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Legionnaires' Disease Outbreaks and Cooling Towers with Amplified *Legionella* Concentrations

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Abstract. This study was designed to investigate the possible association of high colony counts of legionellae from cooling towers and evaporative condensers with Legionnaires' disease outbreaks. We obtained legionellae counts from samples of cooling towers and evaporative condensers that were the likely sources of two different Legionnaires' disease outbreaks and compared these counts with those from cooling towers that were not associated with reports of human disease. Among 675 potential control cooling tower water samples from 258 facilities, 136 facilities had one or more cooling towers that met our criteria for inclusion into the study. Samples taken from buildings where an outbreak had occurred had much higher *Legionella* counts than did samples from other buildings. Colony counts from the two outbreak-associated facilities were significantly higher than colony counts from other facilities [Wilcoxon Rank Sum Test (Exact), $p < 0.01$]. The results of the study suggest that, among cooling towers that test positive for the presence of legionellae, higher colony counts are associated with higher risk of Legionnaires' disease.

Legionellosis, including both Legionnaires' disease and Pontiac fever, is caused by organisms of the genus *Legionella*. An estimated 25,000 to 100,000 cases of Legionnaires' disease occur in the United States annually [1]. Most occur as sporadic cases, and the source of exposure often remains undetermined. Previous studies have shown cooling towers and/or evaporative condensers to be responsible for outbreaks [4, 9]. Several investigators have suggested an increased risk of Legionnaires' disease from exposure to contaminated building sources [2, 10]; however, no relation with *Legionella* counts has been established. The significance of the presence of *Legionella* bacteria in building water systems in the absence of disease is often downplayed owing to the ubiquitous nature of the organism [4, 6, 9, 12].

Published investigations on outbreaks of Legionnaires' disease that have established the exposure source seldom include colony counts of *Legionella* bacteria from the identified exposure source. At present, routine environmental culturing is not recommended when sporadic cases occur because no association has been developed between degree of contamination and disease [4, 6, 9, 12], and sporadic community acquired illnesses are usually not known to be associated with further cases. Thus there is a tendency to wait for a contaminated building water system to be associated with illness before any interventions are initiated [9, 12]. Because a large percentage of cooling towers have been shown to contain *Legionella* [13], further study of the association between legionellae concentrations in cooling towers and Legionnaires' disease is warranted. The need for this information was recognized by Fraser in 1980. He called for studies to demonstrate the risk of legionellosis in relation to concentration of *L. pneumophila* in cooling tower or evaporative condenser water [8]. This study is an initial step in the investigation of such an association.

Materials and Methods

In this study, we compared colony counts in samples taken from cooling towers (or evaporative condensers) that were implicated epidemiologically as the source of an outbreak of Legionnaires' disease with the colony counts in samples taken from cooling towers and evaporative condensers not associated with an outbreak.

All outbreaks of Legionnaires' disease that occurred in the U.S. between 1988 and 1991 were eligible for entry into the study if the implicated source was a cooling tower or evaporative condenser. We identified outbreaks from a listing of outbreaks

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investigated by the Centers for Disease Control (CDC) between 1983 to 1991 [1]. We obtained legionellae counts from one of the outbreak-associated cooling towers from a publication describing the investigation performed by the CDC in a peer-reviewed journal [4]. For the other outbreak, the legionellae counts from the implicated cooling towers were determined by our laboratory. Only those outbreaks with two or more cases of Legionnaires' disease were eligible, and only those outbreaks with patients that showed symptoms of pneumonia, radiographic evidence, and culture-positive sputum were included in the study. We excluded outbreaks that occurred from sources other than cooling towers or evaporative condensers. We identified two outbreaks that met these criteria.

Outbreak description. Outbreak 1, investigated by our laboratory, occurred in a hospital, and the cooling towers were implicated as the source. The outbreak strain was *Legionella pneumophila* serogroup 1, monoclonal subtype 1, 2, 5, 6. The outbreak consisted of three cases that occurred from June, 1989, to August, 1989. All cases were hospitalized patients who were located in a single building at the hospital. The cooling towers, which were connected side by side, were a common exposure source for all cases since all cases were on the same wing, on the same floor, and the air intakes of the building were situated downwind from and faced the cooling tower drift. The potable water system was also a common exposure source to all cases. However, no viable *Legionella pneumophila* were detected from the potable water system after three thorough investigations. The samples from two cooling towers yielded *Legionella pneumophila* serogroup 1, monoclonal subtype 1, 2, 5, 6 and *Legionella pneumophila* serogroup 1, monoclonal subtype 1, 6 at concentrations ranging from 120 colony forming units (CFU)/ml to 3600 CFU/ml (mean = 1917 CFU/ml, n = 6). The high variation in colony counts resulted because one of the towers had much higher counts than the other tower.

