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Determinants of *Legionella pneumophila* Contamination of Water Distribution Systems: 15-Hospital Prospective Study

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ABSTRACT

We conducted a prospective environmental study for *Legionella pneumophila* in 15 hospitals in Pennsylvania. Hot water tanks, cold water sites, faucets, and showerheads were surveyed four times over a one-year period. Sixty percent (9/15) of hospitals surveyed were contaminated with *L pneumophila*. Although contamination could not be linked to a specific municipal water supplier, most of the contaminated supplies came from rivers. Parameters found to be significantly associated with contamination included elevated hot water temperature, vertical configuration of the hot water tank, older tanks, and elevated calcium and magnesium concentrations of the water ($P < 0.05$). This study suggests that *L pneumophila* contamination could be predicted based on design of the distribution system, as well as physicochemical characteristics of the water. [Infect Control 1987; 8(9):357-363.]

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INTRODUCTION

Legionnaires' disease is now recognized as a major nosocomial problem.[1-3] Its presence has been linked to the degree of *L pneumophila* contamination within the hospital water distribution system. We have found *Legionella* contamination of the water supply linked to the presence of Legionnaires' disease within these same hospitals.[4-8] Currently, notable gaps exist in our knowledge of the prevalence of *L pneumophila* contamination of water distribution systems and those environmental factors that predispose to such contamination. Specifically, is the source of incoming water a predisposing factor for *L pneumophila* contamination? Are there physicochemical characteristics of water that might predispose to contamination of water distribution systems by *L pneumophila*? And, do certain types of plumbing and water distribution systems have a predilection for *L pneumophila* colonization?

We, therefore, conducted a 15-hospital prospective study over one year in order to determine the extent of *Legionella* contamination in these hospitals and to elucidate those factors that might predict contamination of these water distribution systems.

METHODS

Hospitals

The 15 study hospitals were all members of the Hospital Council of Western Pennsylvania, an association of hospitals and health care facilities in western Pennsylvania. The Council is a nonprofit, voluntary organization offering programs in administration, professional services, and education for member hospitals. One of the notable programs is the group purchasing program in which hospital goods are purchased collectively at a cost savings. The 15 hospitals enrolled in the study had volunteered their participation in response to a solicitation sent to 45 hospitals by the Hospital Council. None of the hospitals enrolled were known to have cases of legionellosis.

The 15 hospitals were geographically located as follows:

TABLE 1
SURVEY OF WATER DISTRIBUTION SYSTEMS FOR 15 HOSPITALS FOR *L PNEUMOPHILA* CONTAMINATION

Hospital No.	% HWT Samples *Lp*	% Distal Sites *Lp*	Serogroups Represented	Age* Years	HWT (Horizontal [H] Vertical [V])	Water Source
51	0	0	NA	2	H	River
81	0	0	NA	4	H	Lake
63	0	0	NA	4	H	River
31	0	0	NA	4	H	Well
18	0	0	NA	17	H,V	Lake
58	0	0	NA	19	H	Lake
99	0	10	4	15	H	River
38	17	5	1,4	34	H,V	River
78	17	17	4,5	29	H	River
94	41	0	3,6	25	H,V	River
56	50	10	1,3	23	H	River
33	36	38	1	25	H,V	River
41	71	5	1,4,6	15	H,V	Well
90	77	95	1,2,5,6	5	V	River
96	83	25	1	18	V	River

NA = Not applicable.
HWT = Hot water tank.
Lp = L pneumophila.
* *If multiple hot water tanks in one hospital, mean age is given.*

three hospitals in the immediate Pittsburgh area; eight hospitals within a radius of 10 to 30 miles; and four hospitals within a radius of 50 to 80 miles. The bed capacities of the hospitals ranged from 150 to 800, with a mean of 400. In most instances, these hospitals served both metropolitan and urban populations and have teaching programs. The Pittsburgh hospitals are serviced by the City of Pittsburgh Water Authority whose source is the Allegheny River. Of the 12 hospitals outside the city (10- to 80-mile radius), 8 are serviced by independent water authorities and 4 are supplied by the Western Pennsylvania Water Company using the Monongahela River. Also, two of the independent water authorities use the Youghiogheny River as a source of water.

Water Distribution Systems

Of the 15 hospitals surveyed, 7 (47%) had a preventive maintenance program that consisted of cleaning or flushing the tanks on a weekly to annual basis. Two of fifteen (13%) had periodic checks for leaks and 6 of 15 (40%) had no preventive maintenance program.

