Occurrence of culturable *Legionella pneumophila* in drinking water distribution systems

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**Abstract**
A prior study of 12 potable water distribution systems detected *Legionella pneumophila* in only one of 576 samples (0.17%), but the survey was conducted during winter and spring months when water temperatures were generally below 15°C (LeChevallier, AWWA Water Science 2019, e1122). Ten of the utilities agreed to participate in a second round of testing during the summer and early fall months when water temperatures averaged 23°C (average temperatures ranged from 14 to 29°C). *L. pneumophila* was enumerated using the Legiolert assay. A total of 669 samples were analyzed during the study: 50 source water samples, 46 from the plant effluent, and 573 from the distribution system. *L. pneumophila* was detected in two source water samples and not detected in any of the treated plant effluent samples. *L. pneumophila* was detected in 14 distribution system samples, 13 from free chlorinated systems and one from a chloraminated system. All occurrences of *L. pneumophila* were observed when water temperatures were >18°C. Concentrations of *L. pneumophila* were <10 most probable number/100-mL except when chlorine residuals were less than 0.1 mg/L. Based on the results of this study, it is recommended that water utilities maintain at least a 0.1 mg/L chlorine residual, particularly when water temperatures are >18°C.

**KEYWORDS**
distribution, Legiolert, *Legionella*, *Legionella pneumophila*, microbiology

1 | INTRODUCTION

The incidence of Legionellosis, a respiratory infection caused by bacteria of the genus *Legionella*, has increased 550% since 2000 and is now the most commonly reported cause of drinking waterborne outbreaks (Benedict et al., 2017; Centers for Disease Control and Prevention [CDC], 2019). The biggest threat of Legionnaires' disease comes from premise plumbing when the organisms proliferate and become aerosolized. Outbreaks are typically associated with the potable water within building plumbing systems, cooling towers, hot tubs, decorative fountains, and industrial waters (Garrison et al., 2016). Although outbreaks typically occur in building water systems and cooling towers, potable water utilities typically do not monitor for this important pathogen. One outbreak, however, was associated with a community water system storage tank that had a low (<0.2 mg/L) free chlorine residual (Cohn et al., 2015). *Legionella* is regulated under the Surface Water Treatment Rule (SWTR) with a maximum contaminant level goal (a nonenforceable guideline) of zero *Legionella* organisms for drinking water (U.S. Environmental Protection Agency...
[USEPA], 1989). The rule specifies a treatment technique for Legionella control (e.g., filtration and maintenance of a detectable disinfectant residual), and therefore, monitoring for Legionella is not required. Although analytical methods existed for Legionella detection, the USEPA determined that testing was impractical to implement, particularly for small systems.

Water utilities are reluctant to monitor for Legionella, particularly because there are no USEPA or CDC guidelines for responding to positive results. Even some state agencies are hesitant to allow water systems to monitor for Legionella as there is a lack of guidance on how to communicate the results to the public (LeChevallier, 2019). However, recently, protocols have been developed through consultation with one state agency for responding to sporadic occurrences of Legionella pneumophila in drinking water systems that emphasize resampling, serotyping, and flushing to maintain a residual disinfectant (LeChevallier, 2019). For building water systems, the American Industrial Hygiene Association (2015) has recommendations for monitoring culturable Legionella; although the goal is to have zero (below detection limits) Legionella bacteria, the guidelines for potable water (<10 cfu/mL) are acceptable provided that other water quality values (e.g., temperature, disinfectant, etc.) and operational parameters are within normal operating ranges. The European Union (2017) also published proposed guidelines for the prevention, control, and investigation of infections caused by Legionella species.

To date, most monitoring has been performed in building water systems, so there is not much information about Legionella occurrence or concentrations in drinking water distribution systems. When monitoring is performed, it is often difficult to separate the impact of building plumbing from water in the distribution system. Wang, Edwards, Falkinham, and Pruden (2012) detected Legionella spp. using quantitative polymerase chain reaction (qPCR) in two chloraminated systems, although L. pneumophila was detected in only 5.6% of samples, and Legionella spp. concentrations were reduced 45-fold after tap samples were flushed for 3 min. Stagnation (at least 8 h) was found to be important, and none of the postflushed samples were positive for L. pneumophila in one of the systems. Donohue et al. (2014) used qPCR assays to evaluate the occurrence of L. pneumophila and L. pneumophila serogroup 1 in 272 cold drinking water taps collected from 40 sites (32 buildings, eight houses) across the United States. L. pneumophila serogroup 1 was detected in 47% of the taps, but the samples were flushed for only 15 s, so the results are primarily reflective of the building plumbing system. Drinking water fountains were most commonly positive, and taps that were repeatedly positive were more frequently fed chlorinated water (16%) compared with chloraminated water (8.6%). Lu et al. (2016) examined large-volume (90 L) ultrafiltration concentrates from six sites within a distribution system and frequently (57%) detected Legionella spp. by qPCR at an average concentration of 85 cell equivalents per liter. L. pneumophila was detected but at low frequency (6%). Concentrations of Legionella spp. were 0.4–78-fold higher in the distal sections of the distribution system compared with the entry point, suggesting growth within the distribution system.

