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Abundance and Distribution of *Legionellaceae* in Puerto Rican Waters

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Waters in marine and freshwater areas of Puerto Rico were analyzed for the presence of *Legionella* spp. by direct fluorescent antibody assay with guinea pig confirmation. Several species, including *L. bozemanii*, *L. dumoffii*, *L. gormanii*, *L. longbeachae*, *L. micdadei*, and *L. pneumophila*, were widely distributed among all sites. *Legionellaceae*, including *L. pneumophila*, were found in high densities in water collected in the rain forest from epiphytes in trees 30 ft. (about 9.25 m) above the ground. Both interspecific and intersite variations were significant. *L. pneumophila* was the most abundant species at all sites, with average densities of 10^4 cells ml^{-1}, very close to the range which is potentially pathogenic for humans. Densities of *L. pneumophila* were highest in sewage-contaminated coastal waters. These are the highest densities of *Legionella* spp. ever reported for marine habitats. Densities of *L. pneumophila* were positively correlated with concentrations of sulfates, phosphates, and pH. A survey of 88 fatal atypical pneumonia cases at a Puerto Rico hospital showed that 15% of the patients had *L. pneumophila* infections. This study establishes *L. pneumophila* as a relatively common cause of atypical pneumonia in Puerto Rico and suggests natural aquatic habitats as possible sources or reservoirs of pathogenic *Legionella* spp. in the tropics.

Since the isolation of *Legionella pneumophila* in Philadelphia, Pa., at the American Legion convention in 1976 (18), 11 serogroups of *L. pneumophila* and 22 other *Legionella* species have been described (25). Twelve of these species have been implicated in human infections (5, 6).

Most environmental studies on legionellae have concentrated on artificial aquatic habitats that are believed to function as amplifiers (hot-water tanks) or disseminators (e.g., aerosol-producing cooling towers, air conditioners, vaporizers, shower heads [23]) of *Legionellaceae*. Christensen et al. (8) showed that infectivity of *L. pneumophila* was positively correlated with conductivity, growth temperature, and dissolved inorganic carbon and consistently negatively correlated with dissolved oxygen in domestic- and industrial-use waters. In addition, various species of amoebae, ciliates, and cyanobacteria have been found to be associated with legionellae (2, 9, 21, 24, 26). However, only a few studies have ever examined natural nonepidemic waters for the presence of this genus (13, 20). Fliermans et al. (14) demonstrated legionellae in 792 of 793 samples examined in the southeastern United States, demonstrating their ubiquitous nature among natural aquatic environments.

The present study examined the distribution and abundance of *Legionellaceae* in natural aquatic habitats in Puerto Rico.

MATERIALS AND METHODS

Study sites. Twenty-six sampling sites (16 marine, 8 freshwater, and 2 estuarine) were chosen from five different sampling areas for their accessibility and diversity of water quality. Eight sites along San Juan beaches, which receive constant input from storm drains and illegal sewage discharge from the city of San Juan, were sampled (Fig. 1). This area constitutes the major hotel and tourist zone. The Cañas River (five sites) passes through the city of Ponce on the southern coast of Puerto Rico and receives both domestic sewage and effluent from an asbestos cement factory. Two sites were sampled from Mata de La Gata, a mangrove picnic island in the municipality of Lajas described elsewhere by A. López-Torres et al. (Microbiol. Ecol., in press). Mata de La Gata Island is administered by the Department of Natural Resources of the Commonwealth of Puerto Rico, and its waters receive sewage from two latrines. Six sites sampled in Boca Vieja Cove, detailed previously by Biamón and Hazen (3), receive 635,000 gallons (about 2.4×10^6 liters) of untreated effluent daily from the world's largest rum distillery. The Mameyes River watershed, from which five sites were sampled, including the pristine upper third near its origin at an elevation of 1,000 m in the Luquillo Experimental Forest, as well as sites downstream which receive domestic sewage, drains into the Atlantic Ocean (for details, see Carrillo et al. [7]).