Two designs were observed for hot water recirculation: total system volume recirculation (15/15, 100%) and recirculation within individual tanks (7/15, 47%). Recirculation of water throughout the system is accomplished by low volume pumps. The circulation is slow and functions to keep the water warm throughout the system such that the system can deliver hot water on demand to distal sites. Recirculation of hot water within a tank minimizes scale/sediment accumulation. The water is usually mixed by a pipe that leaves the top of the tank, loops outside, and reenters at the bottom.

The capacity of individual hot water tanks ranged from 264 to 6016 gallons (mean = 1500 gallons). The configuration of the tanks is given in Table 1. Tanks were designated as "vertical" if the vertical dimension (height) exceeded the horizontal dimension (width), while tanks were designated as "horizontal" if the horizontal dimension exceeded the vertical dimension.

Specimen Collection and Processing for *Legionella*

Hot water tank specimens were collected from the 15 hospitals in four sampling periods (October 1982, January 1983, April 1983, October 1983) over one year by the same team of investigators. Water was generally collected from tank bottoms in screw-cap bottles in the amount of 20 to 500 mL. Water temperatures (both set-point and actual tank sample) were recorded at time of sampling. Ten distal site (faucet or showerhead) samples from each hospital were collected on swabs in two of the four sampling periods. The same sites and tanks were sampled throughout the study period. One cold water site, usually the cold water tank, was also sampled from each hospital in the first sampling. Water and swabs were plated directly onto selective media containing dyes, glycine, and antibiotics and processed as previously described.[4,5] (This medium is commercially available from Gibco, Madison, WI, and Remel, Lenexa, KS.) Ten to forty milliliters of direct culture-negative water samples were concentrated by centrifugation at 1000 g for 20 minutes, the sediment resuspended at 1.0 mL volume, and then recultured using 0.1 mL per plate. A modified acid-wash treatment was used on water samples overgrown with non-*Legionella* organisms.[9] Ten milliliters of water was centrifuged at 1000 g for 20 minutes, the sediment resuspended at 1.0 mL, 2.0 mL acid-buffer added for 3 minutes, and 0.1 mL of the mixture plated onto buffered charcoal yeast extract and selective media.

Metal Ion Analysis

Hot water tank samples were analyzed for metallic ions. Concentrations of calcium,[10] magnesium,[10] zinc,[11] iron,[12] and lead[13] were determined by atomic absorption spectroscopy. Samples were stored at 2° to 6°C until tested. Analyses were performed on coded samples without knowledge of culture results.

Suspended Solids Analysis

As previously published,[14] hot water tank samples were analyzed for nonfilterable solids. One hundred milliliters of the suspended water sample was passed through a 0.45 micron pore-sized filter in a Gooch crucible. The crucible was placed in a drying oven at 100°C for one hour and then cooled in a dessicator. The solid analysis was calculated from the weight of the nonfilterable residue.

STATISTICAL ANALYSIS

The study was analyzed in two ways. The first analysis was directed at individual hot water tanks (N = 47, two instantaneous heating systems excluded). It was assumed that hot water tanks constitute a unique environment in which parameters specific to an individual tank may be operational in the determination of *L pneumophila* positivity. Outcome measures were presence and quantity of *L pneumophila* isolated for each individual hot water tank. Observations for each tank included chemical content of the water within the tank, temperature of the tank water, age, capacity, and configuration of each tank.

The second analysis was directed at the individual hospital (N = 15). This analysis was based on the assumption that there would be ecological parallels or similarities within each hospital that would be operative for *all* tanks within the same hospital. Outcome measures were presence of *L pneumophila* within the water distribution system at any time. Observations for each hospital were those parameters that would be constant for all tanks within that hospital, including source of incoming water, thermostat set-point, presence of a maintenance program, and geographic location in the state.

All parameters were stored in a data bank housed on the Prophet System (Division of Research Resources, National Institutes of Health). To assess association between outcome measures and individual observations, the Fisher's exact test was used. The Mann-Whitney rank sum test was applied in those instances where normality assumptions could not be met.

