THE EPIDEMIOLOGY OF SPORADIC, COMMUNITY-ACQUIRED LEGIONNAIRES' DISEASE

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ABSTRACT

Legionnaires' disease (LD) is the second most common type of bacterial pneumonia in the United States. It disproportionately affects elderly and immunocompromised individuals and can lead to disability and death. LD is caused by the waterborne bacterium *Legionella* which is found in many aqueous environments and amplified in man-made structures such as cooling towers and building water systems. LD transmission occurs through inhalation or aspiration of *Legionella* contaminated water. The majority of LD cases in the US and worldwide are community-acquired with no known association with other cases, which are referred to as sporadic cases. The environmental source is often unknown, thus hindering targeted control measures. The overall objective of this dissertation is to better define the epidemiology of sporadic, community-acquired LD in order to inform targeted public health interventions. This objective was addressed through three studies. The first was a literature review of environmental sources of sporadic, community-acquired LD. We found that residential potable water, large building water systems and car travel contribute to a substantial proportion of sporadic LD. Cooling towers may also be a significant source, but definitive linkage to sporadic cases is difficult. The second study assessed the prevalence of *Legionella pneumophila* bacteria in Allegheny County cooling towers. We found *L. pneumophila* in almost half of cooling towers

tested; however, the concentration level was relatively low. Facilities were encouraged to develop a water management plan and conduct annual basin water emptying, quarterly cleaning, quarterly *Legionella* testing and diligent inspection of older towers. The third study is a prospective simulation of community-acquired LD spatiotemporal cluster detection is presented in chapter four to demonstrate the utility and performance of this method in Allegheny County. Larger, cooling tower-associated simulated outbreaks were detected. Health departments should consider adopting this method for improved LD outbreak detection, faster investigation initiation and potential disease prevention. Overall, the findings of these three complementary studies are of public health relevance given they inform locally-focused intervention strategies for LD prevention. LD is a costly disease and interventions should be efficiently tailored for local response. Health departments should allocate resources for locally-focused interventions to reduce LD incidence.

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1.0 INTRODUCTION

This dissertation topic stemmed from observations made in Allegheny County, PA, with regard to Legionnaires' disease (LD) epidemiologic trends. These trends are also observed nationally and globally. Most LD is community-acquired with no known association with other cases and the environmental source is unknown. Each chapter of this dissertation contributes to an improved understanding of the epidemiology of sporadic, community-acquired LD in general and more specifically in Allegheny County. The following introductory sections provide necessary context for each dissertation chapter. First, general information about the disease, the bacterial cause, and incidence is presented. Second, sporadic, community-acquired LD is defined in more detail and the importance of cooling towers as an environmental source of sporadic and outbreak-related LD is discussed. Finally, the burden of LD in Allegheny County is presented to rationalize the necessity and advantage of studying LD in this part of the US.

1.1 LEGIONELLOSIS

Legionellosis is an infectious respiratory condition which encompasses both LD and Pontiac Fever. Both conditions are caused by the Gram-negative bacteria, *Legionella*. LD is the second most common form of bacterial pneumonia in the US [1]. Pontiac fever is a less common, milder febrile illness that is usually self-limiting. Henceforth, LD will be the focus of this dissertation.

LD occurs when patients inhale or less commonly aspirate water contaminated with *Legionella* bacteria [2]. The incubation period can range from 2 to 14 days but is most commonly 2 to 10 days. Elderly individuals are susceptible to LD as well as people who are immunocompromised, smokers, or have underlying conditions such as chronic respiratory diseases [2]. Symptoms include high fever, cough, chills, shortness of breath and sometimes headache. About half of LD patients exhibit a productive cough. Neurological and/or gastrointestinal symptoms can also accompany pneumonia in LD patients [2]. LD also causes neurological and/or neuromuscular symptoms and some patients experience post-traumatic stress disorder. Generally, mortality rates in the US range from 5 – 10% for community-acquired cases and about 20% for nosocomial cases, but patients who are immunocompromised experience higher mortality rates. About 12% of community-acquired cases in Europe are fatal [3].

1.1.1 *Legionella*

To date, 58 species of *Legionella* have been discovered [2]. *Legionella pneumophila* accounts for an estimated 90% of *Legionella* infections in the US [4] *L. pneumophila* is also the most virulent [2]. *Legionella* are found naturally in soil and water; however, *Legionella* are amplified under certain conditions in man-made structures which can lead to disease outbreaks [2]. These include warm temperature, stagnant conditions, increased sediment and biofilm. Optimal growth of *Legionella* occurs between 77-107.6℉ [3, 5]. Deactivation was previously thought to start at about 122℉; however, more recent research indicates 123.8℉ could provide a unique growth environment given deactivation of other inhibitory microbes [6]. *Legionella* thrive as intracellular parasites of organisms such as amoebae, ciliated protozoa and slime molds. *Legionella* multiply in biofilm when amoebae are present but only subsist in the absence of amoebae [4].

The following are confirmed sources of LD outbreaks and single cases where the *Legionella* strain that caused patient disease was indistinguishable from the *Legionella* strain found in an environmental source: building water systems potable water, residential potable water, evaporative cooling towers, whirlpool spas, potting soil and compost (*L. longbeachae)*, bath water, indoor and outdoor decorative fountains, wastewater treatment plants, room humidifiers, ice and ice machines, mist machines, air conditioning systems, cooling liquid for machinery, and natural sources like soil and springs [7]. Unconfirmed, but possible sources of LD include water added as windshield wiper fluid in motor vehicles, medical respiratory equipment, water used for cleaning, dental exposures, roof harvested rainfall, construction and excavation, ground and surface water, and rainwater from puddles [7].

Prevention measures for reduced *Legionella* growth have been shown to be effective for various man-made environments (i.e. building water systems, cooling towers) where *Legionella* tend to proliferate; however, eliminating the bacteria from water sources is difficult given the non-sterile state of naturally occurring water sources [4]. In 2016, the US Environmental Protection Agency published 'Technologies for *Legionella* control' which summarized disinfection methods for building water systems such as hotels, hospitals and large apartment buildings [8]. The summarized technologies include chlorine, monochloramine, chlorine dioxide, copper-silver ionization, ultra violet light, and ozone. The methodology as well as advantages and disadvantages were presented for each technology. This document also summarized the utility of point-of-use filters as well as emergency remediation techniques such as superheat-and-flush [8].

1.1.2 Legionnaires' Disease Incidence

A reported increase in LD incidence has been observed in multiple developed countries around the globe [9-11]. In the US, reported LD incidence has more than doubled, from 0.78 cases per 100,000 population in 2003 to 1.58 in 2013 [12]. This change in reported incidence differs by US geographic region, with the Mid-Atlantic region experiencing both the highest incidence rate and largest increase in incidence [9].

Many reasons for this increase have been offered including changes in LD diagnostic testing and increased awareness of the need for LD diagnostic testing among healthcare providers. In clinical practice, urinary antigen detection is the most commonly used diagnostic test for LD; however, it only detects *L. pneumophila* serogroup 1, which is also the serogroup and serogroup that most frequently causes LD. The urinary antigen test is also used most frequently because it is a quick diagnostic tool, urine is a readily-available specimen, and *Legionella* are particularly difficult to culture [2]. A major limitation of the urinary antigen test is that it is culture-independent and therefore provides no bacterial isolate for molecular epidemiologic studies of transmission. Urinary antigen usage for LD diagnostics has increased since the mid-1990s; however, researchers do not believe that this change in diagnostic practice is entirely responsible for the significant increase in LD seen worldwide [13].

Quantitative real-time polymerase chain reaction (qPCR) has also become more widely used in recent years because it produces results quickly and can detect more serogroups and species of *Legionella* than the urinary antigen test. Similar to the urinary antigen test, qPCR also does not produce an isolate for the studies of transmission. Culture is the gold-standard diagnostic test for LD. However, specimens for culture can be difficult to obtain because LD patients often do not produce sputum. Without culture, patients infected with *L. pneumophila* serogroups 2 – 14 and other *Legionella* species cannot be diagnosed. Also, clinical cultures are required in the event of an outbreak to determine *Legionella* strain relatedness among cases and between case isolates and environmental isolates.

Other postulates for the increase in LD incidence include changes in *Legionella* favorable weather conditions, more frequent installation of building cooling towers to increase energy efficiency, and changes in residential water sources from ground to surface water [11, 13-15]. It is likely that a combination of these factors and others led to increased LD incidence given the plethora of LD waterborne sources.

1.2 SPORADIC LEGIONNAIRES' DISEASE

LD cases can be classified into two categories: outbreak and non-outbreak cases. Furthermore, outbreak and non-outbreak cases can be classified as healthcare-acquired or community-acquired. This dissertation will generally focus on non-outbreak, community-acquired LD. A non-outbreak case is defined as a case not attributed to an outbreak investigation and thus has no known association with other cases. An LD outbreak is defined as two or more cases sharing a common exposure during each case's incubation period such as a hotel stay or exposure to a specific water source [9]. The term 'sporadic case' is used in the literature to describe non-outbreak cases [2, 16, 17]. Nevertheless, public health resources, specifically in the US, sometimes prevent investigation of sporadic cases that could uncover common exposures between cases and thus outbreaks. Spatial-temporal cluster detection is discussed in the fourth chapter of this dissertation. This method has the potential to uncover outbreaks among seemingly sporadic cases; however, false alarm signals are also generated using this method.

The majority of cases reported in the US and worldwide are classified as sporadic with no confirmed source of infection [4, 18]. A review article published in 1995 by Bhopal summarized known sources of sporadic disease including home water sources and cooling towers; however, the author emphasized that more large-scale research needed to be conducted before drawing conclusions about the major sources of sporadic LD [16]. Sources of sporadic LD will be reviewed in the second chapter of this dissertation.

1.2.1 Cooling Towers and Legionnaires' Disease

Evaporative cooling towers have been associated with both sporadic and outbreak-related LD [14, 19]. Evaporative cooling is a technique used to cool large air conditioning systems, refrigeration systems and industrial processing systems [20], with the goal of increasing energy efficiency. Cooling towers and evaporative condensers utilize evaporative cooling technology and produce aerosolized water as a byproduct. Cooling towers are classified as open-circuit or closed-circuit. Open-circuit cooling towers are the most common type of evaporative cooling system [20]. Warm water produced by an air conditioning system, for example, is sprayed into the tower and cooled over a large surface area which includes a type of media called fill. A fan also cools the fill area. The cooled water then travels out of the tower and back to the system from which it originated.