The qPCR methods have the advantages of being quick and specific but cannot determine the viability of the organisms detected. This is a big disadvantage for monitoring disinfected water supplies, where the presence of a residual would be expected to inactivate Legionella cells (Kuchi, States, McNamara, Wadowsky, & Yee, 1983). Although viable-but-not-culturable qPCR methods that use ethidium or propidium monoazide technology exist (Chen & Chang, 2010; Delgado-Viscogliosi, Solignac, & Delattre, 2009; Johnson, Jjumba, Bukhari, & LeChevallier, 2018; Mansi et al., 2014), these methods are not commercially available and have not been standardized. In addition, few water utilities have the facilities, equipment, or trained staff for routine molecular analyses. Recently, IDEXX Laboratories Inc. (Westbrook, ME) developed a culture-based assay for the enumeration of L. pneumophila that is easy to use by water utilities (LeChevallier, 2019). The test has been shown by several researchers to result in equivalent or higher counts of L. pneumophila from potable, nonpotable, and water cooling tower samples (Petrisek & Hall, 2018; Rech, Swalla, & Dobranic, 2018; Sartory, Spies, Lange, Schneider, & Langer, 2017; Spies et al., 2018).

LeChevallier (2019) conducted a study of 12 potable water utilities using the Legiolert assay and detected only one positive result in 576 samples (0.17%). L. pneumophila was measured at a concentration of 1 most probable number (MPN)/100 mL in a sample that contained 0.72 mg/L free chlorine (pH 6.95, temperature 18.2°C, total organic carbon of 0.32 mg/L) and was serotyped as belonging to the 2–14 serogroup. A repeat sample collected a week later was negative for L. pneumophila. The study was conducted during winter and spring months when water temperatures were generally below 15°C. It may be possible that the occurrence and concentration of L. pneumophila might be higher during the warmer months when water temperatures are expected to be more favorable for growth (Garrity, Bell, & Tilburn, 2005).

The objective of this study was to conduct a second round of Legiolert testing during the summer and early fall months when water temperatures would be expected to be warmer. Of the original 12 utilities, 10 agreed to participate using a sampling program similar to the one used in the first study. The results provide insights into the seasonal
occurrence of *L. pneumophila* in potable water distribution systems and the impact of treatment and disinfectant residual on the concentration of the organisms in drinking water. A case study demonstrates one utility's response to manage *L. pneumophila* occurrence and communicate the results to the public. The study provides useful information on the occurrence and control of *L. pneumophila* in potable water distribution systems.

2 | MATERIALS AND METHODS

2.1 | Utility information

Of the 12 utilities that participated in the winter/spring study (LeChevallier, 2019), 10 agreed to participate in a second round of sampling during the late summer/fall period when conditions would be most favorable for bacterial growth. The two systems that declined to participate cited laboratory workload and other planned projects that limited their resources to continue with the study.

The 10 utilities represented a good geographic distribution, with five sites along the East Coast and two in the West, as well as three sites in the center of the United States. Sites were also distributed equally, with five sites both in the northern and southern parts of the United States. Table 1 shows the characteristics of the systems. Nine of the systems used surface water, and one had a blend of surface and groundwater. Two systems were unfiltered, one used ultrafilter membrane filtration, and the others used various forms of granular media filtration. For primary disinfection, two systems used ozone and ultraviolet (UV) disinfection, one used UV alone, two used chlorine dioxide, four used prechlorination, and one used prechloramination with sufficient contact time to meet the requirements of the SWTR (USEPA, 1989). Five of the systems maintained a chlorine residual in the distribution system, while the other sites used free chlorine.

2.2 | Sampling sites

The study plan included collecting four raw water, four plant effluent, and approximately 48 distribution system samples from each utility. Some systems collected additional samples or had multiple treatment plants. The participating utilities were instructed to develop a sampling plan for each system that represented a cross section of the distribution. Samples were to be collected from sites with a known history of water quality results. Most systems used their existing Total Coliform Rule monitoring locations, but at least 36 (6.3%) of the 573 distribution system samples were from finished water reservoirs or storage tanks. Some of the utilities had coded sampling locations, so it is possible that additional samples were obtained from storage facilities. Most samples were collected in September through October 2018, but utility 2 examined samples every week from February through October 2018.

2.3 | Analytical methods

Table 2 shows a summary of the analytical methods used in this study. All supplies used for the microbiological analyses were provided at no cost by the manufacturer to ensure that all utilities collected data using the same methodology. All

