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Environmental *Legionella* Assessment in Office Buildings of Continental United States

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Abstract

An environmental assessment of Legionella *bacteria was conducted in five office buildings in the continental United States where no legionellosis was reported. The purpose of this investigation was to (i) determine the presence of* Legionella *bacteria in potable and non-potable water systems, (ii) provide a baseline information for management, and (iii) evaluate the effectiveness of the remedial actions taken. Water samples were collected from all possible water sources in surveyed buildings. The samples were analyzed by both direct fluorescent antibody microscopy (DFA)* and *the bacterial cultural method for the presence of* Legionella *species.* Legionella *bacteria were detected in some samples collected from various water distribution systems in the buildings. Remedial action was taken to eliminate these bacteria, and case-by-case results are presented.*

KEY WORDS:
Cooling tower, *Legionella pneumophila,* Water tank, Chlorination, Superheating, Biocide.

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Introduction

Legionella species are a group of gram-negative, rod-shape bacteria. They are ubiquitous in natural and man-made freshwater environments, and have been implicated as the causative agent for Pontiac fever and Legionnaires' disease (Barbaree et al., 1993). The disease is often acquired by inhaling the aerosol containing virulent bacteria, which is passed into the lung and deposited deeply in the alveoli of the susceptible host (Breiman, 1993). Therefore, the most prevailing mode of transmission of Legionnaires' disease has been hypothesized as aerosolization of the virulent strain of bacteria (Breiman, 1993; Fraser, 1980), with cooling towers as a likely primary amplification reservior. However, there is evidence that potable water systems may be considered as an alternative reservoir (Neill et al., 1985; Muder et al., 1986; Stout et al., 1992).

The Centers for Disease Control and Prevention (CDC), US Public Health Service, does not recommend routine environmental sampling of *Legionella* bacteria unless at least two cases of Legionnaires' disease have been confirmed in the same building (Redd and Cohen, 1987). Most of the environmental sampling for *Legionella* bacteria analyses in the United States has therefore been conducted *after* a Legionnaires' disease outbreak was confirmed (Barbaree et al., 1987). These samplings emphasized the matching of *Legionella* serogroup types in order to detect the origin of the causative bacterium.

As addressed in Miller and Kenepp (1993), *Legionella* assessments in the absence of disease have been undertaken in some cooling towers of North American buildings as a routine surveillance. Moreover, environmental surveys of hospital potable water supplies have been recommended and used to screen for undiscovered nosocomial legionellosis in health facilities (Goetz and Yu, 1991; ASHD, 1993).

Superheating and/or hyperchlorination of the water systems are two common control measures during a Legionnaires' disease outbreak (Barbaree et al., 1993; HSE, 1991; OSHA, 1991; WDHSS, 1987; HDV, 1989). These control measures, combined with biocide treatment, may also be effective in controlling *Legionella* concentrations in the water system where no outbreak is reported but *Legionella* is detected.

An environmental assessment was conducted in five office buildings in the continental United States with an absence of legionellosis. The objective of this study was to: (1) determine the presence of *Legionella* bacteria in both potable and non-potable water systems; (2) provide a baseline information for building managers, and (3) evaluate the effectiveness of the remedial actions taken for controlling *Legionella* population in these water systems.

Material and Methods

Water samples were collected from possible amplification reservoirs such as: cooling tower reservoirs, evaporative coolers, humidifiers, condensate drainage pans in the air handling units, fire sprinkler systems, city main, domestic hot and/or cold water storage tanks, drinking fountains, showers, and hot and/or cold faucets in the kitchen(s) and rest rooms. For non-potable water samples, 250 ml of water were collected. For each potable water system, two samples (pre- and post-flush) were collected in two bottles. The pre-flush sample was collected immediately after the valve or faucet was opened. The post-flush sample was collected after water had been running for one minute.

To detect the culturable legionellae, both direct fluorescent antibody test (DFA) *and* the bacterial cultural method were used. Water samples were processed, plated onto buffered charcoal yeast extract agar (BCYE) and BCYE with antibiotics, and incubated according to Gorman et al. (1983). Cultures were examined under a dissecting microscope for the presence of bacterial colonies resembling *Legionella* (Fields, 1993). The suspicious colonies were counted and then inoculated onto commercially available BCYE biplates with and without L-cysteine (Gorman et al., 1983; Fields, 1993). If the colony grew on one side of the plate but not on the other, it is presumptive *Legionella*. Identification and typing of *Legionella* isolates was then carried out by polyclonal DFA assay (Wilkinson, 1988) using commercially available poly- and mono-specific conjugates (*L. pneumophila* serogroup 1–6, and *L. bozemanii*). Those presumptive *Legionella* isolates failed to react with conjugates used, or reacted strongly with poly-specific conjugate but not with individual conjugates are referred to as *Legionella*-like organisms (Wilkinson, 1988).