Closed-circuit cooling towers and evaporative condensers are structured similarly [20]. They include a heat exchange coil that allows a system coolant to cool over an enclosed pipe surface. Closed-circuit systems cool a water coolant, while evaporative condensers usually cool a refrigerant gas [20]. The material enclosed in the heat exchange coil is cooled using the same mechanism as an open-circuit system. Both fans and a large surface area are used for cooling.

Open-circuit and closed-circuit cooling towers as well as evaporative condensers can provide an environment for *Legionella* growth if water temperatures are warm (68 – 113˚F) and biofilm, rust and/or scale are present [20]. In some systems, water can be stored and recirculated. If not maintained, storage and recirculation of cooling tower or evaporative condenser water can contribute to *Legionella* proliferation [5, 20, 21]. If *Legionella* grows in the system, contaminated water is aerosolized and people can be exposed to the bacterium. In addition to outbreaks, cooling towers have also been linked to sporadic LD [22]. Cooling tower outbreaks may also be difficult to detect because cases are often unaware of cooling tower exposure.

Maintenance strategies are challenging to generalize given the variability in individual cooling systems. The American Society for Heating and Air-Conditioning Engineers (ASHRAE) published Guideline 12-2000, which describes strategies for evaporative cooling system cleaning, disinfection, scale and corrosion control, and appropriate start up and shut down of the system [5]. This document describes various biocide options such as oxidizing and/or non-oxidizing biocides and ultimately concludes that biocide choice should be made by someone with an understanding of water chemistry and microbiology as well as a thorough understanding of the specific system.

In 2015, ASHRAE published 88-2015 which describes risk minimization through development of a building water system water safety plan [21]. If a building water system includes an evaporative cooling system, the cooling system must be addressed in the water safety plan. Other guidelines have been published, but no prescriptive guideline for evaporative cooling system maintenance exists. A study published by Rangel et al. reviewed available guidelines as well as cooling tower-related outbreaks [23]. They suggest that vague guidelines could be contributing to inadequately-maintained cooling towers and thus transmission of *Legionella*. From this point forward, the dissertation will discuss cooling towers rather than the more general category of evaporative cooling systems given that cooling towers are the most common of these systems.

During the summer of 2015, the New York City Department of Health and Mental Hygiene (DOHMH) investigated an LD outbreak caused by a hotel cooling tower that sickened 138 people [19]. As a result of this outbreak, all cooling towers are now regulated in the city and state of New York [24]. These regulations require registration, quarterly certified inspection, annual certified cleaning, quarterly *Legionella* testing and development of an ASHRAE 188-2015 compliant water safety plan. The relationship between LD incidence and cooling tower regulation is unclear [23]. An evaluation of the impact of the DOHMH regulation is currently in progress [19]. These regulations were the first of their kind in the US. Cooling tower registration alone could aide in outbreak investigations and spatiotemporal cluster detection.

1.2.2 Spatiotemporal Legionnaires' Disease Cluster Detection

The 2015 New York City cooling tower-associated outbreak was initially detected using a spatiotemporal cluster detection program [25]. This specific program utilizes a space-time permutation scan statistic which is available in SaTScan^{TM}, a free software program [26, 27]. The space-time permutation scan statistic is appropriate for retrospective and prospective cluster detection and does not require an estimation of underlying population-at-risk.

The space-time permutation scan statistic identifies clusters by cylindrically analyzing a spatial area using thousands or millions of scanning windows or cylinders of various heights and widths. Each scanning window is centered on a census tract centroid. The scanning window height is time and width is spatial area. Numerous scanning windows are created using predetermined parameters. For LD cluster detection, the maximum scanning window height or temporal window is either 30 days or 180 days; the maximum scanning window width or spatial area is the area of 50% of the cases reported in the study period [28]. The study period is one year when using a maximum temporal window of 30 days and two years when using a maximum temporal window of 180 days.

The significance of an LD case cluster occurring in each scanning window is determined by comparing the likelihood ratio test using actual LD case data and the likelihood ratio test results using 999 Monte Carlo simulated datasets. The p-value of the test statistic is calculated by dividing the rank of the likelihood ratio test using the real LD case data by the number of simulations plus one. The reciprocal of the p-value, the recurrence interval (RI), determines significance. The RI is defined as the number of days of analyses required in order to expect the number of clusters at least as unlikely as the observed cluster to be equal to 1 by chance alone [26].

Several New York City LD clusters have been successfully detected by this cluster detection program performed daily by DOHMH [28]. This detection program has the potential to improve surveillance efficiency and relate seemingly sporadic cases. Relating sporadic cases in space and time could help pinpoint a harmful environmental source and lead to remediation efforts and potentially disease prevention.

1.2.3 Allegheny County, PA LD Incidence

Allegheny County, Pennsylvania, located in the Mid-Atlantic region of the US, experiences high rates of LD [29, 30]. Allegheny County encompasses the city of Pittsburgh and the surrounding suburbs. The proportion of Allegheny County residents 65 years and older is larger than the proportion in the US as a whole, and elderly individuals are at higher risk of LD; however, over the last decade, Allegheny County's average LD rate has remained above 4 per 100,000 after age-adjustment. Between 2006 and 2016, the age-adjusted LD rate in Allegheny County ranged from 3.3 to 7.2 per 100,000 [29, 30]. In contrast, the age-adjusted LD rate for the US in 2009 was 1.15 per 100,000 [9].

Over 80% of LD cases reported every year in Allegheny County are not known to be related to outbreaks or healthcare facilities [29, 30]. These community-acquired cases are sporadic and the source of infection is unknown. Although travel has also been identified as a risk factor for sporadic LD [31], fewer than 5% of sporadic Allegheny County cases report traveling during their incubation period. Travel-associated cases are more prevalent in other parts of the US and Europe [29, 32]. Most Allegheny County sporadic case-patients are likely to have acquired the infection in the County. Thus, Allegheny County is an important place to study community-acquired sporadic LD given the necessity to better understand the origins of this disease in this part of the country. The second and third chapters of this dissertation describe community-acquired LD studies specifically designed for Allegheny County. Improving our understanding of the sources of community-acquired LD, especially in Allegheny County, will inform future targeted interventions for disease burden reduction.

2.0 ENVIRONMENTAL SOURCES OF SPORADIC COMMUNITY-ACQUIRED LEGIONNAIRES' DISEASE: A REVIEW

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2.1 ABSTRACT

BACKGROUND: Most Legionnaires' disease (LD) in the US and abroad is community-acquired and believed to be sporadic, or non-outbreak associated. Most patients are exposed to numerous water sources, thus making investigations difficult. Identifying known sources of sporadic community-acquired LD will inform sporadic LD investigations as well as highlight directions for research. The objective is to summarize and rank sporadic LD sources based on the level of linkage between the environmental source and cases.

METHODS: A PubMed search was conducted using the search terms legion* and (origins or source or transmission) and (sporadic or community-acquired). Studies of nosocomial and/or outbreak-associated disease were excluded from this review. Definite, probable, possible and suspect ranks were assigned to sources based on evidence of linkage to sporadic LD.

RESULTS: The search yielded 196 articles and 43 articles were included in the final review after application of exclusion criteria. A total of 28 sources were identified. Of these, eight were assigned definite rank including residential potable water and car air-conditioner water leakage. Probable rank was assigned to five sources including solar-heated potable water and soil. Possible rank was assigned to nine sources including residential potable water and cooling towers. Suspect rank was assigned to 20 sources including large building water systems and cooling towers.

CONCLUSION: Residential potable water, large building water systems and car travel appear to contribute to a substantial proportion of sporadic LD. Cooling towers are also a potentially significant source; however, definitive linkage to sporadic cases proves difficult. The sources of sporadic LD cannot be definitively identified for most cases.

2.2 INTRODUCTION

Legionnaires' disease (LD) is a common form of community-acquired bacterial pneumonia [2]. This waterborne disease is caused by the bacteria *Legionella*, which naturally exist in lakes and rivers and amplifies in building water systems and other man-made structures. Persons develop LD by inhaling aerosolized *Legionella* contaminated water or aspirating potable contaminated water. LD disproportionately affects elderly and immunocompromised individuals. Chronic medical conditions such as diabetes mellitus and COPD are also associated with increased risk, as is current and former smoking [2]. Almost all LD patients require hospitalization and average mortality rates range from 15 to 20% [3].

From 2000 to 2014, the reported annual rate of legionellosis in the US, which includes both LD and the milder Pontiac Fever, increased almost 300% from 0.42 to 1.62 per 100,000 [33]. US surveillance from 2005 to 2009 revealed that only 4% of cases during that time period were associated with known outbreaks. The same trend was observed in other parts of the world [18].

The source of LD outbreaks is commonly pursued in order to stop the outbreak; however, the source of sporadic or isolated cases is rarely pursued [33]. Sporadic LD is defined as an isolated, single case with no known associations with other cases and thus no known associations with outbreaks. The source of LD, as a waterborne disease, can be very difficult to determine given the plethora of water sources to which a person may be exposed during the 10-day incubation period.

LD is a reportable condition in the US that healthcare organizations and providers are required to report to health departments. The intensity of sporadic LD investigations varies by jurisdiction. The Centers for Disease Control and Prevention (CDC) recommends investigation of sporadic community-acquired LD if two or more cases share a common exposure [12]. Jurisdictions can elect to investigate sporadic community-acquired cases; however, limited public health resources often restrict these investigations. CDC also requests that all state and local health departments complete a case report form which includes information on outcome, occupation, travel, healthcare, whirlpool spa exposures and use of respiratory equipment [33]. Through this form, the CDC determines travel, occupational and healthcare-associated risk. In-depth investigation and analysis of sporadic case relatedness is left to state and local health departments.

Knowledge about the sources of sporadic LD assists public health practitioners to efficiently conduct *Legionella* source investigations. The most recent review of sporadic LD sources was published in 1995 [16]. This review summarized known sources of sporadic disease including residential potable water sources and cooling towers; however, the author emphasized that additional large-scale research needed to be conducted before drawing conclusions about the major sources of sporadic LD [16]. The purpose of the current review is to update current knowledge about known sources of sporadic LD.