| Utility code | Population served | Water source | Filtration | Primary disinfectant | Secondary disinfectant | Miles of pipe |
|--------------|-------------------|--------------|------------|----------------------|------------------------|--------------|
| 1            | 200,000           | Surface      | Unfiltered | Ozone and UV         | Chloramine             | 1,000        |
| 2            | >2,000,000        | Surface      | Unfiltered | UV/free chlorine     | Free chlorine          | 6,500        |
| 3            | 1,600,000         | Surface      | Filtered   | Free chlorine        | Chloramine             | 3,200        |
| 4            | 950,000           | Surface      | Sand/GAC filtered | Free chlorine      | Free chlorine          | 4,200        |
| 5            | 750,000           | Surface      | GAC/anthracite/sand filtration | Chlorine dioxide/chlorine | Free chlorine | 200* |
| 6            | 450,000           | Surface      | GAC filtration | Free chlorine       | Free chlorine          | 2,300        |
| 7            | 500,000           | Surface      | Anthracite/sand filtration | Chloramine       | Chloramine             | 1,600        |
| 8            | >2,000,000        | Surface/ground | Multistage filtration | Ozone and UV       | Free chlorine          | 6,500        |
| 9            | 65,000            | Surface      | Direct filtration, anthracite/sand filtration | Chlorine dioxide | Chloramine             | 600          |
| 10           | 500,000           | Surface      | Ultrafiltration/anthracite/sand | Chloramine       | Chloramine             | 1,000        |

Abbreviations: GAC, granular activated carbon; UV, ultraviolet.

*Regional wholesale system services multiple consecutive systems.*
analyses were conducted according to the manufacturer's protocols or according to Standard Methods (2017).

Briefly, the Legiolert method consisted of collecting a 100 mL potable water sample and allowing the temperature to equilibrate to room temperature. The water hardness was adjusted, if necessary, using reagents supplied in the kit. The Legiolert reagent was added to the sample, shaken, and poured into a Quanti-Tray/Legiolert and sealed using a Quanti-Tray Sealer PLUS (IDEXX Laboratories Inc.). The trays were incubated at 39 ± 0.5°C for 7 days and examined for production of a brown color and/or microbial growth (as evidenced by turbidity) relative to a negative control. The number of positive wells was counted, and an MPN/100 mL was determined using a formula provided in Table 4 of 13.

| Parameters to test | Test performed |
|--------------------|----------------|
| Legionella pneumophila (MPN/100 mL) | Legiolert |
| Total coliform / Escherichia coli (presence/absence) | Colilert |
| HPC (MPN/100 mL) | Simplate for HPC* |
| Free/total chlorine | DPD or amperometric titration method |
| Temperature | Thermometer |
| pH | pH strip or electrode |
| Total organic carbon | SM5310B |

Abbreviations: DPD, N,N-diethyl-p-phenylenediamine; HPC, heterotrophic plate count; MPN, most probable number.

*One site used plate count agar.

It is important to maintain adequate humidity during the 7-day incubation to avoid moisture loss within the Quanti-Tray/Legiolert. As a quality assurance/quality control procedure, laboratories were instructed to monitor the weight of the Quanti-Tray/Legiolert before and after the 7-day incubation and verify that not more than 15% weight loss occurred during incubation. If the postincubation Quanti-Trays were <85% of their original weight, the laboratories were instructed to consult the manufacturer for further guidance.

2.5 | Data analysis

An Excel spreadsheet was provided to each participating utility so that a common set of data in a common format was collected. The spreadsheet also contained a look-up table so that the Legiolert MPN was automatically calculated once the number of positive wells was entered. The data from all the sites were compiled into a single file so that summary and descriptive statistics could be completed.

3 | RESULTS AND DISCUSSION

3.1 | Legionella and water quality

A total of 669 samples were analyzed during the study: 50 source water samples (Table 3), 46 from the plant effluent (Table 4), and 573 from the distribution system (Table 5). L. pneumophila was detected only in two source water samples, both at utility 7. The low frequency of detection was surprising given that Legionellae are normal inhabitants of the aquatic environment and are widely distributed in natural waters, including lakes, rivers, and groundwaters, as well as natural soil, potting soil, and compost samples (USEPA, 2016). The nonpotable Legiolert assay only processes 1 mL of source water, so the low frequency of detection may be due to the small volume and the relatively few samples analyzed.

The two source water samples contained L. pneumophila serotype 1 and serotype 2–14 in separate samples. L. pneumophila represents 95.4% of the isolates of sporadic community-acquired Legionellosis associated with waterborne bacteria, with serogroup 1 accounting for 84.2% of the infections and serogroups 2–13 for 7.4% of the cases (Yu et al., 2002). The Legiolert assay is specific for L. pneumophila, so other species of Legionella were not
**TABLE 3** Summary of raw water data

