, 1982. Surface waters were sampled aseptically, concentrated by centrifugation at 5000 x g for 15 min at 4°C and analyzed by DFA. Specific conjugates of *L. pneumophila* serotypes,[1 6] *L. bozemanii, L. dumoffii, L. gormanii, L. longbeachae,* and *L. micdadei* were kindly provided by CDC in Atlanta. A negative control which consisted of preimmune fluorescein isothiocyanate (FITC) labelled rabbit serum was done for each site. Positive controls of the homologous CDC antigens and specific conjugates were used with each batch run. Concentrated samples were counted with the aid of Model 16 + IV FL vertical illuminator, epifluorescent microscope (Carl Ziess, Inc., New York, NY).

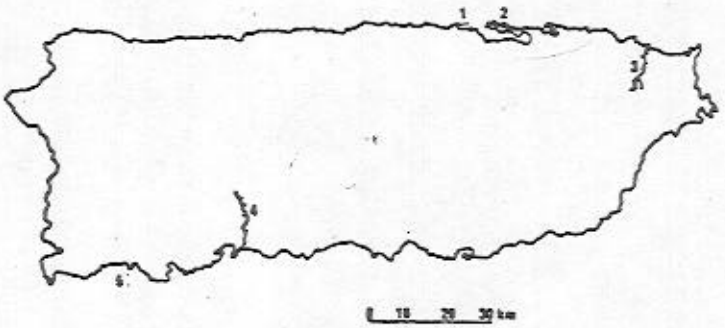

Figure 1. Sampling sites in Puerto Rico (1 = Ensenada de Boca Vieja, 2 = San Juan, 3 = El Yunque, 4 = Ponce, 5 = Parguera).

Simultaneous to sample collection, the following water quality parameters were measured *in situ*: air temperature, water temperature, dissolved oxygen, pH, conductivity, salinity, hardness, turbidity and alkalinity. Other water samples were fixed and analyzed in the laboratory for phosphate, nitrate, total phosphorus, chlorophyll A and sulfate. All water quality analyses were by APHA standard methods.[27] Density of fecal coliforms were determined by membrane filtration of triplicate samples, plating on m-FC media and estimation of the number of colony-forming units after incubation at 44.5°C for 24 hours.

Results and Discussion

All areas of Puerto Rico tested, except Parguera, had significant densities of all of the six species examined (Table I). The variability in densities was large in all species except *L. pneumophila*. This was undoubtedly due to the fact that six serogroups, or strains, were used for *L. pneumophila* examination while only one serogroup was used to examine samples for the other five species. In addition, the large variability also suggests great spatial heterogeniety, as reported before by Fliermans et al.[9] Some samples were negative for one or more species at a given time; however, repetitive samples at some sites revealed all of the species were present. The absence of *L. dumoffii* from the Parguera area is probably just a sampling error due to the small of samples and repetitions in this sampling area.

TABLE I

Density of *Legionella* spp. in Puerto Rico by Sites*

Species	Sampling Sites: Parguera	Boca Vieja	El Yunque	Ponce	San Juan
L. bozemani	1018 ± 1018	82 ± 52	108 ± 63	479 ± 123	3245 ± 1335
L. dumoffii	0	88 ± 88	251 ± 122	744 ± 452	6967 ± 3693
L. gormani	265 ± 265	1766 ± 1766	4953 ± 3132	1248 ± 573	7083 ± 4661
L. longbeachae	692 ± 507	1277 ± 735	117 ± 57	838 ± 253	6696 ± 2926
L. micdadei	4311 ± 2177	3338 ± 1732	575 ± 203	3077 ± 656	1007 ± 258
L. pneumophila (1-6)	2639 ± 552	2119 ± 326	3026 ± 209	8547 ± 411	31036 ± 4272

* Mean cell densities per ml ± one standard error.

TABLE II

Density of *Legionella pneumophila* serogroups in Puerto Rico by Site*

Serogroup	Sampling Sites: Parguera	Boca Vieja	El Yunque	Ponce	San Juan
1	1160 ± 421	349 ± 66	1579 ± 545	1447 ± 113	5051 ± 214
2	787 ± 263	330 ± 64	575 ± 349	956 ± 135	9587 ± 1179
3	0	734 ± 230	627 ± 347	531 ± 56	7206 ± 1120
4	0	265 ± 67	116 ± 68	3192 ± 243	4446 ± 319
5	218 ± 94	223 ± 53	127 ± 65	1659 ± 224	4516 ± 389
6	473 ± 128	217 ± 45	379 ± 209	804 ± 87	4347 ± 508