2.3 METHODS

This narrative review was initiated through a literature search of MEDLINE conducted on August 9, 2017. PubMed was used to identify potential articles for inclusion using the following search criteria:

(legion*[All Fields] AND (“origin”[All Fields] OR ("source"[All Fields]) OR ("transmission"[Subheading] OR "transmission"[All Fields])) AND (“sporadic”[All Fields] OR ("community"[All Fields]) AND “acquired”[All Fields]))

All 196 articles generated through this search were considered for inclusion through title and abstract review (Figure 2.1). Exclusion criteria in the final review included description of sources of waterborne pathogens without specifically discussing Legionella, focus only on clinical or laboratory aspects, inclusion of only nosocomial infections, and studies of outbreak cases. Review articles were also excluded. Full text articles were obtained if the article appeared to meet inclusion criteria (Figure 2.1).

A sporadic LD case was defined as a patient meeting a LD case definition without exposure to a healthcare facility and no known association with an outbreak. A healthcare facility was defined as an acute care hospital, long-term acute care hospital, or long-term care facility, such as skilled nursing, rehabilitation, or assisted living facility.

Through review of full text articles’ citations, other articles not identified through the PubMed search were considered for final selection. Articles included in the final selection were studies that investigated origins or sources of sporadic cases either through full epidemiologic and/or environmental investigations, sometimes including molecular linkage of Legionella isolates, spatial analysis of sporadic cases, or environmental testing alone. Articles that solely described environmental testing were only included if the authors suggested linkage between the source and sporadic disease (Figure 2.1).

Ranking was assigned to a source based on evidence from a single study. A source may be categorized into multiple ranks based on evidence from multiple studies. Definitive rank was assigned when the environmental source was molecularly linked to sporadic LD cases. Probable rank was assigned to a source when human and environmental isolates shared a common Legionella species, but no molecular testing was performed. The types of studies included in the definitive and probable ranks included single case reports and larger investigative studies where intensive environmental sampling was completed. Possible rank was assigned to a source when either epidemiologic or spatial analyses linked sporadic cases to a potential source. The types of studies included in this rank were descriptive case studies, case-control studies, cross-sectional environmental studies, and epidemiologic spatial analyses. Statistical methods included basic descriptive proportions and relative risks as well as ecologic spatial analyses and complex cluster detection methods. Finally, suspect rank was assigned to a source when environmental sampling was conducted and linkage to sporadic cases was only suggested theoretically. Suspect rank was also assigned to a source when linkage between cases and an environmental source was suggested through descriptive case series.

2.4 RESULTS

Of the 196 articles identified, 138 were excluded based on title and abstract screening (Figure 2.1). The full text of 58 articles was reviewed. Through this final review of full text, 12 additional articles were identified through reference review. Of 70 identified total articles, 27 were excluded because confirmed or probable linkage between the source and sporadic disease was not specifically studied and/or the source of disease was not explicitly discussed. We included 43 articles in this sporadic disease source review (Figure 2.1).

A total of 28 environmental sources of sporadic LD of various likelihoods were identified. Studies of eight sources of definite rank demonstrated molecular linkage to sporadic disease cases. These sources included potable water from single family homes and apartment buildings, leaking water from car air conditioning systems, potable water from a construction site, potable water from a dental office as well as the dental unit waterline, hot springs and potable water used in a humidifier (Table 1). Studies of five sources of probable rank described linkage without molecular confirmation between a source and a case. These sources included water from car air conditioning systems, home spas, natural soil, potting soil and water from a home solar-heated hot water tank (Table 2). Of those studies that described a definite rank or probable rank source, only four studied more than one case [34-37].

Studies of nine sources of possible rank described statistical significance between a source and sporadic cases. These sources included water from construction sites, cooling towers, residential potable water, driving, general travel and weather patterns (Table 3). Suspect rank was assigned to 20 studies of sources including construction sites, travel, home potable water, dental office potable water, hot springs, driving and car air-conditioning, rainwater on roads, large building water systems, cooling towers, composting facilities and soil (Table 4).

2.5 DISCUSSION

The source of most sporadic LD cases is difficult to confirm. Nevertheless, as evidenced by this review, many potential sources have been linked to cases. The studies of possible and suspect rank included many more cases, but the types of analyses are less resource intensive compared to the methods required to molecularly confirm sources. Some reasons why it has proven difficult to confirm sources of sporadic disease include limited public health resources to conduct environmental investigations for single sporadic cases, limited availability of clinical isolates, and the common lag in time between when a case is reported to public health, interviewed and when public health conducts an environmental investigation. The original source of exposure may be difficult to pinpoint through sampling if a great length of time has passed since the original exposure.

It vital not only to identify the sources of sporadic LD but also to identify the sources that pose the greatest risk in terms of the number of potential cases. Understanding this aspect of sporadic LD sources ultimately helps public health professionals conduct more efficient environmental investigations and prevent future disease. Published case reports do not answer the question of risk magnitude regarding the source despite the definitive link between a source and single or multiple cases. This is the benefit of some of the less definitive type studies included in this review. For example, Miyamoto et al. reported a definitive molecular link between a sporadic case and a Japanese hot spring [38]. Lin et al. conducted an environmental study of Taiwanese hot springs to determine the prevalence of *L. pneumophila* [39]. Though not conducted in the same country, these studies complement each other by providing a better understanding of the risk of sporadic disease associated with hot springs. Hot springs most likely are not a major source given low environmental prevalence and limited human exposure [39].

The same type of complementary study was conducted in apartment buildings water systems, large building water systems, and construction areas (Table 1). Nevertheless, the link between sporadic cases and construction occupation has been questioned given the older case-control and case series studies that over-sampled construction workers [7]. Apartment buildings and large building water systems likely pose a significant risk especially if patients are immunocompromised. *Legionella* prevalence found in these types of buildings ranged from 12 to 33% [36, 40, 41].

Several large scale case-control studies and spatial epidemiologic studies included in this review shed light on risk magnitude of several sources such as travel, occupation, cooling towers, and potable water. Travel is commonly reported amongst sporadic LD cases as demonstrated by several large case-controls studies, conducted in the US, Netherlands and France [18, 31]. These studies showed elevated relative risks associated with overnight travel. The Netherlands study demonstrated an elevated relative risk of 33 (95% CI 14 – 78) associated with travel abroad [31]. Almost 25% of US LD is travel-associated, the majority being domestic travel [9]. Potential sources of sporadic travel-associated LD include hotel and other large building water systems as well as cooling towers.

Early studies of sporadic cases found that occupation may pose a risk [42-44]. Professional driving was suggested as a sporadic disease risk by a Netherlands case-control study and a Turkish study of professional drivers which found elevated *L. pneumophila* antibodies [31, 45]. Car air conditioning has been suggested as a source given the results of a larger environmental study in Japan and a definitive link between a case and environmental sampling of car air conditioning [37, 46]. A British case-control study found 7-times elevated risk associated with driving through an industrial area and almost 50-times elevated risk associated with using water for windshield wiper fluid rather than commercial wiper fluid [47]. A small sample of cars using water as wiper fluid tested positive for *L. pneumophila*. The authors estimated that 20% of sporadic disease in England and Wales is caused by driving while using water used as wiper fluid [48].

Cooling towers are known sources of outbreaks, but are difficult to pinpoint as sources of sporadic disease. Several retrospective UK spatial analyses identified cooling towers as a probable source of sporadic LD [22, 49, 50]. The distribution of sporadic cases arguably did not appear to be associated with potable water systems, but rather with the dispersion of cooling tower mist. Two studies showed that the risk of sporadic disease increased as a person lived closer to a cooling tower, especially within 3 km [22, 49]. Ricketts et al. suggests that 20% of sporadic disease in England and Wales is due to cooling towers even after adjusting for socioeconomic status. Nevertheless, an earlier spatial study in Nottingham, England did not find a spatial association between cases and cooling towers [51]. This discrepancy may be due in part to differences in Nottingham case characteristics given Nottingham has an unusually high incidence. A significant spatial association between industrial cooling towers and sporadic cases was observed in France which aligned well with the results from the broader UK spatial analyses [17].

The Nottingham spatial analysis suggested that potable water may be associated with sporadic disease. Potable water in single family homes, high rise apartment buildings and other large buildings were also molecularly confirmed as the source of several sporadic cases through studies conducted in the US and the Netherlands. A 1992 Pittsburgh, PA environmental prevalence study found that an average of 6% of homes from six different areas (range 0 to 22%) were positive for *Legionella* [52]. A case-control study conducted in the US found increased risk associated with home plumbing repairs and electric versus gas home hot water tanks [53]. An environmental prevalence study in Germany found that 12% of homes with hot water storage tanks were positive for *L. pneumophila* compared to zero homes with instantaneous water heaters [54]. A similar finding was reported after a Singapore environmental prevalence study of home potable water [55]. Filters for home potable water, especially those with storage tanks, may be appropriate for immunocompromised individuals.

Finally, several natural sources of increased sporadic disease should be considered including soil and rain water. Several *Legionella* species, including *L. longbeachae* and *L. pneumophila,* have been identified in soil in Australia, the Netherlands and the US and poses a risk to those handling soil [7, 56, 57]. The transmission mechanism is not completely clear; however, given the prevalence in soil, it is unlikely that soil significantly contributes to the burden of sporadic LD. *Legionella* was found in only 4% of rainwater water samples tested in the Netherlands [58]. Nevertheless, 36% of rainwater samples from roads in Tokyo were positive for *Legionella* suggesting a risk of LD when driving and inhaling aerosolized road rainwater [59]. Rainfall and humidity were associated with increased sporadic LD in New Jersey [48]. Changes in weather could create more favorable *Legionella* growth conditions in sources such as cooling towers and potable water.

Despite the source research reviewed, sources remain unidentified for most cases. This shortcoming is emphasized by the results of enhanced surveillance conducted in the Netherlands where sources were only identified in 3% of more than 1400 intensive case investigations conducted over a decade [34]. Stout et al. confirmed the source of 8 (40%) of 20 sporadic cases [35].

This literature review includes several limitations. First, publication bias is likely given that that the results of many public health investigations, particularly those that do not identify the source, are not published. As a result, both confirmed sources and investigations that fail to identify the source are underreported in the literature. Second is the lack of depth of studies supporting definite and probable ranked sources. As was mentioned, it is resource intensive to confirm the source of sporadic LD. Most definite and probable ranked sources were confirmed by single case studies. Relative importance is difficult to glean from case studies and thus requires additional, complementary research. Future large scale studies are needed to discover and further explore significant sources of sporadic disease. For example, the findings of studies support lower rank sources, such as cooling towers, should be further explored by larger scale, resource intensive research to more definitively confirm sources which may emphasize the need for enhanced control and prevention of *Legionella* contamination in cooling towers. Also, definite and probable ranked sources supported by smaller studies should be investigated on a larger scale through epidemiologic studies and/or spatial analyses to explore risk magnitude. Spatial analyses of sporadic cases could also relate these seemingly isolated cases and perhaps uncover new environmental sources.