Physicochemical parameters. The average physicochemical parameters of the five sampling areas are presented in Table 1. Measurements were taken in situ for dissolved oxygen and temperature with a model 57 oxygen meter

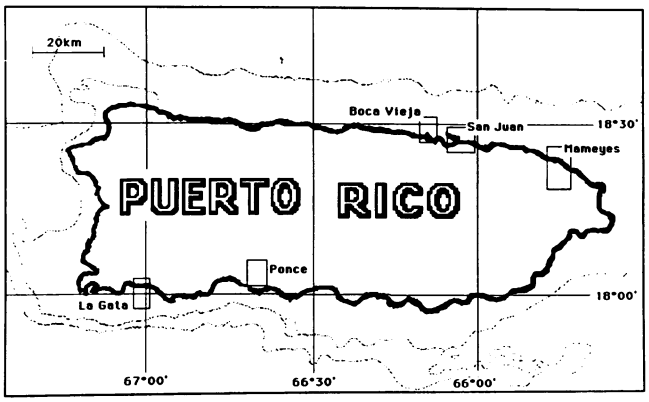

FIG. 1. Map of sampling areas.

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TABLE 1. Average values for physicochemical parameters and fecal coliforms by sampling area[a]

Site(s)	Air temp (°C)	Water temp (°C)	Dissolved O_2 (mg liter^{-1})	pH	Alkalinity (mg of $CaCO_3$ liter^{-1})	Conductivity (10^3 μS cm^{-1})	Hardness (mg of $CaCO_3$ liter^{-1})	Turbidity (% transmittance)
All	25.9 ± 0.3	25.7 ± 0.4	7.28 ± 0.27	7.43 ± 0.08	85.7 ± 7.9	78 ± 26	101.0 ± 16.6	91.4 ± 2.3
Boca Vieja	NA[b]	29.0 ± 1.6	5.21 ± 0.60	7.07 ± 0.93	NA	469 ± 38	NA	NA
La Gata	28.0 ± 1.6	28.8 ± 1.5	7.95 ± 0.29	7.89 ± 0.05	NA	50 ± 0	NA	9.96 ± 2.0
Mameyes	24.4 ± 2.8	23.4 ± 2.9	8.20 ± 2.77	6.93 ± 1.11	47.0 ± 43.0	1.88 ± 5.11	47.5 ± 22.9	95 ± 6.0
Ponce	28.6 ± 2.1	29.6 ± 2.0	5.26 ± 1.75	7.92 ± 0.33	174 ± 65	NA	269 ± 104	68 ± 35
San Juan	28.8 ± 2.8	28.1 ± 1.6	5.53 ± 1.41	7.92 ± 0.03	95 ± 33.6	12 ± 63	NA	98.7 ± 1.27

(Yellow Springs Instrument Co., Yellow Springs, Ohio). Conductivity and salinity were recorded with a model 33 salinity-conductivity-temperature meter (Yellow Springs Instrument Co.). Light intensity was measured with an underwater photometer (Protomatic, Dexter, Mich.), and pH was measured with a model 201 pH meter (Orion Research, Cambridge, Mass.). Immediate field analyses of turbidity, alkalinity, hardness, and ammonia were performed with kits for a Mini-Spectronic 20 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.). Sulfate, nitrate plus nitrite, chlorophyll *a*, phosphates, and total phosphorus were fixed in the field and analyzed in the laboratory as described in *Standard Methods for the Examination of Water and Wastewater* (1).