* Mean density per ml ± one standard error

All sites except Parguera had all six serotypes of *L. pneumophila* (Table II). The density of *L. gormanii* dominated the *Legionella* species in the Yunque area, comprising 54.9% of the total *Legionella* present. Since *L. gormanii* is a river isolate and the sites in El Yunque were along the Mameyes river, a relatively unpolluted watershed, *L. gormanii* would seem to be a normal floral constituent of unpolluted waters in Puerto Rico (Table I). Indeed, *L. gormanii* was high at all sites except Parguera. *Legionella micadadei* dominated Parguera and Boca Vieja, 48.3% and 38.5% respectively. Both of these areas are moderately polluted by sewage. *Legionella pneumophila* dominated the Ponce and San Juan sampling sites, comprising 57.2% of the *Legionella* present in Ponce sites and 55.4% of the *Legionella* in the San Juan samples. All sampling sites had at least 25% *L. pneumophila* present (Fig. 2). Thus in tropical environments *L. pneumophila* would seem to comprise a large proportion of the *Legionella* present.

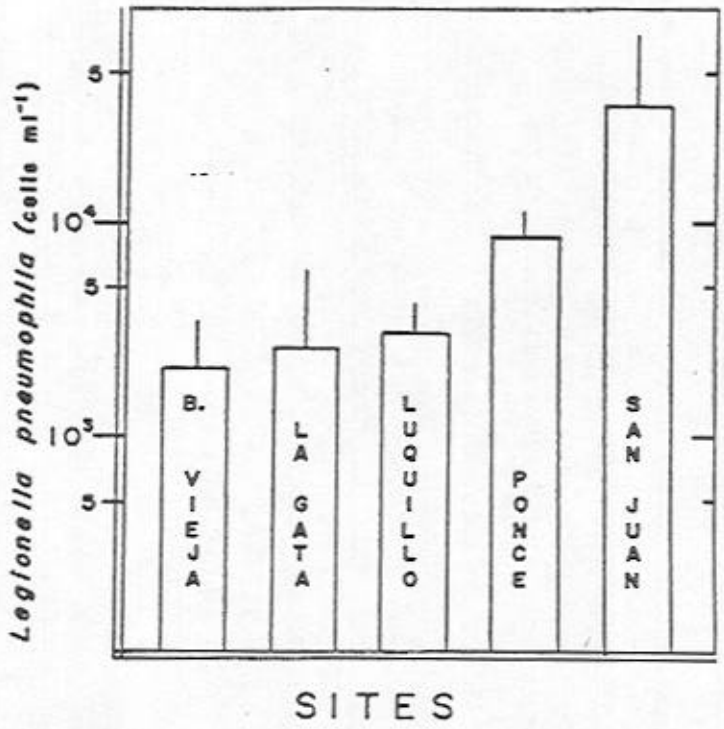

Figure 2. Densities of *Legionella pneumophila* by site (bar = 1 standard erro, Luquillo = El Yunque, La Gata = Parguera).

The relationship between pollution and increased density of *L. pneumophila* is further substantiated by correlation analysis with water quality (Table III). Sulfate and phosphate concentrations and pH of the water at the time of sampling were significantly positively correlated with density of *L. pneumophila*, while ammonia concentrations were significantly negatively correlated (Table III). However, other *Legionella* spp. did not show a similar pattern. Other investigators have shown that densities of *L. pneumophila* are significantly correlated with densities of cyanobacteria, blue-green algae.[25,28] Since we also know that cyanobacteria are inhibited by ammonia, stimulated by phosphates and when in high densities will cause the pH in poorly buffered waters to increase.[29] It seems likely that sewage and other pollutants are stimulating production of cyanobacteria which are in turn stimulating increases in *L. pneumophila*. This is further supported by the lack of significant correlation between densities of *L. pneumophila and densities of fecal coliforms.*

TABLE III

Correlation Matrix for Water Quality and Densities of *Legionella* in Puerto Rico*

	TEMP	DO	PH	TURB	SAL	NH_4	PO_4	TP	SO_4	CHLA	FC	TNLP	TL	TLP
TEMP	1.000													
DO	-.582	1.000												
PH	.457	-.079	1.000											
TURB	.404	.137	.294	1.000										
SAL	.758	-.558	.374	-.044	1.000									
NH_4	.360	.276	-.357	-.012	-.739	1.000								
PO_4	.622	-.489	.258	-.450	-.045	-.229	1.000							
TP	.340	-.346	.076	-.430	.418	.107	.372	1.000						
SO_4	.367	.172	.297	-.387	.434	-.249	.228	.346	1.000					
CHLA	.291	-.055	.066	-.147	.206	-.225	-.073	-.153	.387	1.000				
FC	.300	-.218	.155	-.062	-.053	-.100	.238	.166	.097	-.001	1.000			
TNLP	.310	-.281	.019	.023	.457	-.094	.097	-.019	-.226	-.093	.195	1.000		
TL	.164	-.0.83	.271	-.056	-.147	-.271	.118	-.206	-.004	-.128	.261	.866	1.000	
TLP	.176	-.036	.363	-.145	-.234	-.275	.223	-.129	.417	.016	.049	.451	.813	1.000