LD is a costly illness both in terms of mortality, healthcare expenses and resources for prevention and control measures. A UK study estimated the cost of a LD outbreak at over £455,000 (~ $588,000); only 14% was spent on the investigation and control measures [60]. The remainder was spent on case-patient healthcare. The benefit of prevention measures far outweighs the monetary costs and general psychological impact of sporadic and outbreak-associated LD. Additional research regarding sources of sporadic disease is critical for sporadic LD prevention. A greater understanding of the risk magnitude associated with sources will improve targeted prevention efforts.

Conclusion

A variety of sporadic LD sources have been identified that have the potential to cause isolated incidents and more wide spread clusters. From the literature reviewed, significant sources of sporadic LD are residential potable water, large building water systems, car travel, and cooling towers. Nevertheless, sporadic LD sources remain to be identified as even intense environmental investigations sometimes still do not identify a source. Additional research is required to further understand sporadic disease risk associated with known sources and uncover other significant sources of disease. This research will critically inform prevention and control efforts to reduce sporadic LD incidence.

2.6 TABLES AND FIGURES

Table 2.1. Studies supporting definite rank of environmental sources.

Source	**Reference**	**Year**	**Location**	**Study Population**	**Study Design**	**Molecular Subtyping Method**	***Legionella* species**	**Results Summary**
Potable water from high rise apartments and single family homes, work places	[35]	1992	Pennsylvania, US	20 sporadic cases	Case source investigations	Restriction endonuclease analysis	*Legionella pneumophila*	Source confirmed in 8(40%) of sporadic cases studied. Home potable water should be considered more often as a source of sporadic disease.
Construction area sink	[61]	1997	United Kingdom	One 46 year old male employed at construction site	Case Report	Pulsed-field gel electrophoresis	*Legionella pneumophila*	Source confirmed at construction area where patient worked. Sink where patient drank from was positive.
Hot spring	[38]	1997	Japan	One 71 year old female	Case Report	Repetitive element polymerase chain reaction, arbitrarily primed PCR, ribotyping, restriction endonuclease analysis [57], and macrorestriction endonuclease analysis by pulsed-field gel electrophoresis	*Legionella pneumophila*	Hot spring where patient almost drowned was confirmed as the source. Hospital unit where patient subsequently stayed was negative.

Table 2.1 Continued

Source	**Reference**	**Year**	**Location**	**Study Population**	**Study Design**	**Molecular Subtyping Method**	***Legionella* species**	**Results Summary**
High rise apartment showerhead	[62]	2002	Switzerland	One 58 year old male	Case Report	Pulsed-field gel electrophoresis	*Legionella pneumophila*	High rise apartment where patient lived was confirmed as the source. Hospital unit where patient subsequently stayed was negative.
Car air conditioning	[46]	2002	Kentucky, US	One 54 year old male	Case Report	Heteroduplex analysis	*Legionella pneumophila*	Leaking car air conditioning system was confirmed as the source. The patient had driven for a long distance with the malfunctioning system.
Humidifier	[63]	2012	Israel	One Infant less than 6 months	Case Report	Sequence-based typing	*Legionella pneumophila*	Free-standing cold water humidifier using domestic tap water was confirmed as the source.
Dental unit waterline	[64]	2012	Italy	One 82 year old woman	Case Report	monoclonal antibody typing, sequence-based typing, amplified fragment length polymorphism typing	*Legionella pneumophila*	Patient's home was negative and dental office cold water as well as the high-speed turbine of the dental unit waterline were positive for *L. pneumophila*.
Potable water from single family homes and work places	[34]	2015	Netherlands	1484 sporadic cases	Case source investigations	Amplified fragment length polymorphism genotyping	*Legionella pneumophila* and other species	This study reports the results of intensive environmental investigations and confirmed the source in 41/1484 (3%) of investigations.

Definite rank requires molecular linkage between clinical and environmental isolates.

Table 2.2. Studies supporting probable rank of environmental sources.

Source	Reference	Year	Location	Study Population	Study Design	Linkage Method	*Legionella* species	Results Summary
Potting Soil	[56]	2000	United States	3 sporadic cases in Pacific Northwest	Case Report	Environmental and human testing, no molecular linkage completed	*Legionella longbeachae*	Describes cases of *L. longbeachae* in Oregon, Washington, California associated with potting soil and all had frequent gardening exposures. One patient's potting soil was *L. longbeachae* positive.
Home spa (24 hour always ready bathing system used for home birth)	[65]	2003	Japan	1 infant 4 days old	Case Report	Environmental and human testing, no molecular linkage completed	*Legionella pneumophila*	*Legionella* positive water from home spa used for home birth. No species provided so could not link directly to infant's isolate.
Car air conditioning	[59]	2009	Japan	159 regional transportation company employees and 22 evaporator compartments for car air conditioners from scrap cars sampled	Cross-sectional	Environmental sampling and human exposure survey and antibody testing	*Legionella pneumophila*	Half of cars positive for *Legionella* but competing bacteria prohibited further analysis. Higher antibody titers were found in individuals reporting car air conditioning use. Also transportation industry workers (all types of drivers not just commercial) had higher prevalence of Legionnaires' disease compared to the general population in their region.

Table 2.2 Continued

Source	**Reference**	**Year**	**Location**	**Study Population**	**Study Design**	**Linkage Method**	***Legionella* species**	**Results Summary**
Solar heated home hot water tank	[66]	2016	Turkey	Two sporadic cases	Case Report	Environmental and human testing, no molecular linkage completed	*Legionella pneumophila*	*Legionella pneumophila* found in both clinical and environmental isolates of home water heated by solar panels. Authors suggest water may not get hot enough using solar heat.
Natural Soil	[67]	2005	Regional Victoria, Australia	Late 40s male	Case Report	Environmental and human testing, no molecular linkage completed	*Legionella pneumophila* serogroup 1	*Legionella pneumophila* serogroup 1 found in both clinical isolate and environmental isolates from plant nursery where patient worked.

Probable rank requires demonstration of Legionella in both clinical and environmental isolates without evidence of molecular linkage.

Table 2.3. Studies supporting possible rank of environmental sources.

Source	**Reference**	**Year**	**Location**	**Study Population**	**Study Design**	**Linkage Method**	***Legionella* species**	**Results Summary**
Residential/ occupational construction site exposure, Travel	[44]	1979	United States	100 sporadic cases	Case-control	Statistical	*L. pneumophila* and other *Legionella* spp.	Increased risk for smokers, drinkers, advanced age, male sex, more chronic conditions, living near construction, being a construction worker, recent travel.
Cooling tower	[14]	1991	Scotland	134 sporadic cases and 10,159 lung cancer cases	Case-control	Descriptive spatial analysis of sporadic cases vs. lung cancer cases	*L. pneumophila* and other *Legionella* spp.	Relative risk of sporadic disease was over 3 in people living within 0.5 km of a cooling tower compared with people living more than 1 km away. Dose response was observed.
Cooling tower	[68]	1991	Scotland	378 sporadic cases	Epidemiologic spatial study	Ecologic analysis of sporadic Legionnaires' disease rates by postal code	*L. pneumophila* and other *Legionella* spp.	Legionnaires' disease postal code rates varied across Scotland. If related to home water then would expect to see more uniform rates across geographies but see variations which suggests cooling towers. They suggest that cooling towers are a source of Legionnaires' disease in Scotland.
Non-municipal home water, travel, home plumbing repairs, electric vs. gas hot water heaters	[53]	1996	Ohio, US	146 sporadic cases matched with 146 controls	Case-control	Statistical	*L. pneumophila* and other *Legionella* spp.	Non-municipal home water supply, smoking, and recent residential plumbing repair were independent risk factors in multivariate regression. Travel and electric vs. gas water heaters were univariately associated.

Table 2.3 Continued

Source	**Reference**	**Year**	**Location**	**Study Population**	**Study Design**	**Linkage Method**	***Legionella* species**	**Results Summary**
Home potable water	[51]	2003	Nottingham UK	3714 sporadic cases	Epidemiologic spatial study	Spatial cluster analysis	*L. pneumophila* and other *Legionella* spp.	Proximity of residence to a cooling tower was not identified in this study as a risk factor for acquiring legionnaires' disease and no clustering of cases to suggest an unidentified common source was observed. 39% of sporadic cases had positive homes and this source was deemed the likely source.

Possible rank requires statistical association between cases and source.

Table 2.4: Studies supporting suspect rank of environmental sources.

Source	Reference	Year	Location	Study Population	Study Design	Linkage Method	*Legionella* species	Results Summary
Occupational construction site exposure, Travel	[42]	1979	Great Britain	84 sporadic cases	Descriptive case series	Exposure histories	*L. pneumophila* and other *Legionella* spp.	Most cases were older men, some traveled, mostly resided in cities, no occupation stood out but some exposed to construction, 85% smokers, only small percentage immunosuppressed.
Construction, Travel	[69]	1981	United States	1005 sporadic cases	Descriptive case series	Exposure histories	*L. pneumophila* and other *Legionella* spp.	37% traveled overnight, 23% lived in site of construction, 32% exposed to construction, they suggest same risk factors as outbreaks.
Healthcare occupation, construction, birds, dentist	[43]	1981	Iowa, US	30 sporadic cases	Descriptive case series	Exposure histories	*L. pneumophila* and other *Legionella* spp.	40% of cases were employed in healthcare and others had associations with construction and birds. They suggest bird droppings in cooling towers could be a source. No active farmers were found which was unexpected given soil and LD connection. Also found association with dental extractions.