Multivariate Analysis

To examine the association of *Legionella* positivity and other factors in more detail, the following strategy was used: those factors found significantly associated with *Legionella* positivity using two-way contingency table analysis were all entered as a group into a stepwise logistic regression model in which *Legionella* positivity was the dependent binary variable (BMDP, University of California). The analysis entailed adding variables singly to the model, comparing the best model with the additional variable to the existing model, and retaining the new model if it provided a significantly better description of the data than the preceding model. The probabilities to enter and remove variables were set respectively at 0.10 and 0.15. To further explore the relationship of *Legionella* positivity and the variables selected by the logistic regression, the methods of generalized linear models were used.[15] Both hierarchical and nonhierarchical models were considered. The values for sensitivity, specificity, and predictive value were computed as previously described.[16]

TABLE 2
WATER TANK CONFIGURATION SIGNIFICANTLY ASSOCIATED WITH *L PNEUMOPHILA* CONTAMINATION

Configuration	*Lp* Present	*Lp* Absent	Fisher's Exact Test, *P* Value
Horizontal	8	20	
Vertical	15	4	< 0.05

Data shown is for 47 hot water tanks in 15 hospitals (2 instantaneous steam heating systems not included).
Lp = L pneumophila.

RESULTS

Environmental Survey for *L pneumophila*

Of the 15 hospitals sampled, 9 of 15 (60%) yielded *L pneumophila* during the one-year sampling period (Table 1). Of the 9 positive hospitals, the percent of hot water tanks in a given hospital yielding *L pneumophila* over the four sampling periods ranged from 17% (1/6) to 100% (4/4). Forty-nine percent (23/47, two instantaneous systems excluded) of all hot water tanks yielded *L pneumophila* with 91% (21/23) positive on more than one sampling. Hot water tanks from one hospital failed to yield any *L pneumophila* over the one-year period, but 10% of distal sites including faucets and showerheads yielded the organism. *L pneumophila* was isolated from cold water sites of only two hospitals (#38 and #41 in Table 1).

Serogroup 1 was seen in 67% (6/9) of the positive hospitals, but all serogroups tested (1 through 6) were found in at least one hospital (Table 1). Multiple serogroups were isolated from four tanks in three hospitals.

The concentration of *L pneumophila* recovered from positive samples ranged from 10 to 3000 colony-forming units (CFU) per milliliter. No significant association could be established for concentration of *L pneumophila* versus type of water distribution system and physicochemical characteristics of water.

Water Source

The 15 hospitals received their water via ten different water companies and no single source could be implicated as significantly more contaminated. Table 1 shows that most of the water suppliers of contaminated hospitals received their water from rivers—the Youghiogheny, Monongahela, and Allegheny ($P < 0.09$, Fisher's exact test).

Plumbing System Characteristics

Configuration of the hot water tanks was found predictive of *L pneumophila* contamination (Table 2). Vertical tanks were significantly more likely to be contaminated than horizontal tanks ($P < 0.05$, Fisher's exact test). No association was found for tanks with recirculation versus those without for the presence of *Legionella* contamination. Two hospitals used an instantaneous steam heating system (Figure) in addition to conventional hot water tanks, and no evidence of *L pneumophila* contamination was found in these hospitals.

Table 3 shows the thermostat set-point temperature of the hot water tanks was significantly associated with the presence of *L pneumophila*; tanks with set-point temperatures exceeding 60°C were more likely to be free of *Legionella*. We also observed that thermostat set-point only roughly correlated with the actual temperature of the water when sampled. More detailed analysis of 102 samplings taken from 47 tank samples in which the *actual* temperature was entered for statistical analysis (as opposed to thermostat set-point) confirmed that higher temperatures were significantly associated with the negative cultures ($P < 0.05$, Fisher's exact test, data not shown).

The age of the individual tank was significantly associated with the presence of *L pneumophila* (Table 4). Newer tanks were more likely to be free of *L pneumophila* contamination ($P < 0.05$, Mann-Whitney rank sum test). Tanks less than five years old were generally free of *L pneumophila* ($P < 0.05$, Fisher's exact test).

Application of a periodic preventive maintenance (as described in the Methods section) by the hospital had no apparent effect on the presence of *L pneumophila* in the water distribution system. We also point out that appearance, cleanliness, and overall upkeep of the system was not associated with the presence or absence of *L pneumophila* contamination.

Water Quality

Higher concentrations of calcium and magnesium in tank water were significantly associated with *L pneumophila* positivity of that water ($P < 0.05$, Mann-Whitney rank sum test). No association was found for copper, zinc, iron, and suspended solid concentrations (Table 4).