Outbreak 2 was associated with an evaporative condenser at a retirement hotel between June 10 and July 22, 1988 [4]. The evaporative condenser was epidemiologically implicated as the source of the outbreak, which included six cases [4]. The association was also supported by microbiological air sampling. The outbreak strain was *Legionella pneumophila* serogroup 1, monoclonal subtype 1, 2, 5, 6. The outbreak strain was demonstrated in the evaporative condenser water at concentrations greater than 9000 CFU/ml [4].

We compared the colony counts in samples from these outbreak-associated towers with counts in control samples from other cooling towers. All samples sent to our laboratories for analysis from cooling towers or evaporative condensers from 1988 to 1991 were eligible if the building was not reported to be associated with an outbreak. Cooling tower water samples (n = 675) were analyzed for *Legionella* concentrations from 258 buildings with no previously known association with Legionnaires' disease. Because of the similarity between cooling towers and evaporative condensers with respect to design, operation, and potential for exposure, evaporative condensers were considered as cooling towers in this study. Cooling tower samples that tested negative for *Legionella* were excluded from the comparison group. If we had several samples from cooling towers or evaporative condensers from the same building, we used the mean concentration.

Control water samples were collected and sent to the laboratory, next day (AM) delivery. All samples were processed on the day of arrival by the laboratory for viable *Legionella*. All samples, including the one outbreak sample sent to our laboratory, were analyzed in duplicate. Samples were analyzed with Buffered Charcoal Yeast Extract agar (BCYE) with selective antibiotics and glycine as previously described [5]. Increased sensitivity was obtained by filter concentration of 100 ml through a 0.2 micron Nuclepore polycarbonate membrane followed by resuspension in 1 ml of filtered sterile water for further analysis. Samples were treated by the method of Bopp et al. [3] as previously described only if a significant number of competing non-legionellae bacteria were detected.

Typical colonies on BCYE agar were isolated, purified, and tested for cysteine requirement. Cysteine-requiring cultures were analyzed for immunofluorescent reactions against poly- and monovalent *Legionella* antisera to determine species and serogroups.

Monoclonal subtyping was performed on the environmental and clinical isolates by the CDC by methods previously described [11].

We used an exact version of the Wilcoxon Rank Sum Test to test the null hypothesis of no difference between colony counts of cooling towers associated with an outbreak and those of other towers. We restricted this analysis to the warmer cooling tower operational months of May, June, July, August, September, and October. As well, frequency tables of the mean numbers of *Legionella* in control cooling tower samples per facility were created. The mean numbers of *Legionella* from the control towers were compared with numbers in "outbreak" towers to study differences between the two groups.

For additional descriptive analysis we constructed histograms to summarize the relative frequency of the mean CFU/ml from control facilities. We classified counts as "high" if the mean CFU/ml was greater than or equal to 1600, the upper 5 percentile of mean CFU/ml from control building cooling tower samples. All counts less than 1600 were considered "low." We assessed the association between outbreaks and "high" counts, using Fisher exact confidence limits. The upper 5 percentile was chosen arbitrarily for purposes of analysis, not to suggest this as a guideline level.

Results

We processed 675 cooling tower samples from 258 facilities that met our criteria. We processed 12 control cooling tower samples from 8 facilities in 1988, 65 control samples from 21 facilities in 1989, 190 control samples from 71 facilities in 1990, and 408 samples from 158 facilities in 1991. We excluded 122 of these comparison facilities because the colony counts were zero. Of the 122 excluded facilities, 8, 11, 30, and 73 were excluded in the years 1988, 1989, 1990, and 1991 respectively. None of these excluded facilities was known to be associated with an outbreak. The final comparison population was 286 cooling tower samples from 136 facilities. As shown in Fig. 1, mean colony counts from the comparison cooling towers were substantially lower than those from the outbreak-associated towers.