Table 2.4 Continued

Source	Reference	Year	Location	Study Population	Study Design	Linkage Method	***Legionella* species**	Results Summary
Sinks and showerheads in high rise apartments	[36]	1985	United States	95 people living around the University of Chicago hospital	Cross-sectional	Environmental sampling and human (apartment residents) antibody testing	*Legionella pneumophila*	30 (32%) residences were positive, median 200 CFU/mL. Less positives with hot water >60C. No association between water test result and resident antibody level maybe because lower pathogenicity strains or healthier subjects.
Travel	[70]	1990	Italy	42 sporadic cases	Descriptive case series	Exposure histories	*L. pneumophila* and other *Legionella* spp.	Of the travel associated cases studied, 36% were sporadic and not associated with outbreaks.
Dental office water	[71]	1995	United States	Environmental samples of 28 dental offices	Cross-sectional	Environmental testing only	*L. pneumophila* and other *Legionella* spp.	*Legionella* detected in 68% of water samples from dentist offices, but also detected in 61% of comparable community sites. Dental office occupational exposure may be of concern.
Ground water	[72]	2004	US and Canada	114 environmental samples of ground water and biofilm	Cross-sectional	Environmental testing only	*Legionella spp.*	Of ground water samples tested, 29% and 28% were positive by culture and PCR respectively. Found in both water and biofilm.
Hot spring	[39]	2007	Taiwan	55 environmental samples of 19 hot springs	Cross-sectional	Environmental testing only	*L. pneumophila*	21% positive but only 11% positive for *L. pneumophila*. Authors did not conclude that immunocompromised should not use hot springs, but suggested further research given their limited sample size.

Table 2.4 Continued

Source	Reference	Year	Location	Study Population	Study Design	Linkage Method	*Legionella* species	Results Summary
Long distance driving and car air conditioning	[45]	2007	Turkey	79 long-distance male professional drivers	Descriptive case series	Exposure histories and antibody testing	*L. pneumophila*	Bus driver seropositivity rate was 19% and 0% for driver's assistants. Environmental samples from air conditioning units from buses driven by antibody positive bus drivers were all negative.
Home potable water	[54]	2008	Germany	452 environmental samples of home water	Cross-sectional	Environmental testing only	*L. pneumophila* and other *Legionella* spp.	Houses with hot water storage tanks and recirculation were 12% *Legionella* positive but ones with instantaneous hot water heaters were not positive. Water below 46C were frequently positive. Filters may be appropriate in certain circumstances.
Rainwater on roads	[59]	2009	Tokyo, Japan	45 environmental samples of rainwater	Cross-sectional	Environmental testing only	*L. pneumophila*	Rainwater was 36% positive by culture and serogroup 1 detected in 37% of positives. They observed a type of amoeba that can resuscitate non-viable Legionella and they found it in their rain samples. Perhaps they were more positive but the state of legionella were unculturable and maybe amplify in certain warm, sunny weather conditions.

Table 2.4 Continued

Source	**Reference**	**Year**	**Location**	**Study Population**	**Study Design**	**Linkage Method**	***Legionella* species**	**Results Summary**
Building water systems, cooling towers	[41]	2010	Italy	97 sporadic cases and 533 potential sources	Cross-sectional	Environmental testing and epidemiologic surveillance	*L. pneumophila* and other *Legionella* spp.	58% of buildings (33% community bldgs. such as apartments, hotels, offices) were positive 1 - 10 CFU/mL, 32% *L. pneumophila* serogroup 1. Water sources tested including 1 liter samples from fountains, cooling towers, and cold and hot water supply from various sites in the bldg. Incidence in certain parts of Italy have increased so they emphasize the need for increased control measures.
Home potable water storage tanks vs. instantaneous heaters	[55]	2011	Singapore	49 environmental samples of home water tanks and instantaneous heaters	Cross-sectional	Environmental testing only	*L. pneumophila* and other *Legionella* spp.	Homes with storage tanks were 21% positive, instantaneous heaters 3% positive but concentrations were generally under 100 CFU/mL so the authors conclude only moderate risk of outbreaks due to homes.
High rise apartments potable water	[40]	2012	Hong Kong	77 environmental samples of home water	Cross-sectional	Environmental testing only	*L. pneumophila* and other *Legionella* spp.	22% of 77 households *Legionella* positive from 0.1 to 639 CFU/mL, mean ~100 CFU/mL. Higher counts in biofilms. 21% positive hot water storage tanks and 3% positive instantaneous heaters.

Table 2.4 Continued

Source	Reference	Year	Location	Study Population	Study Design	Linkage Method	*Legionella* species	Results Summary
Composting facilities	[73]	2013	Switzerland	88 environmental samples of compost and bioaerosols	Cross-sectional	Environmental testing only	*L. pneumophila* and other *Legionella* spp.	63% of facilities positive for both *Legionella* and FLA, only 6% Legionella positive only and 28% FLA only. But only 10% of bioaerosol pools positive for Legionella.
Biological waste water treatment plants	[74]	2014	Norway	130 environmental samples of treatment plants	Cross-sectional	Environmental testing only	*L. pneumophila* and other *Legionella* spp.	16% positive for *Legionella*, 9% positive for *L. pneumophila* by culture. By PCR, 99% of samples were positive for *Legionella* and 46% for *L. pneumophila*. None of the strains identified matched previous outbreaks.
Rainwater and natural soil	[58]	2014	Netherlands	97 environmental samples of rainwater and natural soil	Cross-sectional	Environmental testing only	*L. pneumophila*, *L. longbeachae*, and other *Legionella* spp.	30% of soils and 4% of rainwater were positive for *Legionella*. 33% of soil positives were *L. pneumophila* compared to 66% of rainwater positives. The authors suggest these as alternative sources of sporadic disease.
Garden Soil	[75]	2016	Netherlands	177 environmental samples of garden soil	Cross-sectional	Environmental testing only	*L. pneumophila*, *L. longbeachae*, and other *Legionella* spp.	12% of soil samples were positive and of those 32% were *L. pneumophila*. Multivariable analysis found no soil variables significantly associated with *Legionella* positivity. None of the sequence-based types most often detected in humans in Netherlands were found in soil. They conclude that

Table 2.4 Continued

								garden soil is probably not the cause of most sporadic disease in Netherlands.
Home showers	[76]	2017	United Kingdom	99 environmental samples of 82 home showers	Cross-sectional	Environmental testing only	*L. pneumophila* and other *Legionella* spp.	6% of households were *Legionella* positive. 31% positive by PCR. Risk of PCR increased with older homes, older showers, and the frequency of use.

Suspect rank requires evidence from environmental prevalence that suggests risk of sporadic Legionnaires' disease or requires evidence from descriptive case series.

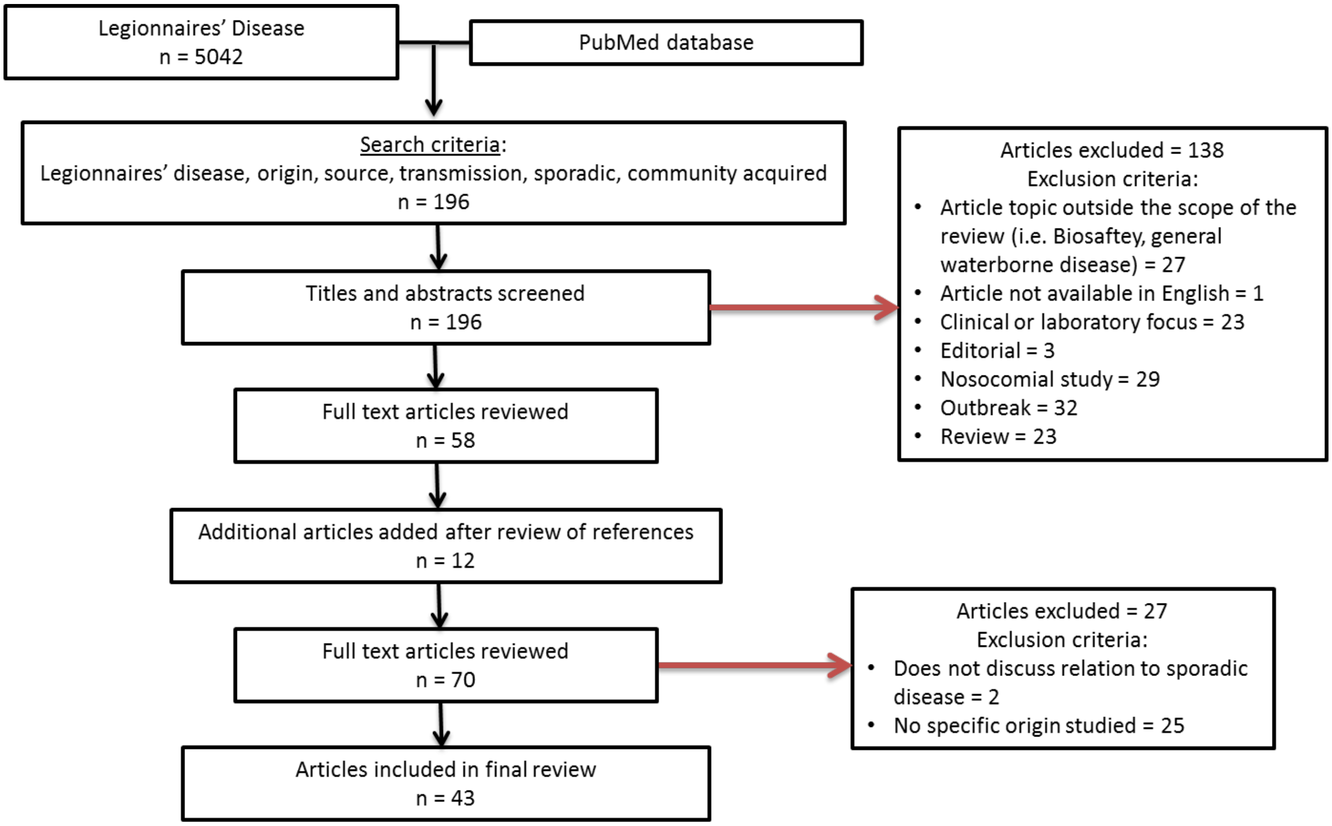

Figure 2.1 Literature search strategy for sporadic Legionnaires' disease sources.

PubMed search performed August 9, 2017.

3.0 COOLING TOWER MAINTENANCE PRACTICES AND LEGIONELLA PREVALENCE, ALLEGHENY COUNTY, PA, 2016

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Public Health, Pittsburgh, PA, USA; and **Janet E. Stout, PhD**; Special Pathogens Laboratory, Department of Civil and Environmental Engineering, University of Pittsburgh Swanson School of Engineering, Pittsburgh, PA, USA.

3.1 ABSTRACT

BACKGROUND: Cooling towers have been linked to outbreak related and non-outbreak related legionellosis. Proper cooling tower maintenance and disinfection are imperative for legionellosis prevention but not monitored in Allegheny County, PA, a high incidence area.

