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

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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 symptomology 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 and Jones and Herbert. 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 30 other cases (5 fatal) were reported from people who had been near the hotel during the same period. Characteristically, pneumonia and acute diffuse alveolar damage were observed. This epidemic due to its 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. 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 rickettsiologically techniques combined with indirect immunofluorescence (IFA) resulted in the isolation of the etiological agent Legionella pneumophila, a previously unknown fastidious, gram-negative bacteria.

Subsequent examination of patient sera, collected during the epidemic, for antibodies to L. pneumophila demonstrated seroconversion, 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. 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.

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 an unrelenting 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, e.g., nosocomial infections. Malaise, lethargy, headache and profound weakness are demonstrated in more than 50% of all cases. Chills and rigor that are also observed in 75% of all patients during the first three days. More than 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 mortality rate runs as high as 30% contrasting with a 7% rate in the treated patients. Immunocompromised patients have mortality rates greater than 50%.

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).

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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. 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. 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 fourfold increase in titer to greater than 1:128 in sera from acute to convalescent phase as determined by indirect immunofluorescence. Validation of IFA with epidemic sera has a sensitivity of >78% and a specificity of 99% but does not consistently show a four-fold rise between acute and convalescent sera until at least three weeks after the onset of legionallosis. 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. DFA against sputum and pleural aspirate has a 50-60% sensitivity. With a high fatality rate 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. 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. Tetracycline gives variable results while penicillin, cephalirin, cefazolin, carbenicillin, aminoglycosides and chloramphenicol have little effect on the disease's course. In fact cases treated with ampicillin seem to have a higher fatality rate. 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. oaksridgensis, L. gormanii, L. jordanis, L. longbeachae, L. Botemani, L. wadsworthii, L. dumoffii and L. micdadei. Of the above, all except L. gormanii, L. jordanis and L. oaksridgensis 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. Legionella oaksridgensis has not been implicated in human pathogenicity but presents a pathogenic pattern very similar to the other Legionella species when injected into guinea pigs.

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.

The mode of transmission of this airborne pathogen has been traced to aerosols produced by evaporative condensers and cooling towers. 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. Recent studies have demonstrated that airborne spread of L. pneumophila from infected to healthy guinea pigs does not occur.

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, and they estimate that among the flu and pneumonia infections that Legionella is responsible for 250,000 cases each year in the USA.

Since its discovery, four cases of Legionnaires' disease have been retrospectively diagnosed in Puerto Rico by IFA (Dr. C.H. Ramirez-Romero, 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. 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. Isolation of L. pneumophila from guinea pigs was attained in 15% of the injected samples confirming the identity of the organism. Seven physicochemical parameters describing the habitats of the aforementioned isolates had the following ranges: temperature, 5.7 to 63°C; conductivity, 18 to 106 uhos; pH, 5.5 to 8.1; dissolved oxygen, 0.3 to 9.6 ppm; chlorophyll A, 0.7 to 24.2 mg/l; phosphate 0.2 to 18.8 mg/l; and water clarity, 1 to 4 m secchi disk readings. 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 10^1 - 10^8 viable cells per ml has been suggested as potentially pathogenic to humans.

With the exception of the apparent association of L. pneumophila with blue-green algae 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 its apparent thermophilic character, the fact that this bacterium has 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. Fierens (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 habitats 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 (Catano), San Juan Beach, Canas River (Ponce) and Mata de la Gata Island (Parguera), for detailed descriptions of sampling sites see Baymon and Hazen. A.J. Lopez-Torres, MS thesis, University of Puerto Rico, Rio 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, 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 Zeiss, Inc., New York, NY).

![Figure 1. Sampling sites in Puerto Rico](image)

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. 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 heterogeneity, as reported before by Fiermans et al. 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**

<table>
<thead>
<tr>
<th>Species</th>
<th>Parguera</th>
<th>Boca Vieja</th>
<th>El Yunque</th>
<th>Luquillo</th>
<th>Ponce</th>
<th>San Juan</th>
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</thead>
<tbody>
<tr>
<td>L. bozemanii</td>
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<tr>
<td>L. micdadei</td>
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*Means ± 1 standard error per site 1 for standard error.*

**Table II**

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<tr>
<th>Serogroup</th>
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<th>Boca Vieja</th>
<th>El Yunque</th>
<th>Luquillo</th>
<th>Ponce</th>
<th>San Juan</th>
</tr>
</thead>
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<td>349 ± 66</td>
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<tr>
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<tr>
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<td>116 ± 68</td>
<td>3192 ± 243</td>
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<td>5</td>
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<td>223 ± 33</td>
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<td>1659 ± 224</td>
<td>4514 ± 389</td>
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<td>6</td>
<td>473 ± 128</td>
<td>217 ± 45</td>
<td>379 ± 209</td>
<td>104 ± 87</td>
<td>4347 ± 308</td>
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</tr>
</tbody>
</table>

*Means ± 1 standard error per site 1 for 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 micdadei* 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.
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 IV). 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

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.26 It seems likely that sewage and other pollutants are stimulating production of cyanobacteria which 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.

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, i.e. $10^4 - 10^6$ 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. 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 pulmonías 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**

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References


LISTA DE ANUNCIANTES

- LA CRUZ AZUL DE PUERTO RICO
- ROCHE LABORATORIES
- Bactrim DS
- Valium
- Dalmane
- PFIZER LABORATORIES
- Procardia
- THE UPJHON COMPANY
- Motrin
- JAMES
- Seguros de responsabilidad profesional para médicos y cirujanos
- SEGUROS DE SERVICIOS DE SALUD
- Triple S
- BANCO DE PONCE
- U.S. ARMY