Multivariate Analysis

Those factors found to have significant association with *Legionella* positivity ($P < 0.05$) when each was considered separately through the use of two-way contigency table methods were as mentioned above: age of tank, tank configuration, tank water temperature, calcium concentration, magnesium concentration, and source of the water. When these factors were used in the logistic regression model, the factors that remained significantly associated with positivity were source of water and calcium concentration. The water tanks are classified accordingly in Table 5-A. The results of further investigation using hierarchical and nonhierarchical models in the generalized linear models program and using Table 5-A as input indicated that the best model was the one that included only the interaction terms of water source and calcium concentration. The statistic from generalized lin-

TABLE 3
WATER TANK TEMPERATURE SIGNIFICANTLY ASSOCIATED WITH *L PNEUMOPHILA* CONTAMINATION OF HOT WATER TANKS

Thermostat Set-Point	*Lp* Present	*Lp* Absent	Fisher's Exact Test, *P* Value
< 60°C (140°F)	9	3	< 0.05
≥ 60°C (140°F)	0	3	

Data shown is for L pneumophila *positivity in 15 hospitals. Mann-Whitney rank analysis for 47 individual hot water tanks is also significant,* P *< 0.05.*

Fisher's exact test analysis for actual recorded temperature *of 102 water tank samples is also significant,* P *< 0.05.*

Lp = L pneumophila.

TABLE 4
FACTORS ASSOCIATED WITH PRESENCE OF *L PNEUMOPHILA* IN 47 HOT WATER TANKS IN 15 HOSPITALS

	Median (range) Values in Hot Water Tanks				
	Lp Present		*Lp* Absent		Mann-Whitney *P* Value Rank Sum
Age	16 years	(5-37)	11 years	(1-42)	< 0.05
Calcium	30 mg/L	(3-48)	21 mg/L	(1-31)	< 0.05
Magnesium	10.2 mg/L	(1.1-20.4)	5.5 mg/L	(1.1-288)	< 0.05
Copper	1.0 mg/L	(0.07-20.2)	0.85 mg/L	(0.11-345)	NS
Zinc	2.85 mg/L	(0.11-18.6)	.49 mg/L	(0.08-15.2)	NS
Iron	0.16 mg/L	(0.09-25.9)	0.22 mg/L	(0.13-8.68)	NS
Suspended solids	206 mg/L	(13-761)	102 mg/L	(51-300)	NS
Capacity	850 gallons	(480-6020)	846 gallons	(110-3450)	NS

Lp = L pneumophila.
NS = Not significant, P *> 0.05.*

ear models program for testing the interaction model was the lack of fit chi-square = 3.404 with 2 degrees of freedom, $P = 0.182$, indicating no lack of fit. Of the four combinations of water source and calcium concentration, the combination of river source of water and high calcium concentration differed from the other three combinations, which did not differ from each other. These latter three were collapsed into a single class and used as a reference against which to compare the observations from the river source of water and high calcium concentration in order to obtain the odds ratio given in Table 5-B. The combination of river source and high calcium concentration also gave the highest sensitivity and predictive value when compared with individual parameters, which were significant via univariate analysis (Table 6).

DISCUSSION

This survey is the most comprehensive and detailed for *L pneumophila* contamination of hospital water distribution systems yet reported. Because *L pneumophila* contamination can be seasonal, culturing at only one or two points in time will not provide an accurate measure of contamination. This study examined hot water tanks and distal sites (showerheads and faucets) in 15 hospitals four times over the one-year study period. Thus, not only was the major source of the organism being cultured (hot water tanks), but the sites relevant to the individual patient were also surveyed. The high frequency of culturing provided an index of consistency. The same investigators were involved in obtaining samples from each hospital and the collection methods were standardized. State-of-the-art culture methodology was employed, including the use of selective dye-containing media (superior and more efficient than guinea pig inoculation),[17-19] large volume centrifugation, and acid treatment for specimens contaminated with resistant water bacteria.[9] As a result, we were able to obtain a detailed overview of water contamination.

We found that a surprisingly high percentage (60%) of the 15 hospitals surveyed were contaminated with *L pneumophila* with most of the hospitals showing consistent contamination throughout the study. This same culture protocol had previously revealed environmental contamination in four other Pittsburgh hospitals.[5-8] In three of these hospitals, nosocomial legionellosis had never been documented prior to environmental culturing. When specialized laboratory tests for *Legionella* were subsequently introduced into these hospitals, these three hospitals were discovered to have a significant incidence of nosocomial legionellosis.[6-8] Although no attempt was made to link water contamination to disease in the 15 hospitals in this study, these findings have obvious implications for the detection of occult nosocomial legionellosis, given our previous experience. Because the percentage of contaminated hospitals was fairly high in this study, we wonder if surveys elsewhere might show similar frequencies. If so, the possibility arises that underdiagnosed nosocomial legionellosis may become more apparent as clinician awareness increases and as specialized laboratory testing for *Legionella* becomes more readily available.