Bacteriological analysis. Grab samples were taken from surface waters, stored in 180 ml of presterilized Whirl-Pak bags (NASCO, Ft. Wilkinson, Wis.) and 10-liter sterilized Nalgene bottles, and transported to the laboratory on ice. All manipulations of material thought to contain legionellae were done inside a glove box with negative air pressure (Kewaunnee Scientific Engineering, Adrian, Mich.). Water samples for inoculation into guinea pigs were pressure concentrated (N_2) through a 0.45-μm-pore-size, 47-mm-diameter, HA-type membrane filter (Millipore Corp., Bedford, Mass.). Paired samples from the washed filters were injected intraperitoneally into guinea pigs. Moribund animals were sacrificed, and *L. pneumophila* recovery was confirmed by direct fluorescent antibody (DFA) staining of isolates obtained by culturing macerated spleen and liver tissue from moribund animals on CCVC medium at 37°C and 2.5% CO_2 for 48 to 96 h (4). Viable cell counts for presumptive *Legionella* densities in water were done by filtering samples through 0.45-μm-pore-size, 47-mm-diameter, HA-type membranes (Millipore). These membranes were placed on CCVC selective medium and incubated at 37°C and 2.5% CO_2 atmosphere for 48 to 96 h. Acid pretreatment was also used on selected samples (4). Colonies consistent with *L. pneumophila* morphology were examined by DFA assay.

For an index of human fecal contamination of the samples taken, fecal coliform densities were determined for every sample. Determination of fecal coliform densities was performed by membrane filtration of triplicate samples, plating on m-FC medium, and incubation at 44.5 ± 0.1°C for 24 h in a block-type incubator (1).

Direct cell counts were done by concentrating 10-liter samples at 5,000 × *g* for 15 min at 4°C. Concentrated samples were analyzed by the DFA assay with specific conjugates of *L. pneumophila* serotypes 1 to 6, *L. bozemanii*, *L. dumoffii*, *L. gormanii*, *L. longbeachae*, and *L. micdadei*. A negative control consisted of preimmune fluorescein isothiocyanate-labeled rabbit serum. Positive controls of the homologous antigens and specific conjugates were run with each batch processed. All sera and antigens were provided by the Centers for Disease Control, Atlanta, Ga. DFA-stained specimens were counted with the aid of a model 16+ IV FL vertical illuminator epifluorescence microscope (Carl Ziess Inc., New York, N.Y.).

Histological analyses were done on 88 patients who had died from undiagnosed pneumonia or acute respiratory distress syndrome at the Medical Center of Puerto Rico, University of Puerto Rico, during 1984. The study was done in a blind fashion (without examination of case histories) by examination of thin-sectioned lung tissue samples, numbered by patient, which had been fixed in Bouin solution and sliced in paraffin. The sections were mounted and stained with DFA against *Legionella* spp., the source of the DFA, and the technique was as described above.

Data analysis. The data were analyzed with prepared programs for Apple II and IBM 370-148 computers. Factorial analyses of variance were used to test for differences between sites and species. Multiple correlation and regression analyses were used to determine relationships among the parameters measured. Heteroscedastic data were made more homoscedastic by using the appropriate transformation before analysis. Any probability of less than or equal to 0.05 was considered significant (27).

RESULTS

L. pneumophila was found at all of the sites sampled. Samples from the Mameyes River were sent to Carl B. Fliermans (E. I. du Pont de Nemours & Co., Inc., Aiken, S.C.), who confirmed our DFA density estimates for these samples (10^4 to 10^5 cells ml^{-1}) and confirmed the viability and pathogenicity of the *L. pneumophila* present by recovering serogroup 1 organisms from moribund guinea pigs. The selective media used in this study did not give reliable results because of high background growth of both bacteria and yeasts. Several variations of the media were tried, but none gave satisfactory results. DFA counts were relied upon for the remainder of the study. Though it could be argued that the DFA assay may measure only dead cells or unrelated cross-reacting bacteria, we subsequently showed (T. C. Hazen, unpublished data) that all of the sites tested had viable and pathogenic *Legionella* spp. by the guinea pig assay.