By restriction of the analysis to the warmer cooling tower operational months, outbreak samples ranked first and eighth out of 101 total samples. Using the Wilcoxon Rank Sum Test (Exact), outbreak

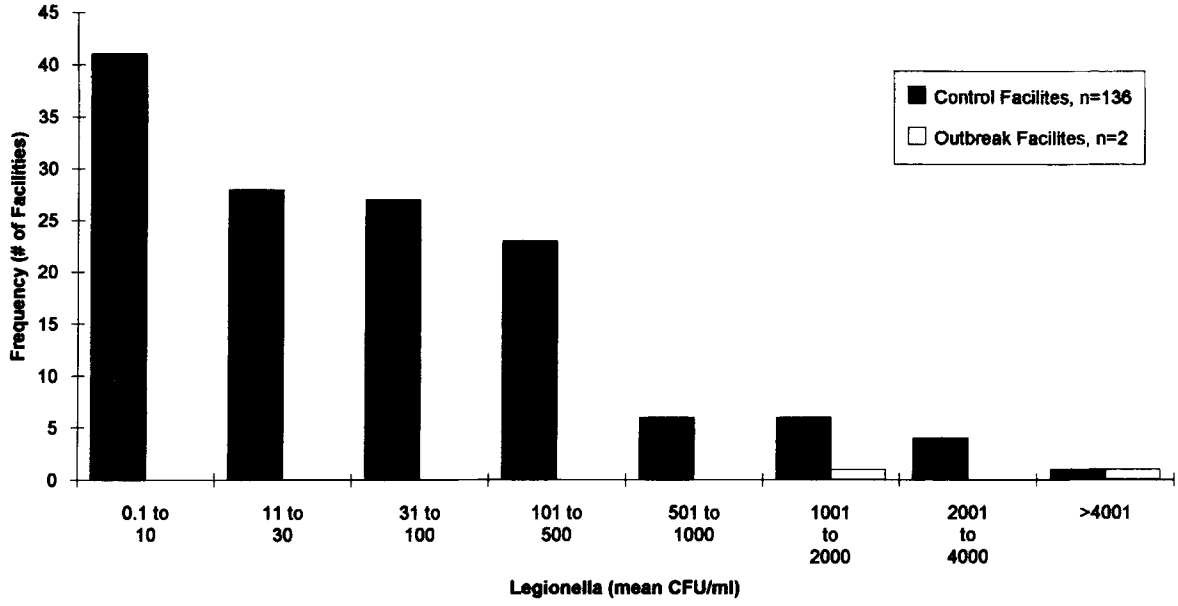

Fig. 1. Distribution of *Legionella* colony counts in positive facilities.

Table 1. Wilcoxon Rank Sum Test (Exact), p-value = 0.0063 controlling for cooling tower operational months May through October

Mean CFU/ml per facility	Number of outbreak facilities	Number of control facilities
≥0 and <100	0	68
100–499	0	18
500–999	0	5
1000–1499	0	0
1500–2999	1	5
≥3000	1	3

samples had significantly higher colony counts than the control samples, p-value = 0.0063. Table 1 shows the distribution of *Legionella* counts in cooling tower water samples, restricted to cooling tower operational months. Analysis of the unrestricted data was also statistically significant.

Legionella concentrations from each of the different outbreak-associated cooling towers were above 1600 CFU/ml, the upper 5 percentile of the comparison group. Thus, only 8 of 136 facilities with no outbreak (10 of 286 positive samples) had mean colony counts equal to or above 1600 CFU/ml compared with 2 of 2 facilities associated with an outbreak. The odds ratio was infinite with a 95% Fisher Exact lower confidence limit of 2.6, again indicating that high colony counts are very strongly associated with having had an outbreak (Fisher Exact P-value = 0.005).

Discussion

The results of our study suggest a strong relation between high *Legionella* counts in cooling towers with Legionnaires' disease. An extensive epidemiologic investigation to identify a common source was conducted for one of the outbreaks, as previously described (outbreak 2). Although an extensive epidemiologic investigation was not performed in outbreak 1, all three cases were located on the same side of the building that faced the cooling towers, the air intakes faced the cooling towers, the cooling towers were the only source at the facility to contain the same *Legionella* subtype as the outbreak strain, and no further cases occurred once the cooling towers were decontaminated. This further suggests that the cooling towers determined to be the cause for outbreak 1 were the actual exposure source of the outbreak.