| Utility code | n positive Legiolert | Total coliform (100 mL avg) | Escherichia coli (100 mL avg) | HPC (mL avg) | HPC (mL min) | HPC (mL max) | Temp. (°C) | Temp. min (°C) | Temp. max (°C) | pH | pH min | pH max | TOC min (mg/L) | TOC max (mg/L) |
|--------------|----------------------|-----------------------------|------------------------------|--------------|--------------|--------------|-----------|----------------|----------------|----|---------|--------|----------------|----------------|
| 1            | 1                    | 0                           | 1                            | —            | —            | —            | 6.00      | —              | —              | 6.60| —       | —      | —               |                |
| 2            | 18                   | 0                           | 58                           | 12           | 19           | 0            | 107       | 13.30         | 3.70           | 20.60| 7.57    | 7.08   | 8.31           | 2.15           |
| 3            | 3                    | 0                           | —                            | 205          | —            | —            | 21.70     | 19.00         | 23.00         | 7.75| 7.49    | 7.94   | 2.92           | 2.26           |
| 4            | 4                    | 0                           | 10                           | 360          | 299          | 440          | 24.60     | 24.20         | 25.20         | 6.96| 6.90    | 7.02   | 1.78           | 1.60           |
| 5            | 3                    | 0                           | 1,500                        | 235          | 171          | 299          | 27.30     | 27.20         | 27.40         | 6.73| 6.67    | 6.79   | 3.76           | 3.71           |
| 6            | 2                    | 0                           | —                            | 5,071         | 148          | 4,100        | 7,400     | 19.20         | 18.70         | 19.80| 7.19    | 7.07   | 7.28           | —              |
| 7            | 6                    | 0.7                         | 13,307                       | 5,550         | 3,700        | 7,400        | 27.30     | 27.20         | 27.40         | 6.73| 6.67    | 6.79   | 3.76           | 3.71           |
| 8            | 3                    | 0                           | —                            | 347          | 0            | 71           | 19.70     | 18.00         | 19.20         | 8.00| 7.94    | 8.00   | 2.43           | —              |
| 9            | 3                    | 0                           | —                            | —            | —            | —            | 22.90     | 21.00         | 25.00         | 7.20| 7.10    | 7.30   | 1.60           | 0.90           |
| 10           | 7                    | 0                           | 86                           | 2041         | 1,370        | 3,920        | 21.50     | 12.30         | 25.60         | 8.30| 8.20    | 8.40   | 10.20          | 9.50           |

Abbreviations: —, not determined; avg, average; HPC, heterotrophic plate count; min, minimum; max, maximum; temp., temperature; TOC, total organic carbon.

**TABLE 4** Summary of plant effluent data

| Utility code | n positive Legiolert | Total coliform / (100 mL avg) | Escherichia coli / (100 mL avg) | HPC (mL avg) | HPC (mL min) | HPC (mL max) | Temp. (°C) | Temp. min (°C) | Temp. max (°C) | pH | pH min | pH max | TOC min (mg/L) | TOC max (mg/L) |
|--------------|----------------------|-------------------------------|---------------------------------|--------------|--------------|--------------|-----------|----------------|----------------|----|---------|--------|----------------|----------------|
| 1            | 2                    | 0                             | 0                               | —            | —            | —            | 2.30      | 2.00           | 2.50           | 9.50| 9.00    | 9.90   | 8.20           | 8.30           |
| 2            | 9                    | 0                             | 0                               | 0            | 0            | 0            | 0.80      | 0.50           | 1.20           | 12.00| 3.10    | 18.90  | 7.50           | 7.80           |
| 3            | 9                    | 0                             | 0                               | 0            | 0            | 0            | 2.40      | 2.00           | 3.00           | 22.90| 21.00  | 25.00  | 7.20           | 7.30           |
| 4            | 5                    | 0                             | 0                               | 0            | 0            | 0            | 1.50      | 1.30           | 1.60           | 27.40| 27.00  | 28.00  | 8.40           | 8.50           |
| 5            | 3                    | 0                             | 0                               | 0            | 0            | 0            | 2.00      | 1.90           | 2.20           | 1.70| 1.90    | 22.30  | 8.00           | 8.10           |
| 6            | 2                    | 0                             | 0                               | 0            | 0            | 0            | 1.20      | 1.10           | 1.30           | 25.70| 25.10  | 26.20  | 7.60           | 7.70           |
| 7            | 2                    | 0                             | 0                               | 449          | 392          | 507          | 4.10      | 4.00           | 4.10           | 29.60| 29.30  | 29.80  | 8.10           | 8.10           |
| 8            | 6                    | 0                             | 0                               | 1.90         | 1.80         | 2.00         | 1.60      | 1.80           | 15.40          | 14.30| 16.50  | 7.70   | 7.50           | 7.90           |
| 9            | 1                    | 0                             | 0                               | 1.88         | —            | —            | 20.00     | 8.88           |                |      |         |        |                |                |
| 10           | 7                    | 0                             | 2                               | 3.80         | 3.60         | 4.00         | 22.10     | 14.10          | 26.30          | 8.90| 8.80    | 9.10   | 4.20           | 3.80           |

Abbreviations: —, not determined; avg, average; HPC, heterotrophic plate count; min, minimum; max, maximum; temp., temperature; TOC, total organic carbon.
examined. Source water values for total coliforms, *Escherichia coli*, and heterotrophic plate count (HPC) levels (Table 3) showed good water quality and the relatively low source water values of *E. coli* demonstrated limited fecal contamination—well below the criteria for source water used as a potable water supply (Bordner, Winter, & Scarpino, 1978).