L. pneumophila was found to be the most abundant of the *Legionellaceae* in Puerto Rican waters, with an average density of 4.7×10^4 cells ml^{-1} (Table 2; Fig. 2). *L. pneumophila* represented 48.5% of the *Legionellaceae* found. *L. pneumophila* densities were higher in the San Juan and Ponce areas. *L. gormanii* predominated in the Mameyes River, with an average density of 4.9×10^3 cells ml^{-1}, while *L. micdadei* was the most abundant *Legionella* species at Boca Vieja Cove (38.5%) and La Gata Island (48.3%). Except for La Gata, where *L. micdadei* had the highest

Salinity (mg ml^{-1})	NH_4 (mg liter^{-1})	Nitrites plus nitrates (mg liter^{-1})	P_i (mg liter^{-1})	Total P (mg liter^{-1})	SO_4 (mg liter^{-1})	Chlorophyll *a* (mg liter^{-1})	Fecal coliforms (CFU ml^{-1})
10.12 ± 2.51	2.84 ± 0.32	0.77 ± 0.10	9.46 ± 1.59	10.79 ± 1.58	31.13 ± 3.94	163 ± 73	50.6 ± 14.8
NA	NA	0.27 ± 0.25	14.1 ± 23.9	25.3 ± 23.5	NA	144 ± 102	10.2 ± 14.9
33.5 ± 0.70	6.13 ± 2.4	0.94 ± 0.89	3.79 ± 2.67	0.52 ± 1.00	NA	264 ± 383	0.76 ± 1.5
1.76 ± 4.49	4.55 ± 0.93	0.46 ± 0.59	4.09 ± 4.6	8.18 ± 16	35.6 ± 37.8	105 ± 238	37.1 ± 153
NA	3.00 ± 1.64	1.89 ± 1.74	18.2 ± 25.2	26.0 ± 17.0	40.7 ± 10.1	22.3 ± 17.0	148 ± 213
27.3 ± 2.1	0.36 ± 0.33	0.66 ± 0.71	7.45 ± 2.09	6.55 ± 9.23	NA	93.3 ± 103	103 ± 213

[a] All values are means ± 1 standard error.
[b] NA, Not available.

densities, San Juan had the highest densities of the five remaining species. Abundance and distribution did not always coincide, densities of *L. gormanii* were highest in San Juan, yet it was not the predominant *Legionella* species in that sampling area (Table 2; Fig. 2).

Significant differences in the average species densities ($F = 4.33$, df = 5 and 44, $P < 0.01$) and sampling sites ($F = 4.01$, df = 4 and 44, $P < 0.01$) were observed. Subsequently, each sampling area was independently analyzed by using the bacterial densities of each specific sampling site as the analysis of variance variables. In San Juan, densities of each species ($F = 11.42$, df = 5 and 15, $P < 0.0001$) and sampling sites ($F = 7.53$, df = 7 and 15, $P < 0.001$) were responsible for the highly significant variation observed. The Boca Vieja densities also varied in a highly significant manner as a function of species ($F = 11.42$, df = 5 and 25, $P < 0.0001$) and sampling site ($F = 7.53$, df = 5 and 25, $P < 0.0001$). For the sampling areas of Ponce, Mameyes, and La Gata, the distribution pattern of *Legionellaceae* could be significantly explained by interspecific variability ($F = 7.01$, df = 5 and 9, $P < 0.01$; $F = 10.15$, df = 5 and 9, $P < 0.005$; and $F = 5.97$, df = 5 and 15, $P < 0.005$, respectively) but not by intrasite variability within each sampling area. Thus, the sampling areas of San Juan and Boca Vieja Cove had significant intrasampling area variation, while Ponce could be considered homogeneous in terms of *Legionellaceae* distribution. The Mameyes River and La Gata were also found to constitute homogeneous sampling areas. Interspecific differences in distribution were found in all sampling areas and can themselves explain the significant variation in the densities of *Legionellaceae*.

L. pneumophila densities in San Juan were significantly different from those in the Ponce area (Student-Neuman-Keuls multiple range test, $P < 0.01$; Fig. 3). Ponce densities were higher than those at the Mameyes River (SNK test, $P < 0.01$), while the densities at the Mameyes River, La Gata, and Boca Vieja sites did not differ significantly from one another.