TABLE 5
A. DISTRIBUTION OF OBSERVATIONS BY WATER SOURCE, CALCIUM CONCENTRATION AND *LEGIONELLA* POSITIVITY FOR INDIVIDUAL HOT WATER TANKS*

	Calcium	*Lp* Present	*Lp* Absent
Nonriver source	High	0	4
	Low	2	5
River source	High	18	7
	Low	0	4

B. COLLAPSED FORM OF PART A (above)

	Lp Present	*Lp* Absent
River source plus high calcium	18	7
Other conditions	2	13

Odds Ratio = 16.71.
95% Confidence Interval (2.97, 93.78).

**N = 40; in 7 tanks, calcium concentrations were not available.*

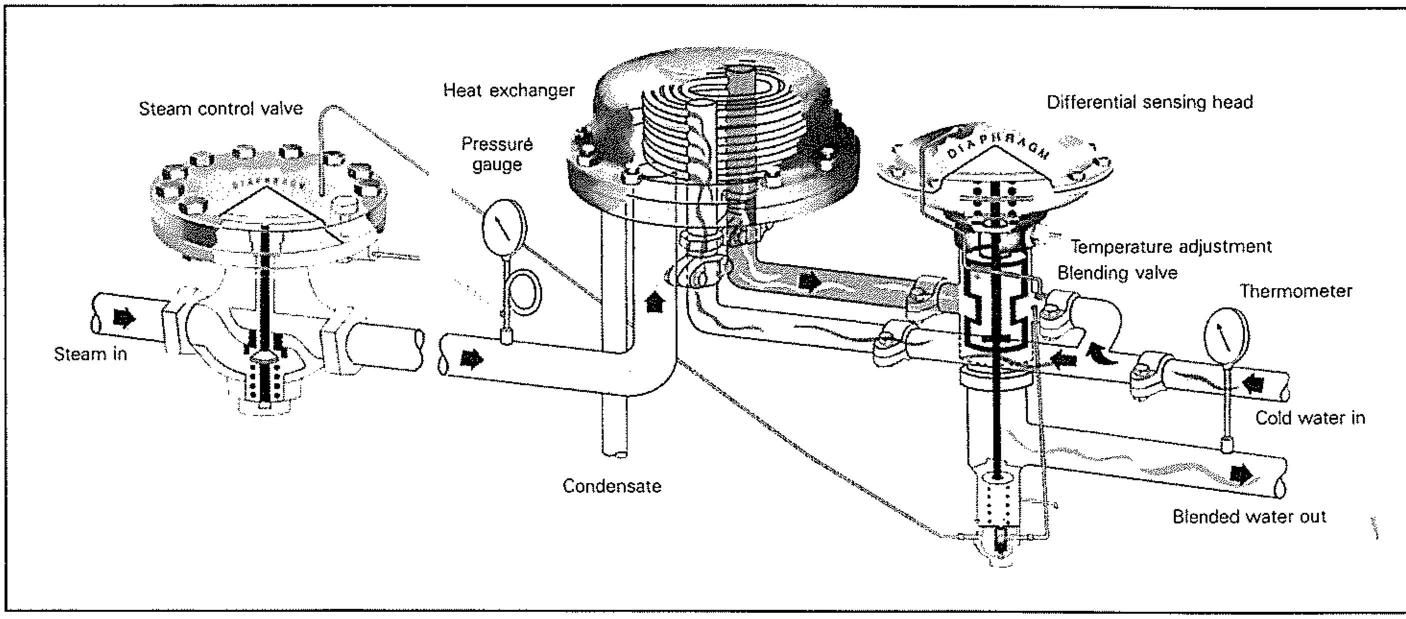
High calcium = calcium ≥ 15 mg/L.
Low calcium = < 15 mg/L.

Lp = L pneumophila.