*L. pneumophila* was not detected in any of the treated plant effluent samples (Table 4), in part because average free chlorine residuals ranged between 0.8 and 1.8 mg/L and average total chlorine residuals ranged between 1.8 and 4.1 mg/L for the five utilities that practiced chloramination. In addition, all total coliform samples were negative, and HPC levels were typically nondetectable. Maintenance of a chlorine residual in potable water systems is typically effective for controlling *Legionella* spp. (Jjemba, Johnson, Bukhari, & LeChevallier, 2015; Kim, Anderson, Mueller, Gaines, & Kendall, 2002); however, there are situations where the bacteria can be shielded from the disinfectant (as in a biofilm or amoebae cyst), and therefore complete eradication of the organism is difficult. Kuchta et al. (1983) reported a CT value of 0.5 min-mg/L at 21°C and pH 6.0 for 2-log (99%) reduction of *L. pneumophila* and values ranging from 1 to 6 min-mg/L and <3 to 9 min-mg/L for pH 7 and pH 7.6, respectively. In comparison, *Legionella* spp. in protozoa cysts survived a 25-fold greater chlorine disinfectant after 18 h (Kilvington & Price, 1990). Two of the utilities used ozone for primary disinfection, and ozone (and other disinfectants such as chlorine) can increase the levels of bio-degradable organic matter (BOM) in the treated water (Volk & LeChevallier, 2000). Biological filtration can remove the BOM, but the filters can promote the growth of macroorganisms, including amoebae, nematodes, and crustaceans—particularly if the filters are not properly back-washed or operated with very long filter run times (Loret & Greub, 2010). These macroorganisms could serve to protect *Legionella* from subsequent disinfection and to inoculate the distribution system and building plumbing. Additional research is needed to better understand how biological treatment operations might impact *Legionella* occurrence or survival.

A total of 573 distribution system samples were collected, including at least 36 (6.3%) from finished water reservoirs (Table 5). *L. pneumophila* was detected in 14 samples (2.4%): in 13 samples of free chlorinated systems (4.1% of 317 samples) and once in the chloraminated systems (0.39% of 256 samples). None of the samples were positive for total coliform bacteria or *E. coli*, indicating that these indicator organisms are not valid predictors of *L. pneumophila* occurrence.

Of the 10 systems examined, five had at least one positive *L. pneumophila* sample, with an average concentration...
(including nondetects) ranging from 0.09 to 14.8 MPN/100 mL. For the 14 distribution system samples that were positive for *L. pneumophila*, individual sample concentrations ranged from 1 to 522 MPN/100 mL. Figure 1 shows the relationship between *L. pneumophila* cell densities and free chlorine residuals. All values greater than 10 MPN/100 mL occurred when free chlorine residuals were less than 0.1 mg/L. There was no relationship between *L. pneumophila* occurrence and free chlorine residuals when cell densities were less than 10 MPN/100 mL. The data suggest that, to prevent elevated concentrations of *L. pneumophila*, utilities should maintain at least a 0.1 mg/L free chlorine residual in all parts of the distribution system. However, the data also show that low concentrations of *L. pneumophila* may occur even when free chlorine residuals are more than 1.0 mg/L. The one chloraminated sample that was positive demonstrated *L. pneumophila* at 4 MPN/100 mL, with a residual of 3 mg/L total chlorine. It is not clear why *L. pneumophila* is able to persist at high disinfectant residuals. Perhaps the cells were entrapped in the cysts of free-living amoebae or inside protozoa hosts where they are protected from disinfection (Dupuy et al., 2011) or protected within pieces of biofilm or suspended sediment. Additional research is needed to understand the survival of low levels of *L. pneumophila* within disinfected potable water supplies.

Figure 1 also shows the detected serotypes of *L. pneumophila*. Serotype group 2–14 was associated with the elevated levels of *L. pneumophila* at free chlorine residuals <0.1 mg/L, suggesting growth of the strains within the distribution system. These samples all came from a single sampling point and taps upstream and downstream of the original site. As described in the case study, this area was a stagnant zone of the distribution system, and water temperatures were 24–28°C. The results indicate that *L. pneumophila* can colonize and grow within drinking water distribution systems under the right temperature conditions and low disinfectant residuals. As described later, *L. pneumophila* was no longer detected after the area was flushed, and free chlorine residuals were maintained at levels >0.1 mg/L.

The difference in the occurrence of *L. pneumophila* between free chlorine and chloraminated systems could be explained by the greater stability of the chloramine residual within the distribution system. Only 3.5% of the 258 distribution system samples collected in the five chloraminated systems had disinfectant residuals <0.5 mg/L, and none were <0.2 mg/L, while 31.2% of the samples from free chlorinated systems were <0.5 mg/L, 10.7% were <0.2 mg/L, and 4.4% were <0.1 mg/L (Table 6).