Results

Since the DFA method sometimes produces false-positive results, positive results presented here are those confirmed by the bacterial cultural method. Negative results indicate that no *Legionella* colonies were detected from the cultural method at the dilution used in the analysis. *Legionella* concentrations are reported as colony forming units per milliliter of water sample (CFU/ml).

Legionella concentrations in water samples varied among samples and buildings. Negative results were obtained from all 47 samples collected from various water systems in Building A (Table 1). However, *Legionella* was detected in both potable and non-potable samples collected from Buildings B and C. *Legionella* was detected only in samples collected from non-potable water systems in Buildings D and E. The results of mitigation in Buildings B, C, D, and E are presented below:

Potable Water Systems

Case 1: Domestic Hot Water Tank 1 in Building B

Both pre- and post-flush samples collected from hot water tank 1 had *L. pneumophila* serogroup 1 (Table 2). Moreover, *Legionella* bacteria were also detected from one of the selected rest room hot water faucets (Table 2). The hot water in this particular rest room was supplied by hot water tank 1 (3,500 gallon capacity, electric unit).

The tank was superheated (150–160°F for 3

Table 1 Total number of samples collected from each building and number of samples with *legionella* bacteria detected by the cultural method.

Building	Samples collected (#)	Samples with *Legionella* detected (#)	*Legionella* in potable water system (#)	*Legionella* in non-potable water system (#)
A	47	0	0	0
B	31	14	9	5
C	34	6	2	4
D	28	3	0	3
E	25	2	0	2

Table 2 *Legionella* concentrations (CFU/ml*) in water samples collected from hot water tank #1 and one hot water faucet in a rest room of Building B.

	Day 1	Day 23	Day 42
Tank, pre-flush	120 Lp#1@	680 Lp#1	Negative**
Tank, post-flush	20 Lp#1	5 Lp#1	Negative
Faucet, pre-flush	50 Lp#1	Negative	–
Faucet, post-flush	6 Lp#1	Negative	–

* CFU/ml: Colony forming units per milliliter of water sample.
** Negative: No *Legionella* colonies were detected from the cultural method at the dilution used in the analysis.
@ *Legionella pneumophila* serogroup #1.

hours) and all outlets were flushed with hot water for at least 15 minutes. The particular faucet was then re-sampled on Day 23 and negative results were revealed. Follow-up samples were also collected on Day 23 from the valve located at the base of hot water tank 1. *Legionella* concentrations of the pre- and post-flush samples were 680 and 5 CFU/ml, respectively. It was later revealed that the valve under the hot water tank was not flushed after the superheating and therefore *Legionella* bacteria proliferated in the piping systems between the tank and the valve. The tank was again superheated and all final distribution outlets *and* the valve under the hot water tank were flushed. *Legionella* was not detected in the re-sampling on Day 42.

Case 2: Domestic Cold Water Tanks in Building C
Two of the domestic cold water tanks (located at the penthouse with 6,000 and 8,000 gallons capacity) contained *Legionella*-like organisms in the pre-flush samples (< 1 and 155 CFU/ml). However, negative results were obtained from the corresponding post-flush samples. This indicates that the source of *Legionella* was in the drain lines, not in the tank itself. The drain lines on these particular tanks each had a portion of "dead legs" (piping that has been cut off but still contains water and is connected to the system) where the water was able to stand undisturbed. A back-flow preventer (a valve which prevents water in the drain line from going back up to the tank) was moved closer to the main supply line on tank 1, in order to prevent water stagnation in the long piping (about 2 feet) between the tank and the preventer. A pipe elbow was removed from the drain line on tank 2 and the line was placed on an angle to allow drainage and prevent the accumulation and stagnation of water. Both water tanks were then chlorinated to 5 ppm, maintained for approximately 8 hours, and flushed for a short period of time. Re-sampling of both water tanks after chlorination revealed negative results.

Non-potable Water Systems

Case 3: Cooling Towers and a Chiller in Buildings D and E
Legionella pneumophila serogroup 1 was detected in three different cooling tower reservoirs of Building D. *Legionella pneumophila* serogroup 1 and *L. bozemanii* were detected in samples collected from a cooling tower reservoir and a chiller drain line in the mechanical room of Building E. The sample concentrations ranged from 50 to 140 CFU/ml. The cooling towers were "shock" treated as recommended in the Wisconsin protocol (WDHSS, 1987). They were low-pressure steam-cleaned and chlorinated to 25 ppm for 24 hours. The residual chlorine level was maintained at 10 ppm for another 24 hours. Then, the cooling towers were drained, refilled with water, and treated with biocide (active ingredient of poly [oxyethylene (dimethyliminio) ethylene-(dimethyliminio) ethylene dichloride]). The chiller was chlorinated in the same manner as the cooling tower, and the biocide was circulated through the piping system connected to the chiller. All systems were re-sampled, and negative results were revealed.