Serotype 2 was the most abundant (23.6%) of the *L. pneumophila* serotypes enumerated, but several serotypes seemed to be present in each sampling area (Table 3; Fig. 3). The San Juan area contained the highest densities of all six serotypes. A two-way analysis of variance was performed to determine any differences in the serotype distribution pattern as a function of serotype density or sampling area. A significant variation in the distribution pattern of serotypes was found to depend on the sampling area ($F = 6.47$, df = 4 and 44, $P < 0.001$) but not on intraserotypic variation per se. Once again, the sites in the San Juan area differed in a highly significant manner with respect to densities of *L. pneumophila* serotypes ($F = 10.80$, df = 7 and 15, $P < 0.0001$). When all of the sampling areas were combined, no serotype dominated overall.

The ubiquitous nature of *Legionella* spp. was further demonstrated in the rain forest when we found that water samples taken from between bracts of epiphytic plants (*Guzmania berteroniana*) growing 30 ft. (about 9.25 m) above the ground on trees had densities from 10^1 to 10^3 cells ml^{-1}. Fecal coliform densities in this same water ranged from 10^3 to 10^6 cells ml^{-1}.

Correlation of water quality and *Legionella* densities. The average measurements of 15 physicochemical parameters and fecal coliform densities for each sampling area are summarized in Table 1. Significant positive correlations were found between *L. pneumophila* densities and pH ($r = 0.313$, df = 44, $P < 0.02$), phosphates ($r = 0.322$, df = 44, $P < 0.05$), sulfates ($r = 0.417$, df = 44, $P < 0.005$), and total non-*L. pneumophila Legionella* spp. Salinity ($r = -0.234$, df = 44, $P < 0.05$) and ammonia ($r = -0.575$, df = 44, $P < 0.001$) were significantly negatively correlated with *L. pneumophila* densities, though salinity was barely significant because of large variation. No correlation was observed between fecal coliform densities and densities of *L. pneumophila* or between *L. pneumophila* and chlorophyll *a*. Non-*L. pneumophila Legionella* spp. correlated positively with *L. pneumophila* ($r = 0.866$, df = 44, $P < 0.0001$).

TABLE 2. *Legionella* species by sampling area

Species	Mean DFA density (cells ml^{-1}) (% for site) at:						% of total DFA density[a]
	La Gata	Boca Vieja	Mameyes	Ponce	San Juan	All sites	
L. bozemanii	1,018 (11.4)	82 (0.9)	108 (1.2)	479 (3.2)	3,245 (5.8)	4,932 (100)	5.05
L. dumoffii	0 (0)	88 (1.0)	251 (2.8)	744 (5.0)	6,967 (12.4)	8,050 (100)	8.25
L. gormanii	265 (2.9)	1,766 (20.4)	4,953 (54.9)	1,248 (8.4)	7,083 (12.6)	15,315 (100)	15.7
L. longbeachae	692 (7.7)	1,277 (14.7)	117 (1.3)	838 (5.6)	6,696 (11.9)	9,620 (100)	9.86
L. micdadei	4,311 (48.3)	3,338 (38.5)	575 (6.4)	3,077 (20.6)	1,007 (1.8)	12,308 (100)	12.6
L. pneumophila	2,639 (29.6)	2,119 (24.4)	3,026 (33.5)	8,547 (57.2)	31,036 (55.4)	47,367 (100)	48.5
All species	8,925 (100)	8,670 (100)	9,030 (100)	14,933 (100)	56,034 (100)	97,592 (100)	

[a] The percentages of the total DFA density produced by all of the *Legionella* spp. at La Gata, Boca Vieja, Mameyes, Ponce, and San Juan, respectively, were 9.14, 8.88, 9.25, 15.3, and 57.4%.