* All underlined values are significant for when $r > .200$ (here $P < .05$). TEMP = temperature, DO = dissolved oxygen, TURB = turbidity, SAL = salinity, NH_4^+ = ammonium, PO_4 = phosphate, TP = total phosphorus, SO_4 = sulfate, CHLA = chlorophyll, FC = fecal coliforms, TNLP = species of *Legionella* other than *L. pneumophila*, TL = all *Legionella* species, TLP = *L. pneumophila* alone.

The densities of *L. pneumophila* observed in the water of Puerto Rico are above or only slightly below the densities considered as potentially pathogenic to humans, ie. 10^5 - 10^6 cells ml^{-1}.[24] In addition, other species of *Legionella* of lesser pathogenicity are also present in most waters. We have also isolated and confirmed the pathogenicity of *L. pneumophila* environmental isolates using the guinea pig model. Since only four retrospective cases of Legionnaires' disease have been reported to date and none of these were fatal, then either a large number of Legionnaires' disease cases are going unreported in Puerto Rico or the pathogenicity of the strains present in Puerto Rico is quite low. It seems highly likely that many cases of legionellosis are going unreported in Puerto Rico considering that the CDC reports that legionellosis represents 10% of all atypical pneumonias in the United States.[1] The possibility for transmission in Puerto Rico is great considering that municipal potable water sources are reservoirs, that wave action along the coasts creates aerosols for considerable distances in-land and that hotels, industries and government office complexes use large evaporative cooling towers for air-conditioning the year-round. We are currently studying the pathogenicity of environmental isolates of *Legionella* and we hope by this paper to increase clinician's awareness of Legionnaires' disease possibility in Puerto Rico. Effective diagnosis of legionellosis is imperative for good patient prognosis, since only Erythromycin has been shown to be effective against the acute disease.

Resumen: La legionellosis es común entre las pulmonias atípicas en los Estados Unidos de América. Paradójicamente, los pacientes infectados de la misma fuente pueden padecer de una enfermedad parecida a la influenza llamada fiebre de Pontiac. En este artículo se resumen brevemente ambas enfermedades. Sólo se han informado cuatro casos de Legionellosis en Puerto Rico. Mediante el uso de inmunofluorescencia directa hemos demostrado la presencia de 6 especies de *Legionella* y de 6 serotipos de *Legionella pneumophila* en altas densidades en aguas puertorriqueñas. Queda demostrado por primera vez la presencia al *L. pneumophila* en playas tropicales. Las densidades encontradas de *L. pneumophila* se aproximan a las consideradas como potencialmente patogénicas para humanos y correlacionan positivamente con contaminación orgánica e inorgánica, siendo las de San Juan las más altas observadas en el país. La patogenicidad de las cepas puertorriqueñas de *L. pneumophila* se demostró utilizando el modelo de conejillos de India. Dado el alto potencial de exposición a este patógeno y la dificultad del diagnóstico, sospechamos que muchos casos de legionellosis en Puerto Rico pasan desapercibidos.

Acknowledgments

We are extremely grateful to Dr. Carl B. Fliermans for preliminary identifications and pathogenicity testing, cultures, supplies, technical assistance and training. We are also grateful for the generous technical assistance which we received from Arleen J. López-Torres, María Guerra, Carlos Aranda, Enid Elías and Laura Valdés-Collazo. Drs. William Bibb and Hazel Wilkinson from the Centers for Disease Control in Atlanta were very helpful. We thank the CDC for generously suppling antisera and control reagents.