Case 4: A Cooling Tower in Building B
The cooling tower (6,000 gallon capacity) reservoir contained 5 CFU/ml of *L. pneumophila* serogroup 4. One "biocide" (active ingredients of disodium cyanodithioimidocarbonate and potassium N-methyldithiocarbamate) had been routinely used in this system. The concentration of this "biocide" was then increased two-fold. However, *Legionella* was still detected after the treatment. It was later revealed that this particular "biocide" was actually an algaecide which was effective against algae but not gram-negative bacteria. A different microbiocide (active ingredient of isothiazolinone) was then pumped into the sump tank. The system was then chlorinated (10 ppm), flushed, and the valve was changed. Re-sampling did not show the presence of *Legionella*.

Case 5: A Cooling Tower System in Building C
The reservoir and overflow of the cooling tower (600 ton capacity) had *L. pneumophila* serogroup 1 and *Legionella*-like organisms with concentrations of 90 and 25 CFU/ml, respectively. The system was then mechanically cleaned, chlorinated at 5 ppm for approximately 8 hours, flushed, and then treated with a biocide (active ingredients of sodium hypo-

chloride and sodium hydroxide). Results from re-sampling of both reservoir and the overflow indicated that *Legionella* was not present.

Case 6: "Kathabar" Systems in Building C
The Kathabar system is a large, closed loop within the cooling tower itself. It is a dehumidification system which replaced the air-conditioning. The system contains a lithium chloride solution (or Kathene) which acts as a desiccant to absorb moisture from the air that enters the system.

Fifteen CFU/ml of *Legionella*-like organisms were detected in two kathabar systems. Due to the potential reaction between lithium chloride and chlorine, they were not hyperchlorinated. Instead, they were treated with a biocide (active ingredients of sodium hypochloride and sodium hydroxide) twice. No *Legionella* was detected in the kathabar systems at re-sampling.

Discussion

Despite the absence of Legionnaires' disease, *Legionella* were detected in some of the water systems in this study. With a proper maintenance program, *Legionella* concentrations can be controlled and the potential for exposure can be significantly reduced. In our study, no *Legionella* was detected in samples collected from Building A, and most samples collected from Buildings D and E had negative results. These results indicate that at the time of sampling, prior maintenance programs in these buildings were effective in controlling *Legionella* concentrations in the sampled water systems.

A proper maintenance program for non-potable water systems usually involves biocide treatment (HSE, 1991; HDV, 1989; WDHSS, 1987). It is important that maintenance personnel need to be aware of what kind of biocide is being applied to a building's non-potable water system. For example, if maintenance personnel had known that the "biocide" used in the cooling tower systems of Building B was actually an algaecide, the remedial actions would have been different (Case 4). Chlorination (10 ppm) of the system along with treatment of a non-oxidizing biocide such as isothiazolinone (Case 4), or mechanical cleaning accompanied with hyperchlorination (Case 3) effectively lowered the concentrations of *Legionella*.

Avoiding water stagnation is one of the preventive measures for controlling *Legionella* bacteria in water systems (Barbaree et al., 1993; HDV, 1989; HSE, 1991; WDHSS, 1987). "Dead legs" in the pipeline and improper drainage of drain lines cause water accumulation, and could provide a breeding niche for *Legionella* bacteria (Buildings B and C). These situations can be improved by re-designing the pipeline device so that water stagnation will not occur.

Results from pre- and post-flush samples collected from the water storage tanks give a good indication whether the amplification site is in the tank itself or in the pipeline. A careful test results interpretation will lead to a proper, effective, and timely remedial action. Chlorination (5 ppm for 8 hours) of the domestic cold water tank accompanied by removal of dead legs and/or re-positioning the pipelines, effectively lowered *Legionella* concentrations in the potable water system in Building C (Case 2).

Superheating of the hot water tank and flushing of all final distribution outlets with heated water effectively reduced *Legionella* concentrations in the water system (Case 1). It is important, however, that outlets directly under the hot water tank should be flushed after the superheating treatment in order to remove *Legionella* and prevent their proliferation in the pipelines (Building B).

Due to the ubiquitous nature of the *Legionella* bacteria in the natural environment, some *Legionella* bacteria are expected to re-occur after several months of treatment (Breiman et al., 1990; Joly and Alary, 1993). It is unfortunate that the treated water systems in the buildings we studied were not available for further monitoring of *Legionella*.

Routine preventive maintenance programs have been implemented to maintain clean water systems and to control *Legionella* population in Great Britain and Australia (HSE, 1991; HDV, 1989). In the United States, environmental sampling for *Legionella* assessment has been a controversial issue. The CDC does not recommend sampling unless two confirmed cases are detected. However, in the absence of knowledge of disease, environmental sampling has been conducted by industrial hygienists and hospital personnel in residence, office buildings, industrial plants, and health care facilities. As a public health concern, a *pro-active* approach is recommended to control *Legionella* in the water systems and prevent the occurrence of legionellosis.

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