METHODS: To investigate cooling tower maintenance and *Legionella* positivity, the Allegheny County Health Department (ACHD) performed a survey regarding the presence and maintenance of cooling towers and tested cooling towers for *Legionella pneumophila.* ACHD surveyed healthcare facilities, senior apartment buildings, and county-owned buildings. Associations between maintenance practices and *Lp* were assessed using Wilcoxon rank-sum tests and multivariable linear regression.

RESULTS: Of 408 building managers contacted, 377 (92%) completed the survey of which, 56 (15%) had a cooling tower. Among 42 cooling towers sampled, 20 (48%) tested positive for *Lp*. Factors associated with positivity included larger tower capacity, year round usage, hospital status and older tower age. Only cooling tower age was associated with *Lp* after stepwise regression.

CONCLUSIONS: Despite maintenance practices, many cooling towers were *Lp* positive. ACHD recommends that facilities develop an ASHRAE compliant water management plan and conduct annual basin water emptying, quarterly cleaning, quarterly *Legionella* testing and diligent inspection of older towers.

3.2 INTRODUCTION

Legionnaires' disease (LD) is the second most common cause of bacterial pneumonia in the United States, accounting for 2 – 9 % of community-acquired pneumonia cases [2, 77]. Developed countries around the globe have experienced an increase in LD incidence since the 2000s [9-11]. From 2000 to 2014 in the United States, legionellosis incidence, which includes LD and the milder, less commonly reported Pontiac Fever, increased 286% from 0.42 to 1.62 annual cases per 100,000 people [3]. This trend persists even after age-adjustment [9].

The majority of cases reported in the US and worldwide occur sporadically with no identified source [4, 18]. The most common sources are speculated to be home potable water, travel-associated potable water, and evaporative cooling towers [16, 22]. Through spatial analysis of LD in England and Wales, 20% of sporadic cases were estimated to be attributed to cooling towers [22].

Transmission of LD occurs through inhalation or aspiration of water containing *Legionella*. *Legionella* is a waterborne pathogen found in many aqueous environments and proliferates in warm, stagnant water. *Legionella* commonly inhabit amoeba as intracellular parasites and thrive in biofilms formed on surfaces [2]. Conditions for proliferation are commonly found in evaporative cooling towers. Prevalence of the bacteria in these structures has ranged from 2 – 87% and variations exist likely due to sample selection, maintenance practices and possibly local cooling tower regulations [78-82].

Both large and small LD community outbreaks have been caused by cooling towers. A 2014 review article described 19 outbreaks attributable to cooling towers with case counts ranging from 7 to 449 cases and 6.3% average case fatality rate [83]. A hotel cooling tower in the South Bronx neighborhood of New York City caused a 2015 outbreak which sickened 138 people and killed 16. Clinical *Legionella* isolates matched the strain of *Legionella* found in the cooling tower [25]. In response to this outbreak, both the City and State of New York issued regulations requiring cooling tower registration, inspection and *Legionella* testing [24].

In the United States, reported LD incidence has more than doubled since 2000. US LD incidence in 2003 was 0.78 per 100,000 and increased to 1.58 per 100,000 in 2013 [12]. The highest incidence of legionellosis in the US consistently occurs in the Mid-Atlantic region. Allegheny County, Pennsylvania, which is part of this Mid-Atlantic region, experiences rates four times higher than the US age-adjusted rate [29]. Over two-thirds of LD cases reported annually in Allegheny County are of unknown origin. These cases are unrelated to outbreaks or healthcare facilities. Cooling tower-related LD has not been identified recently in Allegheny County, but has occurred in the past. Investigating the conditions of cooling towers is an important component of LD prevention, especially in an area with a high burden of the disease. The purpose of this survey is to assess *Legionella* prevalence in Allegheny County cooling towers and identify areas of improvement for cooling tower maintenance and *Legionella* contamination prevention in Allegheny County.

3.3 METHODS

Cooling Tower Maintenance Survey

Buildings selected for the survey included those that house populations who are susceptible to LD. These buildings included hospitals, skilled nursing facilities, assisted living facilities, personal care homes and senior apartment buildings identified through Pennsylvania's Department of Health and Department of Human Services. Allegheny County senior apartment buildings were identified through a Google search using search terms ‘senior apartment AND Allegheny county.’ City and county owned buildings in Allegheny County, PA, were also surveyed and identified through the Allegheny County Housing Authority, the Housing Authority of the City of Pittsburgh, and the Allegheny County Facilities Management Department.

A questionnaire was completed over the phone or sent via email or fax based on facility preference. The questionnaire (Appendix) began with vetting questions to ensure the most knowledgeable persons at the facility completed the survey. Survey questions were based on guidelines from the American Society of Heating and Air-Conditioning Engineers [5], the Cooling Technology Institute [8] and the World Health Organization (WHO). Structural questions addressed building size, number of cooling towers, cooling tower location, and name of water authority. Maintenance questions addressed use of water treatment professional, cooling tower cleaning and inspection procedures, water filtration, basin emptying, biocide treatment and monitoring, record keeping, bacterial load testing, and *Legionella* testing. Finally, facilities were asked to consent to testing by the Allegheny County Health Department (ACHD) cooling tower basin water testing for *Legionella*.

Cooling Tower Sampling

At consenting facilities, ACHD staff selected a single, random cooling tower for testing if the facility had multiple. The cooling tower’s make, model, serial number, year installed, and size (tonnage) were recorded. Basin water temperature was measured using a digital probe thermometer. Basin water pH was measured using test strips. Basin water free and total chlorine were measured using test strips (range 0 to 10ppm at increments of 0, 0.5, 1, 2, 4, 10ppm). Basin water was collected in sterile 125mL plastic bottles. Bottles were filled to 30mL with basin water and a drop of sterile 0.1 N sodium thiosulfate was added to the bottle immediately after water collection using a sterile, disposable, transfer pipette. Water samples were sent to the ACHD Public Health Laboratory on the same day as sample collection. Water samples were stored at 5˚C until processing.

Microbiological Methods

Water samples were cultured for *Legionella pneumophila [84]* within four days of collection at the ACHD Laboratory. Each specimen was plated onto GVPC agar directly after acid treatment and heat treatment. Specifically, *Legionella* Acid Buffer was added to each sample for 15 minutes at room temperature. Samples were heat treated at 50°C for 30 minutes using water bath before plating. Plates were incubated at 35°C and read at 3 and 7 days. Any identified colonies were picked and plated on SBA and GVPC agar and incubated overnight at 35°C. Isolates that grew on GVPC agar were tested with Oxoid *Legionella* Latex Test kit [Oxoid Ltd, Wade Road, Basingstoke, Hants, RG24 8PW, UK] and confirmed positive for *Lp* serogroups 1, 3, 5, 6, Poly 1-14, or b-m with Direct Fluorescent Antibody test. [Monoclonal Technologies, Inc. (m-TECH, 16335 New Bullpen Road, Alpharetta, GA 30004) Rabbit Anti-*Legionella* IgG Fluorescein Labeled].

Whole genome sequencing and phylogenetics

Genomic DNA was extracted at the University of Pittsburgh, School of Medicine, Infectious Disease Epidemiology Research Unit, using the Qiagen DNAeasy Tissue Kit on a QIAcube according to manufacturer's instructions (Qiagen, Germantown, MD). The DNA was eluted in 10mM Tris/1mm EDTA, and sequenced according to the method of Baym et al. (PMCID: 4441430) using Illumina Nextera genomic libraries on a MiSeq v2 (500-cycle) kit.

Fastq Reads were trimmed and assembled using SPAdes v3.9.0 (PMID: 25422674). Assemblies were annotated using Prokka v0.1.1 (PMID: 24642063). The sequencing depth ranged from 36X-94X. The assemblies had a median of 96 contigs per sample with an average assembly length of 3.7Mbp and an average N50 of 200,000bp. Sequence types (ST) were identified using SRST2 (PMID: 25422674). Reads were aligned to reference assembly, LEG551, using BWA-MEM v0.7.12-r1039 (http://bio-bwa.sourceforge.net/). For ST2329 pairwise comparisons, LEG443 was used as the reference genome. SNPs were identified using GATK HaplotypeCaller v3.5 with a ploidy of 1 (PMID: 20644199). SNPs with low mapping quality ($MQ < 20$), strand bias ($FS > 60.0$), low variant confidence ($QD < 2$), only seen near the ends of reads ($ReadPosRankSum < -8.0$), or low depth ($DP < 5$) were filtered using GATK VariantFiltration. A phylogenetic tree of aligned SNPs was generated using RAxML v8.2.9 with 100 bootstrap replicates under the generalized time-reversible model (GTRCAT) and Lewis correction for ascertainment bias (PMID: 24451623). Phylogenies were visualized using the python package ETE3.

Statistical Analysis

Descriptive statistics for the sample were presented using either the proportion or median. The outcome variable for this analysis, cooling tower *Lp* level (colony forming units/mL), was analyzed as a continuous variable. Each predictor variable was coded into two categories. Unadjusted analyses were performed to compare the distribution of *Lp* level between categories for each survey variable using Wilcoxon rank-sum test. Stratification by hospital status was employed to examine association among hospital and non-hospital facilities. A multivariable linear regression model was created for the continuous outcome variable. Log transformation of the outcome variable was considered for improved model fit. Predictors that were univariately associated (p value < 0.1) with *Lp* level were considered for the multiple regression model using a forward stepwise approach with an alpha level=0.05 for entry and remaining in the final model. Interaction terms and confounding variables were assessed for inclusion in the final model. Epi Info® 7.1and SAS® 9.4 were used for data management and analysis respectively.

3.4 RESULTS

Survey response

Among 412 facilities approached, 377 (93%) completed the survey. The response rate by facility type ranged from 78% to 100%; the majority of facility types had response rates above 90%. Of those participating facilities, 56 (15%) reported having a cooling tower on the premises (Table 1). Hospitals more frequently had cooling towers (78%), followed by skilled nursing facilities (20%), and senior apartment buildings (17%). Very few personal care homes and city or county-owned buildings had cooling towers (Table 1).

Cooling Tower Sampling

Of the 56 cooling towers identified, 42 (75%) facilities agreed to ACHD testing. *Lp* was detected in 20 (48%) cooling tower basin water specimens. Of 17 hospitals tested, 12 (71%) were positive (Table 2). In addition, one (20%) skilled nursing facility, four (36%) senior apartment buildings, and three (43%) county-owned buildings were positive. Neither of the two personal care facilities tested were positive. Of those positive, the median concentration level was 35 CFU/mL with a range from 10 – 2,000 CFU/mL. *Lp* counts above 100 were found in three (12%) hospitals and one (9%) senior apartment building (Table 2). Of the 19 (95%)

isolates assigned a serogroup, 14 (74%) isolates were identified as serogroup 1, 4 (21%) isolates were identified as serogroup 5, and one (5%) isolate was identified as serogroup 6.