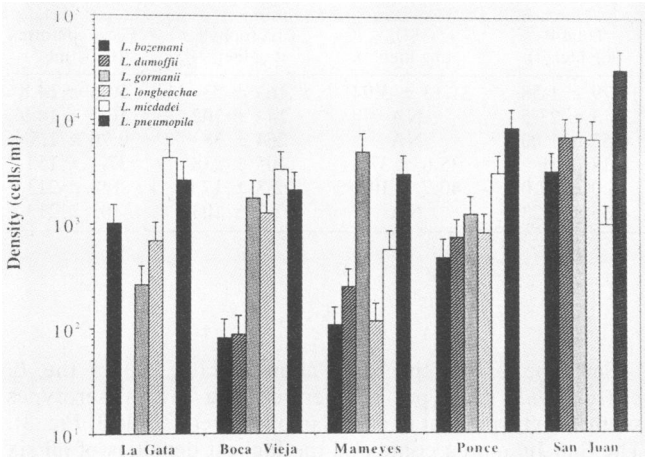

FIG. 2. Density of *Legionella* spp. by site (mean ± 1 standard error).

However, they differed radically in the physicochemical parameters with which they correlated: positively with water temperature ($r = 0.310$, df = 44, $P < 0.05$) and salinity ($r = 0.457$, df = 44, $P < 0.002$) and negatively with dissolved oxygen ($r = -0.301$, df = 44, $P < 0.05$) and sulfates ($r = -0.320$, df = 44, $P < 0.05$).

Pneumonia incidence. Of 88 patients with fatal undiagnosed pneumonia at the Medical Center of Puerto Rico during 1984, 13 (15%) were positive for cells staining with DFA against *L. pneumophila* polyvalent serotypes 1 to 4. After screening and scoring of all tissue specimens, the case history of every patient with a positive sample was examined. Every patient who was positive also showed a typical legionellosis case history and symptoms, i.e., starting with fever and a nonproductive cough, increasing lower lung congestion, and gradual deterioration of condition until death (19).

DISCUSSION

Abundance and distribution. *L. pneumophila* was ubiquitous in the five areas sampled, in densities fluctuating from 10^1 to 10^5 cells ml^{-1}. These densities are higher than those reported by Fliermans et al. (15) in natural aquatic habitats in the southeastern United States (10^1 to 10^3 cells ml^{-1}). The densities at some sites in Puerto Rico were within the range that is considered potentially pathogenic to humans: 10^5 to 10^6 cells ml^{-1} (12). Although the pathogenicity of isolates from the Mameyes River was established, the percentage of metabolically active or viable cells in our samples was not determined.

The highest *L. pneumophila* densities found were those at the San Juan sites (10^4 cells ml^{-1}). Second to San Juan in *L. pneumophila* density was Ponce, which differed from San Juan in that all Ponce sites were freshwater sites receiving heavy loads of domestic and industrial sewage, including the effluent of a cement factory. La Gata, the Mameyes River, and the sites in Boca Vieja Cove did not differ significantly in their *Legionella* counts. These sites make up a group of habitats that range from pristine freshwater (Mameyes River site 1) to heavily polluted marine habitats (sites 1 and 2 of Boca Vieja Cove). Thus, *L. pneumophila* was widely distributed in high densities in Puerto Rico and showed a marked predilection for the heavily populated cities of San Juan and Ponce. This is in accordance with the cosmopolitan distribution postulated by Fliermans (11), who believes that humans have created habitats that act as amplifiers and disseminators of *L. pneumophila*.

The fact that no *L. pneumophila* interserotypic distribution difference was observed in our study suggests that (i) DFA counts are indeed independent serotypes of the same species and/or (ii) the serotype (a surface antigen) plays no environmental adaptive role for *L. pneumophila* in Puerto Rican natural aquatic habitats. That is, all six serotypes seem to be distributed in a random fashion because this distribution is determined by *L. pneumophila* characteristics that are species specific but have little to do with the surface protein for which each serotype is recognized (antigen F1) (16).

