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Occurrence of *Legionella* Species in Tropical Rain Water Cisterns

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ABSTRACT.—**Direct fluorescent antibody staining of concentrated water samples from ten cisterns in the U.S. Virgin Islands demonstrated the presence of *Legionella pneumophila* serogroups 1-6, *Legionella micdadei* and *Legionella gormanii*. These potential pathogens were found in concentrations high enough to suggest that cistern water being used for drinking and bathing could be a source for *Legionella* disease in tropical areas.**

INTRODUCTION

The U.S. Virgin Islands are a group of small islands at 18°N, 65°W, with a tropical oceanic climate. Since ground water sources are either nonexistent or minimal, and there are no rivers or reservoirs, residents must rely upon desalination, reverse-osmosis treatment, and rain water cisterns for potable and domestic-use purposes. More than 75% of the 140,000 inhabitants of the Virgin Islands rely upon rain water cisterns. Rain water is collected from rooftop run-off and stored in concrete, steel, fiberglass, or plastic-lined boxes which may be either above or below ground. All cisterns are enclosed, creating a dark humid chamber with a temperature slightly less than ambient. Dogs, cats, birds, people, trees and their associated bacteria may contaminate both the catchment area and the cistern itself. These conditions are ideal for pathogen survival and since these water sources are used for human consumption without treatment, they could pose a significant health threat (Canoy and Knudsen, 1984).

Recently an outbreak of Legionnaires' disease involving 28 cases was reported from the U.S. Virgin Islands (Schlech et al., 1985). This outbreak was traced to a hotel whose bathing water tested positive for *Legionella*. Previous studies by our laboratory have shown that *Legionella* spp. are common in fresh and marine waters in Puerto Rico (Ortiz-Roque and Hazen, 1987). The present study was undertaken to ascertain if these organisms are present in rain water cisterns in the Virgin Islands.

MATERIALS AND METHODS

Water samples (20 L) were collected from 10 cisterns (designated A–J) in 20 L Nalgene bottles and fixed with 1 ml of 37% formaldehyde. The cisterns were chosen randomly during the normal course of water quality sampling at the Caribbean Research Institute. Samples were concentrated by centrifugation at 5000 × g for 15 min at 4°C in a Beckman J2-21 high-speed centrifuge to a final volume of 80 ml. Aliquots of 0.01 ml of the concentrated sample were transferred to 8 well toxoplasmosis slides, air-dried, and heat fixed. Each well was incubated with 0.01 ml of fluorescein-isothiocyanate (FITC) conjugated antibody to various *Legionella* strains, along with positive and negative controls in a humid chamber for 30 min at 37°C. After incubation the slides were washed with phosphate-buffered saline and blot-dried. The slide was covered with FA mounting fluid (Difco Laboratories, Detroit, Mich.) and examined with a 100× objective using an epi-

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TABLE 1. *Legionella pneumophila* densities in tropical cistern water.

Sero-group	Number of fluorescing cells/ml (by cistern)									
	A	B	C	D	E	F	G	H	I	J
1	632	210	237	0	738	105	0	0	ND*	ND
2	148	190	674	0	554	125	0	0	ND	ND
3	227	190	316	20	410	462	0	0	ND	ND
4	21	221	316	0	205	398	0	0	ND	ND
5	227	200	379	0	369	398	0	0	75	28
6	ND	ND	ND	ND	ND	ND	ND	ND	145	205

* ND—not done.

fluorescence microscope (American Optical, Buffalo, N.Y.). All typically fluorescing cells (as compared to positive controls) were counted and concentration on a per ml basis determined. All antisera and antigens were obtained from the Centers for Disease Control, Atlanta, Georgia. Details of techniques are as described before (Ortiz-Roque and Hazen, 1987).

RESULTS AND DISCUSSION

Direct fluorescent antibody staining revealed that all *L. pneumophila* serogroups tested were present (Table 1). In addition samples were positive for 2 of 5 other species of *Legionella* tested (Table 2). Two of the ten samples were negative for *Legionella* spp. Two samples (I and J) were tested for species other than *L. pneumophila*. They tested positive for *L. gormanii* and *L. micdadei* as well as *L. pneumophila* serogroups. Only 6 of the 13 described serotypes of *L. pneumophila*, and only 6 of the 12 species of *Legionella* were used in this study, due to DFA availability at the time of the study. Thus the estimated densities of *Legionella* spp. and the estimated densities of all serogroups of *L. pneumophila* in the cisterns is minimal. The actual densities of both groups is probably much higher.

TABLE 2. *Legionella* spp. densities in tropical cistern water.

Cistern	*Legionella* species (fluorescent cells/ml)					
	L. pneumophila polyvalent (1–4)	*L. bozemanii*	*L. dumoffii*	*L. gormanii*	*L. longbeachae* (group 1)	*L. micdadei* (group 1)
I	118	0	0	0	0	322
J	322	0	0	8	0	128

The densities for *L. pneumophila* in the waters of Puerto Rico range from undetectable to more than 10,000 cells/ml (Ortiz-Roque and Hazen, 1987). The average density for *L. pneumophila* in the cisterns was 266 ± 36 fluorescing cells per ml. These densities are well below the densities considered to be potentially pathogenic to humans, i.e. 10^5–10^6 cells per ml (Fliermans et al., 1979, 1981). However, considering that far fewer cells may constitute an infectious dose when aerosolized by shower heads (Meyer, 1983), the densities found in the present study could be a potential source of infection.

It seems likely that many cases of legionellosis are going unreported in the Virgin Islands and Puerto Rico, considering that the Centers for Disease Control (CDC) report that legionellosis represents 4% of all cases of atypical pneumonia in the United States (Meyer, 1983). Ortiz-Roque and Hazen (1987) recently reported that an autopsy analysis of fatal pneumonia cases in Puerto Rico indicates that more than 50 cases of legionellosis occur each year in Puerto Rico with a 25% fatality rate, yet only four retrospective cases have ever been reported in Puerto Rico. The possibility for transmission in the tropics is great considering that municipal and domestic water sources are potential reservoirs, and that hotels, industries and government office complexes use large evaporative cooling towers for air-conditioning year round.

LITERATURE CITED

Canoy, M. J., and A. N. Knudsen. 1984. Microbial water quality of cistern water, St. Thomas, USVI Water Resources Report (CRI).

Fliermans, C. B., W. B. Cherry, L. H. Orrison, and L. Thacker. 1979. Isolation of *Legionella pneumophila* from non-epidemic related aquatic habitats. Appl. Environ. Microbiol. 37:1239–1242.

———, ———, ———, S. J. Smith, D. L. Tison, and D. H. Pope. 1981. Ecological distribution of *Legionella pneumophila*. Appl. Environ. Microbiol. 41: 9–16.

Meyer, R. D. 1983. *Legionella* infections: a review of five years of research. Rev. Infect. Dis. 5:258–278.

Ortiz-Roque, C., and T. C. Hazen. 1987. Abundance and distribution of Legionellaceae in Puerto Rican waters. Appl. Environ. Microbiol. 53:2231–2236.

Schlech III, W. F., G. W. Gorman, M. C. Payne, and C. V. Broome. 1985. Legionnaires' disease in the Caribbean. Arch. Intern. Med. 145:2076–2079.

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