Survey Results and Univariate Analyses

Among the 42 facilities with ACHD water testing, the majority of cooling towers had treatment programs administered by a water treatment professional, were treated with at least one biocide, were tested at least annually for biocide level and *Legionella*, had an automatic biocide feed, and had the tower basin cleaned and emptied of stagnant water at least annually (Table 3). Only 31% of cooling towers were inspected more frequently than monthly. All cooling towers were cleaned at least annually, but only 21% were cleaned greater than twice a year as most cooling towers were cleaned at the beginning and the end of the cooling season, which is generally April to October. Only 21% of facilities with a cooling tower had a cooling tower water management plan and of those, most qualified as corporate plans (Table 3). It was difficult to verify whether a facility diligently followed a corporate plan that was not developed specifically for their tower(s). Average age of cooling towers was 13 years old, ranging from less than a year to 38 years (Table 4). Average tonnage or capacity of the cooling tower was 422 tons, ranging from 29 to 14,950 tons (Table 4).

In unadjusted analyses, increased *Lp* concentration was associated with larger tower capacity, year round usage, hospital status, multiple towers, late summer tower sampling, older tower age, water management plan existence, and roof location (Tables 3 and 4). Non-consequetive water authority supplier (i.e. does not purchase water from another water authority) was associated with decreased concentration (Table 3).

The average cooling tower basin water temperature during ACHD testing was 76°F (62 – 88°F). The average pH during testing was 7 (6 - 11). Average free and total chlorine levels were

< 0.5 ppm (0 – 4 ppm) and < 0.5 ppm (0 -10 ppm) respectively. None of these water quality measurements were significantly associated with *Lp* concentration (Table 4).

When stratifying by hospital status, year round usage and older tower age were univariately associated with increased concentration in hospital cooling towers, whereas larger tower capacity was univariately associated with increased concentration in non-hospital cooling towers (Table 5).

Multiple Linear Regression

Cooling tower age was the only predictor significantly associated with the log transformed *Lp* concentration outcome based on stepwise regression methods. As cooling tower age increased concentration level also increased. Year round usage and hospital status were included in the final model to account for potential confounding between tower age and *Lp* level (Table 6).

Whole genome sequencing(WGS)

WGS was performed on 13 isolates. Of those, 12 were *Lp* serogroup 1. The isolates belong to six serotypes (Figure 1). Five isolates belong to ST8 (LEG 322, 349, 507, 551, 590) and four isolates belong to ST2329 (LEG443, 574, 575, and 588). LEG591 belongs to ST2330, a single locus variant of ST8. However, this isolate is unrelated to ST8 isolates having >9,000 SNP differences. ST8 isolates LEG322, 507 and 551 had < 80 SNP differences (Figure 1, Table 7). In a pairwise comparison, LEG443 and LEG574 belonging to ST2329 were closely related with < 40 SNP differences (Table 8). Interestingly, three of the ST2329 isolates came from cooling towers located within a 1.2 miles of each other. No geographic clustering was observed between the ST8 isolates.

3.5 DISCUSSION

Almost half of Allegheny County cooling towers surveyed were positive for *Lp* which causes the vast majority of LD [9]. The most important indicator of concentration level was cooling tower age. WGS identified 6 different ST with the majority belonging to either ST8 or ST2329, a previously undescribed ST. We observed no apparent geographic clustering. ST8 is commonly found in cooling towers and has been linked to outbreaks internationally, but not in the US [85].

Previous studies have found a wide range in the prevalence of *Legionella* in cooling towers outside of outbreak settings. In international prevalence studies of various sample sizes, *Legionella* contamination ranged from 2% to 100% [78-82, 86]. The concentration ranged from < 1 up to 10,000 CFU/mL with most samples under 100 CFU/mL. Concentration fluctuated over time especially in summer months and concentration increased with year round usage [79, 81]. In the US, 196 cooling towers were sampled nationwide for *Legionella* in the summer of 2016 and 84% were PCR positive, while 48% were culture positive. Half of those culture positive towers were positive for *Lp* serogroup 1 [87].

The results of our prevalence survey generally align with previous studies given *Lp* contamination range was broad from 10 to 2,000 CFU/mL and the majority of positive results were under 100 CFU/mL. Nevertheless, the conditions under which prior prevalence studies were conducted differ and should be considered. For example, a prevalence study in New Zealand assessed over 1200 cooling towers and only found 2% positive for *Legionella*. At the time of the study, a cooling tower registry had been in place for several years and the government required *Legionella* testing and reporting of results. This low prevalence could be due in part to strict national cooling tower oversight [80].

The concentration of *Legionella* detected has varied widely in samples collected from cooling towers associated with outbreaks. A 2011 review article summarized 38 cooling tower LD outbreak publications and found that 22% of outbreaks were caused by cooling towers with levels between 100 – 9,999 CFU/mL, while 13% were between 10,000 – 99,000 CFU/mL [23]. A 2014 review of 19 cooling tower outbreaks described levels ranging from 10 to 10,000,000 CFU/mL [83]. The contamination levels we observed were generally lower in comparison to these ranges.

Given this sample of cooling towers in Allegheny County was limited and that the majority were healthcare-associated cooling towers, we expected better cooling tower maintenance in comparison to a more general sample. This was confirmed by our finding that 98% of cooling tower sampled were treated with biocide and all cooling towers were cleaned at least annually; however, despite maintenance practices, age was the most important predictor of concentration level and *Legionella* grew even in well maintained systems. A similar finding related to age was documented in a Greek *Legionella* prevalence study; however, this study sampled cooling towers of a wider maintenance scale and found decreased risk of *Legionella* colonization to be associated with biocide treatment, cleaning greater than every 6 months, and following a risk management plan.

Cooling tower LD outbreaks have mostly been attributed to inadequate maintenance such as lack of or insufficient biocide treatment and lack of cleaning within 6 months of an outbreak [23]. A 2011 cooling tower outbreak review article found that 26% of outbreak-associated cooling towers were described as adequately maintained and 66% neglected or inadequately maintained [23]. Nevertheless, 'adequately' maintained is difficult to define. Of note, outbreaks have also been attributed to 'well maintained' cooling towers [88, 89]. Australia and Japan developed guidelines which mandate testing, inspections and registration, yet Australia continues to experience cooling tower-associated outbreaks [23]. Overall, cooling tower guidelines generally vaguely specify cleaning frequency, biocide type or amount. Most guidelines recommend regular inspections rather than specifying frequency. Occurrence of outbreaks due to ‘adequately maintained’ or ‘well maintained’ cooling towers could be related to guideline inconsistencies [23].

The availability of a clear and comprehensive cooling tower maintenance guideline would be extremely valuable to cooling tower engineering and maintenance personnel. Nevertheless, the lack of specificity in current guidelines may be due in part to the variability of cooling towers themselves. The cooling towers we sampled varied greatly in terms of size, age and overall operation. Given these structural differences, creating a clear and comprehensive guideline appears difficult.

In 2015, the American Society of Heating and Air-Conditioning Engineers [5] updated their guideline (ANSI/ASHRAE Standard 188-2015) for minimizing *Legionella* in building water systems; the guideline describes minimum expectations for maintenance and development of a water management plan to minimize *Legionella* [21]. These guidelines specify that if a building has a cooling tower, the water management plan must address the cooling tower. Less than a quarter of facilities we surveyed had developed a water management plan (Table 3). ASHRAE guidelines do not state specific recommendations related to frequency of cleaning, inspections or testing [5, 21]; facility managers and their water treatment professional decide these specifics. It is noteworthy that our survey indicated contracting with a water treatment professional was associated with decreased concentration level but this finding was not statistically significant. On June 2, 2017, the Centers for Medicare & Medicaid Services published a memorandum requiring that all hospitals, critical access hospitals and long-term care facilities develop a water management plan in compliance with ASHRAE 188-2015. This may increase implementation of water safety and management plans in helathcare facilities.

For facilities with cooling towers, ACHD published the following recommendations: 1) Develop a water management plan, in compliance with ASHRAE 188-2015. 2) Clean cooling towers run year round and test for *Legionella* at least quarterly. Cooling towers that run seasonally should be cleaned and tested for *Legionella* at least before, during and immediately following the cooling season. 3) Collect basin water for routine testing. 4) Clean the basin or sump tank and drain as part of routine cleaning. 5) Inspect older cooling towers and clean diligently given their potential for *Legionella* contamination.

Our study has several limitations that should be considered when interpreting results. The first is our limited sample size. A larger sample size may have improved the robustness of our multivariable linear regression model. We chose to survey buildings that house susceptible populations because these populations are disproportionately affected and LD outbreaks have been associated with cooling towers on these types of buildings [90]. To increase generalizability, we surveyed city and county owned buildings. External validity should nevertheless be considered as the generalizability of these results is suspect. Also, some of the univariate analysis results are not intuitive, such as increased risk associated with water management plans. This is most likely due to our over representation of hospitals. Hospital towers were generally larger and older than non-hospital towers and water management plans were more frequently developed by hospitals. After stratifying by hospital status, we found similar univariately associated variables compared to the overall analysis. The results suggest that that the relationship between *Legionella* and cooling tower year round usage and age was more relevant for hospital cooling towers, whereas tower capacity was more relevant for non-hospitals. Nevertheless, power was limited for this stratified analysis.

Another limitation to consider is survey response accuracy. We required a maintenance supervisor or an engineer to be involved in the completion of the maintenance practice survey; however, whether responses reflected true practice was not possible to confirm. We emphasized when conducting the survey over the phone or when sending the survey via email that all answers would be kept confidential and no punitive action would be taken based on survey response or cooling tower test results.

Strengths of our study include our overall survey response rate and consent for ACHD testing. All samples were collected by the same ACHD personnel and samples were processed at the ACHD public health laboratory rather than commercial labs to ensure consistency of results. In Allegheny County, this prevalence study is an important first step towards understanding the relationship between cooling towers and LD.