As with other infectious organisms, our results suggest that the higher the concentration of *Legionella* in an exposure source, the higher the risk of disease. Previous epidemiologic studies have demonstrated an association of Legionnaires' disease with both duration and proximity of exposure to a legionellae-contaminated source with Legionnaires' disease [4], yet none have attempted to associate the concentration of *Legionella* in the source of exposure.

Our hypothesis—that higher colony counts are associated with higher risk—is biologically plausible. It is plausible to conclude that higher legionellae counts in cooling tower water would result in higher

legionellae counts in air. The concentration of *Legionella* in cooling towers should be a crude measure of potential for exposure, even though the distance from cooling towers to susceptible individuals may vary. Although the infective dose of *Legionella* is not known, if the infective dose required to cause Legionnaires' disease is greater than one CFU, then concentration is important. Because infective dose presumably varies among individuals because of differences in susceptibility, the number receiving an infective dose should increase as the concentration in an exposure source increases. Therefore, it is plausible that the risk should be higher among those exposed to sources with higher colony counts.

In reviewing the literature, we identified additional outbreaks that did not meet our eligibility criteria. These outbreaks were excluded because they occurred outside the United States and/or were Pontiac fever rather than Legionnaires' disease outbreaks and/or were unpublished in the scientific literature and/or occurred in a year when control samples were not collected. In general, colony counts from these outbreaks were high and consistent with our results. For example, colony counts from highly suspected cooling tower samples from three different outbreaks in Australia were high. The mean levels from the cooling towers associated with the Daw Park, Wollongong, and Bumie Tasmania outbreaks were 5500 CFU/ml (Dr. Trever Steele, 1992, personal communication) (n = 2; 1000 CFU/ml and 10,000 CFU/ml), 2000 CFU/ml [7], and 280,000 CFU/ml (Dr. Trever Steele, 1992, personal communication) respectively. An outbreak of Pontiac fever in the U.S. was associated with a cooling tower with colony counts greater than 10,000 CFU/ml (Dr. James Barbaree, 1992, personal communication). An outbreak associated with a cooling tower that occurred in a U.S. prison, however, yielded only 10 CFU/ml (Dr. Barry Fields, 1992, personal communication), and a cooling tower associated with an outbreak currently under investigation yielded a *Legionella* count of 1500 CFU/ml in the laboratories at the CDC (CDC Memorandum Epi-92-64-1, March 22, 1992), and in our laboratory it yielded a *Legionella* count of 2800 CFU/ml (mean = 2150 CFU/ml). A cooling tower outbreak that occurred in 1983 had colony counts of 6000 *Legionella* CFU/ml [9]. These outbreaks were not included in the analysis, but appear to support our findings. Although only two outbreaks met our inclusion criteria, the result is quite stable.

There is a potential for selection bias in the control group, as these were essentially volunteer samples. For example, facilities with low legionellae counts may have preferentially submitted samples. This would have biased the results away from the null, and the true association would be less than the results reported in this study. Alternatively, a bias may exist in the opposite direction, as there may have been a tendency for facilities with indoor air quality health complaints to preferentially submit samples. This would have biased the results towards the null, and the true association would be even higher than the one described in this study. A potential for misclassification bias also exists, since we compared analytic results from two different laboratories. For example, if the laboratory that analyzed the comparison samples and outbreak 1 tended to record lower counts than the laboratory that analyzed outbreak 2, then the true odds ratio would be lower than the odds ratio reported in this study. On the other hand, if the laboratory that analyzed the comparison samples and outbreak 1 tended to record higher counts than the laboratory that analyzed outbreak 2, then the true odds ratio would be higher than the odds ratio reported in this study.

Some potential limitations of our study include (i) the small numbers of outbreaks included, (ii) failure to study other factors that may predict disease risk such as the virulence of the organism, the distance from the target person to the source, and the susceptibility and immune status of the target person, (iii) concentrations from the analyzed sample may not represent concentrations in the source at the time of the outbreak, (iv) lack of randomly sampled comparison facilities, (v) not all outbreaks were investigated, and the total number of outbreaks that occurred in the U.S. is unknown, and (vi) samples from one of the outbreaks [4] could have been handled differently from other samples. These limitations could not be addressed in this study because of its retrospective nature, but would be important to consider in subsequent studies. Despite these limitations, the results of our study suggest that cooling towers with very high colony counts may be substantially more likely to be the source of an outbreak than cooling towers with lower counts.

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