Inter-*Legionellaceae* variability (in which both spatial and interspecific heterogeneities in distribution were taken into consideration) was highly significant. *L. pneumophila* predominated in Ponce and San Juan; *L. gormanii* predominated in the Mameyes River; and *L. micdadei* predominated at Boca Vieja and La Gata. This heterogeneity in species distribution suggests habitat adaptation (rather than random spatial distribution); perhaps *L. pneumophila* adapted to human contaminated areas, *L. gormanii* adapted to the more pristine river waters, and *L. micdadei* adapted to saline environments. These interpretations are limited because they rest solely on DFA counts without concomitant bacterial isolations.

Correlation of *Legionella* spp. and water quality. Little is known about the physiology of *L. pneumophila*, a fastidious, strictly aerobic, and heterotrophic bacterium which seems to be ubiquitous in natural and artificially created aquatic habitats. Fliermans (10) demonstrated that 90% of the cells isolated at temperatures of 25 to 60°C have an active electron transport system. With the FAINT technique, an environmental optimum temperature has been determined to be 65°C, whereas the medium optimum temperature is 45°C (13). In our study, the water temperature averaged 25.7 ± 0.4°C and no correlation was found between temperature and *L. pneumophila* densities, possibly because of the relatively small temperature range.

A positive correlation was found between *L. pneumophila* densities and phosphates. Phosphate concentrations in waters increase because of pollutants such as detergents and can cause algal growth and cyanobacterial proliferation. It has been observed that in vitro photosynthetic products

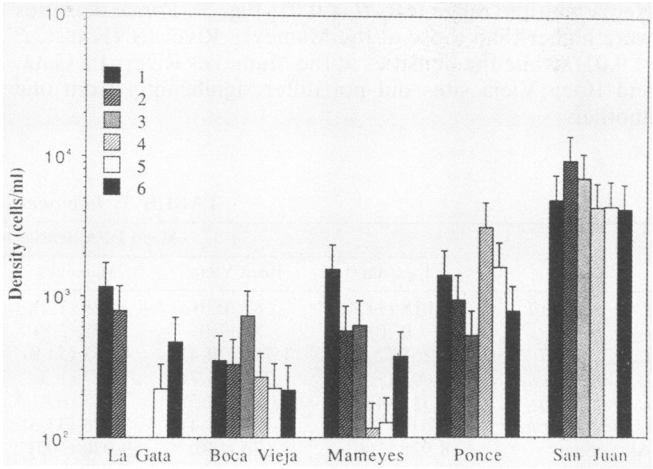

FIG. 3. Density of *L. pneumophila* serogroups by site (mean ± 1 standard error; numbers 1 to 6 represent serogroups).

TABLE 3. *L. pneumophila* serotypes by site

Serotype(s)	Mean DFA density (cells ml^{-1}) (% for site) at:						% of total DFA density[a]
	La Gata	Boca Vieja	Mameyes	Ponce	San Juan	All sites	
1	1,160 (43.9)	349 (16.5)	1,579 (46.4)	1,447 (16.8)	5,051 (14.4)	9,586 (100)	18.5
2	787 (29.8)	330 (15.6)	515 (16.9)	956 (11.1)	9,587 (27.3)	12,235 (100)	23.6
3	0 (0.0)	734 (34.6)	627 (18.4)	531 (6.18)	7,206 (20.5)	9,098 (100)	17.5
4	0 (0.0)	265 (12.5)	116 (3.4)	3,192 (37.1)	4,446 (12.6)	8,019 (100)	15.5
5	218 (8.26)	223 (10.5)	127 (3.73)	1,659 (19.3)	4,516 (12.8)	6,743 (100)	13.0
6	473 (17.9)	217 (10.2)	379 (11.1)	804 (9.35)	4,347 (12.4)	6,220 (100)	12.0
All	2,639 (100)	2,119 (100)	3,403 (100)	8,590 (100)	35,153 (100)	51,901 (100)	

[a] The percentages of the total DFA density produced by all of the *L. pneumophila* serotypes at La Gata, Boca Vieja, Mameyes, Ponce, and San Juan, respectively, were 5.08, 4.08, 6.56, 16.5, and 67.7%.

from cyanobacteria of the species *Fischerella*, *Phormidium*, and *Oscillatoria* enhance *L. pneumophila* growth (2, 24). Thus, phosphates might play an important role in *L. pneumophila* ecology by providing cyanobacteria with a substrate for photosynthetic products. Increases in cyanobacteria (which are inhibited by ammonia) can cause the pH of poorly buffered waters to increase, which could also explain the positive correlation between *L. pneumophila* density and pH.