Chloramine residuals are also known to penetrate biofilms better than free chlorine (LeChevallier, Cawthon, & Lee, 1988; Lee, Wahman, Bishop, & Pressman, 2011; Pressman, Lee, Bishop, & Wahman, 2012). Lee et al. (2011) and Pressman et al. (2012) used microelectrodes to demonstrate that monochloramine had greater penetration than free chlorine in nitrifying biofilms. Numerous studies have shown that chloramines are more effective for *Legionella* control than free chlorine (Flannery et al., 2006; Heffelfinger et al., 2003; Kool, Carpenter, & Fields, 1999; Marchesi et al., 2012, 2013; Moore et al., 2006). Kool et al. (1999) examined 32 hospital-acquired (nosocomial) outbreaks of Legionnaires’ disease from 1979 to 1997 and found that the odds of a nosocomial *Legionella* outbreak were 10.2 (95% confidence interval [CI] 1.4–460) times higher in systems that maintained free chlorine than in those using a chloramine residual. Heffelfinger et al. (2003) surveyed 152 hospitals and found that hospitals supplied with drinking water disinfected with monochloramine were less likely (odds ratio = 0.20, 95% CI 0.07–0.56) to have hospital-acquired Legionnaires’ disease than other hospitals. Flannery et al.

### Table 6: Comparison of average water quality values for the five free chlorinated systems and the five chloraminated systems

| Parameter                              | Free chlorine systems | Chloramine systems |
|----------------------------------------|-----------------------|-------------------|
| Number of samples                      | 317                   | 258               |
| Number of *Legionella pneumophila* positive samples | 13                    | 1                 |
| % positive *L. pneumophila* samples    | 4.1                   | 0.39              |
| Average *L. pneumophila* (MPN/100 mL) | 3.04                  | 0.02              |
| Average disinfectant residual (mg/L)  | 0.82                  | 1.98              |
| Average temperature (°C)              | 23.1                  | 22.6              |
| Average pH                             | 8.04                  | 8.02              |
| Average total organic carbon (mg/L)    | 1.77                  | 3.1               |

Abbreviation: MPN, most probable number.
(2006) showed a 93% reduction in the occurrence of Legionella spp. in building plumbing systems in San Francisco after the utility converted from free chlorine to chloramines. Moore et al. (2006) reported that Legionella colonization was reduced in 96 buildings (public buildings and individual homes) by 69% (to six positive buildings) within a month after conversion from free chlorine to chloramination. In this study, both the frequency of occurrence and the average cell concentration of L. pneumophila was 10 times lower in chloraminated systems than in free chlorinated systems (Table 6), mirroring the results found in the literature.

Johnson et al. (2018) suggested a different mechanism by which monochloramine could be effective for Legionella control. They found that amoebae in five free-chlorinated reclaimed water systems were mostly (50–95%) in the active trophozoite phase; however, in the chloraminated system, 87% of the mesophilic amoebae and 66% of the thermophilic amoebae were in the cyst phase. They hypothesized that the penetration of chloramines into the biofilm might trigger the amoebae to form cysts rather than outright kill the protozoa. In the water environment, L. pneumophila amplifies in the vacuoles of infected protozoa, and this amplification occurs only in the trophozoite stage. Schoen and Ashbolt (2011) reported that a critical density of free-living protozoan hosts in biofilm required to propagate sufficient Legionella to cause an infection was $3.1 \times 10^4$ to $7.8 \times 10^7$ host/cm$^2$—suggesting that it may be possible to manage Legionella risk by limiting the free-living trophozoite population. Additional research is needed to evaluate this hypothesis, but understanding the relationship between the protozoan host life stage, the Legionella bacterium, and the disinfectant is critical to designing control strategies for water systems.

None of the 36 storage reservoir samples examined in this study were positive for L. pneumophila; however, most of the reservoirs examined were from just one system (utility 2). Elevated storage tanks may be prone to high water temperatures where water stratification may prevent mixing and subsequent loss of a disinfectant residual (Peter & Routledge, 2018). Nitrification is a common problem in storage tanks in chloraminated systems (AWWA Manual M56, 2006). Lu, Struwing, Yelton, and Ashbolt (2015) detected Legionella by qPCR in 66.7% of municipal drinking water storage tank sediments from 18 sites. All were chlorinated systems. Diverse Legionella spp., including L. pneumophila, L. pneumophila sg1, and L. anisa, were identified. At least one outbreak has been associated with a community water system storage tank that had low (<0.2 mg/L) free chlorine residuals (Cohn et al., 2015). Additional monitoring of drinking water storage tanks and reservoirs may be warranted.

Data from the prior study conducted during the winter/spring months (LeChevallier, 2019) was combined with information from this study to evaluate the relationship between temperature and the occurrence concentration of L. pneumophila in potable water distribution systems. A total of 1,087 data points were analyzed (Figure 2). The highest concentrations of L. pneumophila were observed in water samples with temperatures of 24–28°C (Figure 2) and low free chlorine residuals (Figure 1). A temperature range of 25–45°C is considered favorable for L. pneumophila growth with an optimum at 36°C (Schulze-Röbbecke, Rödder, & Exner, 1987, Garrity et al., 2005). However, Legionella spp. can also survive at temperatures below 20°C and even below freezing (Borella, Guerrieri, Marchesi, Bondi, & Messi, 2005). Sharaby et al. (2017) reported that environmental strains of L. pneumophila exhibited a superior capability for growth at lower temperatures (25 and 30°C) than did clinical strains. Moreover, as the virulence of L. pneumophila is temperature dependent, with maximum virulence between 37 and 42°C (Edelstein, Beer, & DeBoynton, 1987; Mauchline, James, Fitzgeorge, Dennis, & Keevil, 1994), the virulence of strains growing in drinking water systems needs to be evaluated. Because L. pneumophila enters the human lung directly from the environment, the temperature of growth needs to be considered in the risk analysis.