References

1. *Meyer RD:* Legionella infections: a review of five years of research. Reviews of Infectious Disease 1983: 5(2): 258-278.
2. *Jones GL, Hebert GA:* Legionnaires': the disease, the bacterium and the methodology. Center for Disease Control, Atlanta, GA. 1979: p 173.
3. *Baine WR, Rasheed JK:* Aromatic substrate specificity of browning by cultures of Legionnaires' disease bacterium. Ann Inter Med 1979: 90(4): 619-620.
4. *Fraser DW, Tsai TR, Orenstein W, et al:* Legionnaires' disease. Description of an epidemic pneumonia. New Eng J Med 1977: 297(22): 1189-1197.
5. *McDade JE, Shepard DC, Fraser DW, et al:* Isolation of a bacterum and demonstration of its' role in other respiratory disease. New Eng J Med 1977: 297: 1197-1203.
6. *Lattimer GL, Thodes LV III, Salveti JS, et al:* The Philadelphia epidemic of Legionnaires' disease: clinical, pulmonary and serologic findings two years later. Ann Intern Med 1979: 90(4): 522-526.
7. *McDade JE, Brenner DJ, Bozeman M:* Legionnaries' disease bacterium isolated in 1947. Ann Intern Med 19[illegible] ; 90(4): 658-661.
8. *Tobin JC, Bartlett CLR, Watkins SA, et al:* Legionnaires' disease: further evidence to implicate water storage and distribution systems as sources. Br Med J 1981: 282: 573.
9. *Fliermans CB, Cherry WB, Orrison LJ et al:* Ecological distribution of *Legionella pneumophila*. Appl Environ Microbiol 1981: 41(1): 9-16.
10. *Wilkinson HW, Fikes BJ, Cruce DD:* Indirect immunofluorescence test for serodiagnosis of Legionnaires' disease: evidence for serogroup diversity of Legionnaires disease bacterial antigens and for multiple specificity of human antibodies. J Clin Microbiol 1979: 9(2): 379-383.
11. *Wilkinson HW, Cruce DD, Broome CV:* Validation of immunofluorescence assay with epidemic sera. J Clin Microbiol 1981: 13(1): 139-146.
12. *Broome CV, Cherry WB, Winn WC, MacPherson BR:* Rapid diagnosis of Legionnaires' disease by direct immunofluorescent staining. Ann Inter Med 1979: 90(1): 1-4.
13. *Cherry WB, Pittman B, Harris PP et al:* Detection of Legionnaires' disease bacteria by direct immunofluorescent staining. J Clin Microbiol 1978: 8(2): 329-338.
14. *Zimmerman SE, Morris French LV, Allen SD, Wilson L, Kohler RB:* Immunoglobulin M antibody titers in the diagnosis of Legionnaires' disease. J Clin Microbiol 1982: 16(6): 1007-1011.
15. *Horwitz MA, Silverstein SC:* The intracellular multiplication of Legionnaires' disease bacteria in human monocytes es reversibly inhibited by erythromycin and rifampin (abstract #301). Program and abstracts of the 21st Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, DC 1981.
16. *Meenhorst PL, van der Meer JWM, Borst J:* Sporadic cases of Legionnaires' disease in the Netherlands. Ann Inter Med 1979: 90(4): 529-532.
17. *Cherry WB, Gorman GW, Orrison LH, et al: Legionella jordanis* a new species of Legionella isolated from water ans sewage. J Clin Microbiol 1982: 15(2): 290-297.
18. *Orrison LH, Cherry WB, Tyndall RL, et al.: Legionella oakridgensis:* unusual new species isolated from cooling tower waters. Appl Environ Microbiol 1983: 45(2): 536-545.
19. *Miller RP:* Cooling towers and evaporative condensers. Ann Inter Med 1979: 90(4): 667-670.
20. *Wadosky RM, Yee RB, Mezmar L, Wing EJ, Dowling JN:* Hot water systems as sources of *Legionella pneumophila* in hospital and nonhospital plumbing fixtures. Appl Environ Microbiol 1982: 43(5): 1104-1110.
21. *Katz SM, Habib WA, Hammel JM, Nash P:* Lack of airborne spread of infection by *Legionella pneumophila* among guinea pigs. Infect Immun 1982: 38(2): 620-622.
22. *Fliermans CB:* More on legionella testing. American Society for Microbiology News 1982: 48(9): 396-397.
23. *Fliermans CB, Cherry WB, Orrison LJ, et al:* Isolation of *Legionella pneumophila* from non-epidemic related aquatic habitats. Appl Environ-Microbiol 1979: 37(6): 1239-1242.
24. *Fliermans CB, Soracco RJ, Pope DH:* Measure of *Legionella pneumophila* activity *in situ*. Curr Microbiol 1981: 6: 89-94.
25. *Tison DL, Pope DH, Cherry WB, Fliermans CB:* Growth of *Legionella pneumophila* in association with blue-green algae (cyanobacteria). Appl Environ Microbiol 1980: 39(2): 456-459.
26. Biamon EJ, Hazen TC: Survival and distribution of *Aeromonas hydrophila* in near-shore coastal waters of Puerto Rico receiving rum distillery effluent. Water Res 1983: 17(3): 319-326.
27. *APHA:* Standard Methods for the Examination of Water and Wastewater. 14th Edition. American Public Health Association. Washington, DC. 1975.
29. *Fogg GE:* Algal Cultures and Phytoplankton Ecology. University of Wisconsin Press, Madison, WI. 1965.