Although the highest DFA counts were found in the San Juan area and *L. pneumophila* density was negatively correlated with salinity, this finding was probably spurious since a more significant positive correlation (−0.23 versus 0.46) was observed for total *Legionellaceae*. Fliermans (11) found that, in temperate areas of the United States, as salinity levels increased to saltwater concentrations, neither *Legionella* isolates nor samples positive for DFA could be obtained. Because of the strong positive correlation of total *Legionella* sp. density with salinity and the high densities of *Legionella* spp. at strictly marine sites (La Gata, Boca Vieja, and San Juan), if salinity has a deleterious effect on the *Legionellaceae* some other factor(s) must counter for it in the marine tropical aquatic habitats studied in Puerto Rico.

The presence of *L. pneumophila* in the water pooled by epiphytes along the Mameyes River was an interesting finding which implied rainfall, aerosols from the river, or both as a source of the bacterium. Turner et al. (25) reported *L. pneumophila* and *L. micdadei* in water collected from rainfall in southeastern Pennsylvania, indicating that rain may act as a disseminator of these organisms. Indeed, finding *Legionellaceae* 30 ft. above the ground at densities of 10^1 to 10^3 cells ml^{-1} suggests that this habitat could present a great potential source of natural infections since aerosols containing *Legionellaceae* could easily be created by winds in the rain forest.

L. pneumophila was found in Puerto Rico in densities several orders of magnitude higher than those in corresponding natural aquatic habitats in the United States (10^5 versus 10^3 cells ml^{-1}). This holds true for both polluted and pollution-free habitats. It is possible that the high concentrations of organic matter in the watersheds of wet tropics which, according to Lugo and Holdridge (17), act as carbon sinks through aquifers and rivers, along with the abundance of solar energy, provide *Legionella* spp. and other microorganisms with the substrates to reach higher densities, species variability, and an array of relationships yet to be discovered.

Considering the great potential for *L. pneumophila* infections in Puerto Rico, it was puzzling that only four cases of Legionnaires disease were informally admitted by local physicians (C. Ramirez-Rhonda, personal communication). Either the mechanisms of transmission, infectivity, and susceptibility were different in Puerto Rico than in other countries or many cases of Legionnaires disease were undiagnosed and unreported. The latter served as the working hypothesis for a recently completed research project aimed to determine the incidence of fatal Legionnaires disease in Puerto Rico (C. Ortiz-Roque, Abstr. 4th Congreso Estudiantil de Investigacion Cientifica 1986, N15, p. 9).

Of 88 patients with pneumonia at the Medical Center of Puerto Rico during 1984, 13 (15%) were positive for Legionnaires disease. This finding establishes *L. pneumophila* as a relatively common etiological agent of fatal pneumonias in Puerto Rico and stresses the importance of early diagnosis and treatment for Legionnaires disease in our tropical milieu.

Our results are in agreement with those of Schlech et al. (22), who described continued transmission of Legionnaires disease during 1981 and 1982 affecting 27 tourists visiting St. Thomas and St. Croix in the U.S. Virgin Islands, only 50 miles from Puerto Rico. Potable water was found to be the most probable source of *L. pneumophila*. In addition to *L. pneumophila*, *L. bozemanii*, and *L. longbeachae* in various water sources, new *Legionella* species were isolated (*L. santicrucis*, *L. cherrii*, and *L. steigerwaltii*). Thus, the abundance and species variability of the genus *Legionella* in the Caribbean was corroborated in the present study.

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