L. pneumophila was not detected when water temperatures were less than 18°C (Figure 2). This information could be used to guide the development of sampling programs for water utilities. Prior studies have found that coliform growth within drinking water distribution systems increased when water temperatures exceed 15°C (LeChevallier, Welch, & Smith, 1996; Volk & LeChevallier, 2000). It would be unnecessary for water systems to expend laboratory resources for Legionella monitoring when water temperatures were <15°C, but the utilities could focus these efforts when water temperatures were warm (>15°C), especially in...
areas of the distribution system where disinfectant residuals are low (<0.2 mg/L). This is not to say that *L. pneumophila* cannot be detected at temperatures <15°C, only that the frequency of detection and concentrations will be low. Should the USEPA include Legionella monitoring in the next round of the Unregulated Contaminant Monitoring Rule (USEPA, 2019), resources and expense could be minimized if testing was targeted to periods when water temperatures were >15°C.

In the winter/spring study, LeChevallier (2019) reported that four (0.7%) distribution samples failed to be confirmed by serotyping. The bacteria that grew in these samples were identified by 16s analysis as *Brevundimonas vesicularis*, *Sphingomonas koreensis*, *Ochrobactrum intermedium*, *Brevundimonas diminuta*, and *Elizabethkingia anopheles*. In this second round of monitoring, four source water samples and three potable water samples failed to be confirmed by serotyping. These organisms were identified as *Elizabethkingia* spp., *Serratia marcescens*, and *Stenotrophomonas maltophilia*. One sample was delayed in shipping and could not be subcultured (but was counted as a “not confirmed” sample). The true positivity rate in this study (rounds 1 and 2) was lower than the Legiolert specificity reported by Sartory et al. (2017), where 14 of 290 premise water samples (4.8%) did not serotype as a *L. pneumophila* strain, or by Spies et al. (2018), who reported that the Legiolert method had a specificity for *L. pneumophila* of 97.9% (2.1% nonconfirming) for premise samples, both of which are comparable to the 95.3% specificity for the ISO 11731 method. Legiolert nonpotable evaluations have primarily been conducted with cooling towers, for which the true positivity rate was reported to be 95.1% (Rech et al., 2018). The differences observed in this study for both the potable and nonpotable evaluations compared with other published results are likely derived from both the novel sample types evaluated and the paucity of overall positive samples. Because of this, it is recommended that utilities serotype all positive Legiolert samples, both to confirm that *L. pneumophila* is present and to better understand the public health significance of the specific isolates based on their serotype reaction. Although all serogroups of *L. pneumophila* are known to cause disease, according to the CDC (2019), most cases of Legionnaire’s disease are caused by *L. pneumophila* serogroup 1.

### 3.2 Utility 6 case study

The occurrence of seven positive *L. pneumophila* samples in the distribution system of utility 6 provided an excellent case study to demonstrate one utility’s response to manage *L. pneumophila* occurrence and to communicate the results to the public. Figure 3 shows the timeline for detection of *L. pneumophila* and the free chlorine residuals in the distribution system. During this time, the water temperatures ranged between 22.9 and 31.6°C. Sets of samples were collected several times a week from September 11, 2018, to September 27, 2018, with follow-up repeat samples collected until October 9, 2018. The first positive *L. pneumophila* sample was obtained on September 18 at site 824 at a concentration of 6 MPN/100 mL. Site 824 was in a developing area of the system that had plans for several hundred homes, but only 73 houses have been built. The area is served by a 12 in. (30.5 cm) main, so detention times are currently very long. Because of the 7-day incubation, the positive result was not realized until September 25, and repeat samples were collected the following day as per the response protocol published earlier (LeChevallier, 2019). The sample was also sent to IDEXX Laboratories Inc. for serotyping. The free chlorine residual on the September 18 site 824 sample was 0.12 mg/L, which was low but not unusual for the system that normally maintained an average of 0.4 mg/L within it.

Two days later, a second sample, collected on September 20, was positive for *L. pneumophila* at a concentration of
2 MPN/100 mL, but this sample was from a different part of the distribution system where free chlorine residuals were 0.62 mg/L. Subsequent resampling at this site was negative.

On September 26, four of the nine samples collected that day were positive for *L. pneumophila*, with some of the concentrations as high as 522 MPN/100 mL. Moreover, the free chlorine concentration in the area of site 824 ranged between 0.01 and 0.09 mg/L. In addition to resampling the original site 824, sites upstream and downstream points were analyzed. All three were positive for *L. pneumophila*. In response to the low chlorine residuals, the utility flushed the area around site 824 on September 27 and again on October 2 after the repeat samples were taken. The sites were flushed for 3 h or until a 0.5 mg/L residual was achieved. However, because of the persistent low chlorine residuals (0.03–0.06 mg/L) even after the flushing, the utility installed an automatic flushing valve on October 5 that flushed 7,200 gal eight times a day to achieve a 5-day turnover of water in that zone of the system. The result of all the flushing was that free chlorine residuals increased to 0.27–0.51 mg/L, and samples were no longer positive for *L. pneumophila* (Figure 3). The episode highlighted the need for the utility to focus on areas of low chlorine residuals, and capital was reallocated to make improvements in storage tank mixing to enhance the maintenance of free chlorine residuals throughout the system.