TABLE 6
SENSITIVITY, SPECIFICITY, AND PREDICTIVE VALUE FOR HOT WATER TANK PARAMETERS APPLIED TO 47 HOT WATER TANKS IN 15 HOSPITALS

	Sensitivity	Specificity	+ Predictive Value*	− Predictive Value†
River source + calcium	90	65	72	87
Calcium > 15 mg/L	90	45	65	82
River source	87	31	56	77
Temperature > 60°C	65	83	53	73
Vertical configuration	65	83	79	71

**Predictive value of a positive result.*
†Predictive value of a negative result.



Schematic of an instantaneous steam heating system. This system has no hot water tank (the breeding ground for *L pneumophila*) and heats water under high steam pressure to 88°C (which is bactericidal for *L pneumophila*).

The design of the water distribution system appears important in predisposing to *L pneumophila* colonization. For example, water tanks whose vertical dimension exceeded their horizontal dimension were significantly associated with *L pneumophila* colonization. The reason for this condition remains to be determined, but vertical water tanks have more diverse strata of heating within the tank and sediment accumulation at the bottom of the tank may be thicker.

The temperature of the hot water tanks was a critical factor for *L pneumophila* contamination. The thermostat set-point of the hot water tanks correlated with the presence of *L pneumophila*; lower temperatures were significantly associated with *L pneumophila* contamination. We caution that the set-point temperature may be an imprecise indicator of the temperature at the bottom of the tank (the site of maximal organism accumulation); the actual temperature was usually lower. When the *actual* temperature was used as the dependent variable in a sample of 102 water tank samples, statistical analysis confirmed that water samples at a higher temperature range were significantly less likely to be contaminated by *L pneumophila*. The optimal temperature for survival and propagation of *L pneumophila* in tap water ranges from 32° to 42°C; higher temperatures tend to be inhibitory for *L pneumophila*.[20-23]

Older tanks were significantly associated with contamination by *L pneumophila* (Table 4). The reason for this association is uncertain, but it should be noted that accumulation of scale and sediment would be minimal in a new system. In addition, the amount of deposition and replenishment of *L pneumophila* from incoming potable water would be related to the number of years the water tank was in service.

Two hospitals used an instantaneous steam-heating system in their water distribution systems, and neither had *L pneumophila* cultured from its water supply. Such a system would theoretically be nonconducive to *L pneumophila* colonization because these systems heat water to 88°C which is bactericidal for *L pneumophila*,[22] and because they have no hot water storage tank, a breeding ground for *L pneumophila* (Figure).[4]

We found that concentration of calcium and magnesium correlated significantly with *L pneumophila* contamination of hot water tanks. Calcium and magnesium are the principal divalent metallic cations involved in formation of scale deposits and are primary determinants of water hardness. Scale and sediment formation are dependent on a number of environmental variables including water pressure, temperature, flow rate, and water hardness. We have previously shown that *L pneumophila* localizes and concentrates in areas within the water distribution system laden with scale and sediment. The sediment contributes nutrients utilized by commensal microorganisms that foster the growth of *L pneumophila* as well as providing a physical shelter for the organism.[23]

We emphasize that *L pneumophila* contamination should not be construed as evidence that the water distribution system is being poorly managed. Hospitals with preventive maintenance programs were as likely to be contaminated with *L pneumophila* as hospitals without such programs. It should also be noted that chlorination was maintained in these water distribution systems at a standard level of one to two parts per million; however, this concentration is known to be inadequate in killing *L pneumophila*, a relatively chlorine-resistant microorganism.

The major weakness of this study is that it is confined to a relatively small number of hospitals in one geographic area. Thus, caution should be exercised in any extrapolation to individual hospitals. The predictive value of parameters found significant in this study may be less useful in other geographic areas with lower prevalence of contamination. On the other hand, this study provides a framework for future large-scale studies over a wider geographic area. Parameters found significant in this study should be reassessed prospectively to confirm their validity.

We also emphasize that parameters found significant in this study should not be considered absolute; exceptions can easily be found. For example, one VA Medical Center did not encounter a problem with nosocomial legionellosis until they moved into their *new* hospital building. And, the once highly contaminated hot water tanks in the Pittsburgh VA Medical Center[4] have a horizontal rather than vertical configuration.

It is noteworthy that trends were easily discernible by statistical analysis. Furthermore, the parameters found statistically significant were supported by a plausible biological hypothesis that could explain the observed association. Thus, there seems to be rational and predictable ecology of this organism within water distribution systems. Knowledge of the design of the distribution system as well as physicochemical characteristics of the water can be useful in assessing the risk of *L pneumophila* contamination.

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