Because of the limited area of the distribution system that was affected by the high *L. pneumophila* samples, a decision was made to notify customers door to door to inform them of the situation. Talking points were developed based on the CDC fact sheet on *Legionella* (CDC, 2019), and the chief executive officer and the communications director visited each customer on October 10 to explain what had happened and steps the utility was taking to correct the situation. Where customers were not at home, a door hanger was left with information and a number to call if there were questions. Overall, the customers were appreciative of the outreach, and only one call was received—from a utility employee who wanted to better understand the situation so that he or she could answer any questions from friends or neighbors.

The utility also contacted the health department (which regulates public water systems) to inform it of the sampling results and the actions that the utility took to flush the system and restore free chlorine residuals. The utility also reviewed the plans for the door-to-door outreach to customers and the communication materials. The health department was already aware of the utility’s participation in the sampling program and approved of the actions.

Reflecting on the experience, the director of water quality commented that the proactive detection of *L. pneumophila* in the absence of any illness was a good news story and a boost for the utility’s water quality efforts. If the utility had never conducted the monitoring as part of the study, it would not have been aware of areas of low chlorine and the risk that it might pose to its customers. The episode reinforced the attitude within the entire utility to go beyond just meeting the regulations to produce the highest quality possible for their customers. The support from the health department and the understanding from utility customers produced an overall positive result. The study prompted the utility better focus capital resources on improvements that will maintain water quality throughout the system.

## 4 SUMMARY AND RECOMMENDATIONS

A prior study of 12 potable water distribution systems detected *Legionella pneumophila* in only one of 576 samples (0.17%), but the survey was conducted during winter and spring months when water temperatures were generally below 15°C (LeChevallier, 2019). Ten of the utilities agreed to participate in a second round of testing during the summer and early fall months when water temperatures averaged 23°C (averages ranged from 14 to 29°C). *L. pneumophila* was enumerated using the Legiotolert assay. A total of 669 samples were analyzed during the study: 50 from source water, 46 from the plant effluent, and 573 from the distribution system. *L. pneumophila* was detected in two source water samples and not detected in any of the treated plant effluent samples, suggesting that current treatment was effective for managing the organism in treated supplies. There was also no relationship between *L. pneumophila* and either total coliform bacteria or *E. coli*, indicating that these indicator organisms are not valid predictors of *L. pneumophila* occurrence. Of the 10 distribution systems, five examined had at least one positive *L. pneumophila* sample: 13 of the samples were from free chlorinated systems and one was from a chloraminated system. The difference in the occurrence of *L. pneumophila* between free chlorine and chloraminated systems could be explained by the greater stability of the chloramine residual within the distribution system.

All occurrences of *L. pneumophila* occurred when water temperatures were >18°C. This information could be used to guide the development of sampling programs for water utilities. Systems could focus monitoring efforts when water temperatures were warm (>15°C), especially in areas of the distribution system where disinfectant residuals are low (<0.2 mg/L). Should the USEPA include *Legionella* monitoring in the next round of the Unregulated Contaminant Monitoring Rule (USEPA, 2019), resources and expense could be minimized if testing was targeted to periods when water temperatures were >15°C.
The highest concentrations of *L. pneumophila* occurred when chlorine residuals were less than 0.1 mg/L. The results demonstrate that *L. pneumophila* can colonize and grow within drinking water distribution systems under the right temperature conditions and low disinfectant residuals. *L. pneumophila* was no longer detected at sample sites after the area was flushed, and free chlorine residuals were maintained at levels >0.1 mg/L. The data suggest that utilities should maintain a disinfectant residual of at least 0.1 mg/L in all parts of the distribution system. In addition, the data show that low concentrations of *L. pneumophila* may occur even when free chlorine residuals are more than 1.0 mg/L. The one chloraminated sample that was positive may occur even when free chlorine residuals are more than 0.1 mg/L in all parts of the distribution system. In addition, they should maintain a disinfectant residual of at least 0.1 mg/L. The data suggest that utilities should maintain disinfectant residuals greater than 0.1 mg/L. The occurrence and communication of *Legionella pneumophila* is present and to better understand the public health significance of the isolates.

The occurrence of seven positive *L. pneumophila* samples in the distribution system of utility 6 provided an excellent case study to demonstrate one utility’s response to manage *L. pneumophila* occurrence and communicate the results to the public. If the utility had never conducted the monitoring as part of the study, it would not have been aware of areas of low chlorine and the risk that it might pose to its customers. The episode reinforced the attitude within the entire utility to go beyond just meeting the regulations to produce the highest quality possible for their customers. The support from the health department and the understanding from utility customers produced an overall positive result. The study helped the utility to focus capital resources on improvements that will better maintain disinfectant residuals throughout the system.

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