Conclusion

Cooling towers surveyed in Allegheny County were found to be relatively well maintained in comparison to findings from other *Legionella* prevalence studies and LD outbreak investigations. Nevertheless, *Lp* was detected in almost half of cooling towers tested. Improving maintenance and reducing *Legionella* contamination in Allegheny County cooling towers would likely contribute to a reduction in the overall burden of disease and potential for cooling tower-associated outbreaks.

A detailed cooling tower maintenance guideline would be extremely beneficial for *Legionella* control, although, the creation of such a guideline may not be feasible. At a minimum, ASHRAE Standard 188-2015 should be followed. An important benefit of this prevalence study was increased contact with local water treatment professionals and facility engineers who are tasked with developing maintenance plans. Many times the facility's bottom line may trump implementation of more intensive cooling tower maintenance practices. Through this health department initiative, ACHD encouraged facilities to comply with ASHRAE Standard 188-2015 and improve maintenance practices. Other local and state health departments should note this important benefit and consider conducting a cooling tower *Legionella* prevalence study in their jurisdiction as a component of LD prevention efforts.

Acknowledgments

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3.6 TABLES AND FIGURES

Table 3.1. Survey and sampling response rate by building type (n = 412), Allegheny County, PA, summer 2016.

Facility Type	**Total Buildings**	**Completed Survey**	**Cooling Tower on Premises**	**Completed ACHD Cooling Tower Sampling**
Hospital	27 (7%)	27 (100%)	21 (78%)	15 (71%)
Skilled Nursing	62 (15%)	60 (97%)	12 (20%)	7 (58%)
Assisted Living	1 (<1%)	1 (100%)	0	0
Personal Care	106 (26%)	93 (88%)	3 (3%)	2 (67%)
Senior Apartment	70 (17%)	65 (93%)	11 (17%)	11 (100%)
City or County-Owned Residence	41 (10%)	32 (78%)	0	0
General County-Owned Building	100 (24%)	100 (100%)	9* (9%)	7 (78%)
Total	407	377 (93%)	56 (15%)	42 (75%)

* Two buildings had cooling towers only operational in the winter months, outside of testing window

Table 3.2. *Legionella pneumophila* concentration levels by building type (n = 42), Allegheny County, PA, 2016.

	Concentration (CFU/mL)					
	0	**1 to 9**	**10 to 99**	**100 to 999**	**1000 +**	**Total**
All Building Types	22 (52%)	0	16 (38%)	2 (5%)	2 (5%)	42
Hospital	5 (29%)	0	9 (53%)	1 (6%)	2 12%)	17
Skilled Nursing	4 (80%)	0	1 (20%)	0	0	5
Assisted Living	0	0	0	0	0	0
Personal Care	2 (100%)	0	0	0	0	2
Senior Apartment	7 (64%)	0	3 (27%)	1 (9%)	0	11
City or County-Owned Residence	0	0	0	0	0	0
General County-Owned Building	4 (57%)	0	3 (43%)	0	0	7

Table 3.3. Unadjusted associations between continuous *Legionella pneumophila* level and dichotomous factors(n = 42), Allegheny County, PA, 2016.

		Median and range *L. pneumophila* contamination level (CFU/mL)		
Variable	**Count (%) n = 42**	**Feature Present**	**Feature Absent**	**P-value**
Capacity of Tower > 422 tons	19 (45%)	20 (0 – 2000)	0 (0 – 90)	0.0003
Year Round Use	13 (31%)	40 (0 – 2000)	0 (0 – 100)	0.0015
Hospital	17 (40%)	20 (0 – 2000)	0 (0 – 100)	0.0061
Greater than 1 Cooling Tower onsite	20 (48%)	20 (0 – 2000)	0 (0 – 100)	0.014
Non-consecutive Water Authority Surface Water Supply	33 (79%)	0 (0 – 1140)	20 (0 – 2000)	0.021
August or September ACHD Test compared to June or July	19 (45%)	20 (0 – 2000)	0 (0 – 90)	0.025
Cooling Tower Age > 13 years old	21 (50%)	20 (0 – 2000)	0 (0 – 100)	0.057
Water Management Plan	9 (21%)	20 (0 – 2000)	0 (0 – 1140)	0.068
Located on Roof	22 (52%)	20 (0 – 2000)	0 (0 – 1140)	0.096
Located on the Ground	17 (40%)	0 (0 – 1140)	20 (0 – 2000)	0.12
Inspected > Once per Month	13 (31%)	20 (0 – 1140)	0 (0 – 2000)	0.13
Contract with Water Treatment Provider	38 (90%)	0 (0 – 2000)	50 (0 – 90)	0.14
Use of Drift Eliminator	23 (55%)	0 (0 – 600)	10 (0 – 2000)	0.15
Legionella Test ≥ Annually	22 (52%)	20 (0 – 2000)	0 (0 – 100)	0.15
Use of Both Oxidizing and Non-Oxidizing Disinfectant	17 (40%)	20 (0 – 2000)	0 (0 – 100)	0.22
Tower cleaned > Twice Annually	9 (21%)	0 (0 – 40)	10 (0 – 2000)	0.25
Direct or Open Circuit System	28 (67%)	10 (0 – 2000)	0 (0 – 70)	0.25
Basin Emptying ≥ Annually	28 (67%)	0 (0 – 90)	10 (0 – 2000)	0.25
Use of Non-Oxidizing Disinfectant Only	5 (12%)	0 (0 – 70)	10 (0 – 2000)	0.33
Protected from Sunlight	6 (14%)	30 (0 – 70)	0 (0 – 2000)	0.33
Regular Basin Cleaning	39 (93%)	0 (0 – 2000)	0 (0 – 10)	0.38
Seasonal Chloramination by Water Authority	11 (26%)	0 (0 – 40)	0 (0 – 2000)	0.43
Maintenance and Testing Records Kept	38 (90%)	5 (0 – 2000)	0 (0 – 40)	0.44
Use of Oxidizing Disinfectant Only	12 (29%)	15 (0 – 100)	0 (0 – 2000)	0.45

Table 3.3 Continued

Variable	Count (%) n = 42	Feature Present	Feature Absent	P-value
Test for Bacteria ≥ Annually	34 (81%)	0 (0 – 2000)	15 (0 – 90)	0.51
Year Round Chloramination by Water Authority	11 (26%)	10 (0 – 2000)	0 (0 – 1140)	0.66
Water Filtration	17 (40%)	10 (0 – 1140)	0 (0 – 2000)	0.7
Automatic Biocide Feed	36 (86%)	0 (0 – 2000)	5 (0 – 40)	0.75
Free Chlorine Used by Water Authority	20 (48%)	0 (0 – 1140)	5 (0 – 2000)	0.76
Basin Water Temperature > 77°F	16 (38%)	5 (0 – 600)	0 (0 – 2000)	0.84
Basin Water pH > 7	3 (7%)	0 (0 – 90)	0 (0 – 2000)	0.86
Test for Biocide Routinely	27 (64%)	0 (0 – 2000)	0 (0 – 70)	0.89

Table 3.4. Unadjusted associations between continuous *Legionella pneumophila* level and continuous factors (n=42), Allegheny County, PA, 2016.

Variable	Count or Mean (% or range) n = 42	Regression Coefficient	Confidence Interval	P-value
Cooling Tower Age (years)	13 (38 – < 1 year)	20.5	6.8, 34.1	0.0043
Number of Towers Onsite	2 (1 – 6)	83.4	7.2, 159.7	0.033
ACHD Sampling Month	July (June – September)	62.9	-42.8, 168.7	0.24
Basin Water Temperature (°F)	77 (62 – 88)	5.7	-15.4, 26.8	0.59
Basin Water Total Chlorine Level (ppm)	<0.5 (0 – 10)	-13.7	-88.2, 60.8	0.71
Basin Water Free Chlorine Level (ppm)	< 0.5 (0 – 4)	-23.0	-212.7, 166.7	0.81
Capacity of Tower (tons)	422 (29 - 17950)	0.004	-0.04, 0.05	0.82
Basin Water pH	7.0 (6 - 11)	1.4	-152.7, 155.5	0.99

Table 3.5. Significant unadjusted associations with *Legionella pneumophila* level stratified by hospital status.

Variable	Count (%) n = 17	Feature Present	Feature Absent	P-value
Hospitals (n=17)				
Year Round Use	10 (59%)	60 (0 to 2000)	0 (0 to 40)	0.014
Cooling Tower Age > 13 years old	12 (71%)	50 (0 to 2000)	0 (0 to 20)	0.038
Non-hospitals (n=23)				
Capacity of Tower > 422 tons	7 (28%)	10 (0 to 100)	0 (0 to 70)	0.0098

Table 3.6. Multivariable linear regression model of independent factors and log-transformed *Legionella pneumophila* continuous outcome.

Independent Predictor	Inclusion Criteria	Coefficient	Confidence Interval	P-value
Cooling Tower Age	Independent predictor after stepwise procedure	0.07	0.006, 0.1	0.03
Year round usage	Confounder between tower age and outcome	0.6	-0.4, 1.6	0.2
Hospital status	Confounder between tower age and outcome	-0.6	-1.8, 0.6	0.3

Table 3.7. Pairwise SNP differences among 13 serogroup 1 *L. pneumophila* isolates using LEG551 as reference genome.

	LEG551	LEG507	LEG322	LEG590	LEG349	LEG591	LEG444	LEG508	LEG589	LEG441	LEG574	LEG588	LEG575	LEG443
LEG551	0	46	80	1132	2895	9295	14339	19729	23481	72682	184434	184600	186239	186832
LEG507		0	57	1110	2879	9262	11652	19640	21099	70670	184328	183338	184977	186615
LEG322			0	1157	2919	9304	11665	19677	21151	70702	184380	183389	185033	186710
LEG590				0	1818	10360	11666	20013	21466	70517	184017	183033	184690	186300
LEG349					0	9795	11685	21752	23205	71761	182183	181266	182879	184474
LEG591						0	10528	25695	27973	73999	173203	173409	174160	175491
LEG444							0	12331	14057	16769	23116	23733	24558	23160
LEG508								0	1998	68029	177310	177875	178033	178962
LEG589									0	69156	177032	178793	179230	178547
LEG441										0	179130	180010	181095	180368
LEG574											0	1897	2176	485
LEG588												0	1480	3778
LEG575													0	2135
LEG443														0

Table 3.8. Pairwise SNP differences among 4 ST2329 isolates using LEG443 as reference genome.

	LEG443	LEG574	LEG575	LEG588
LEG443	0	38	1330	4257
LEG574		0	1144	1247
LEG575			0	400
LEG588				0

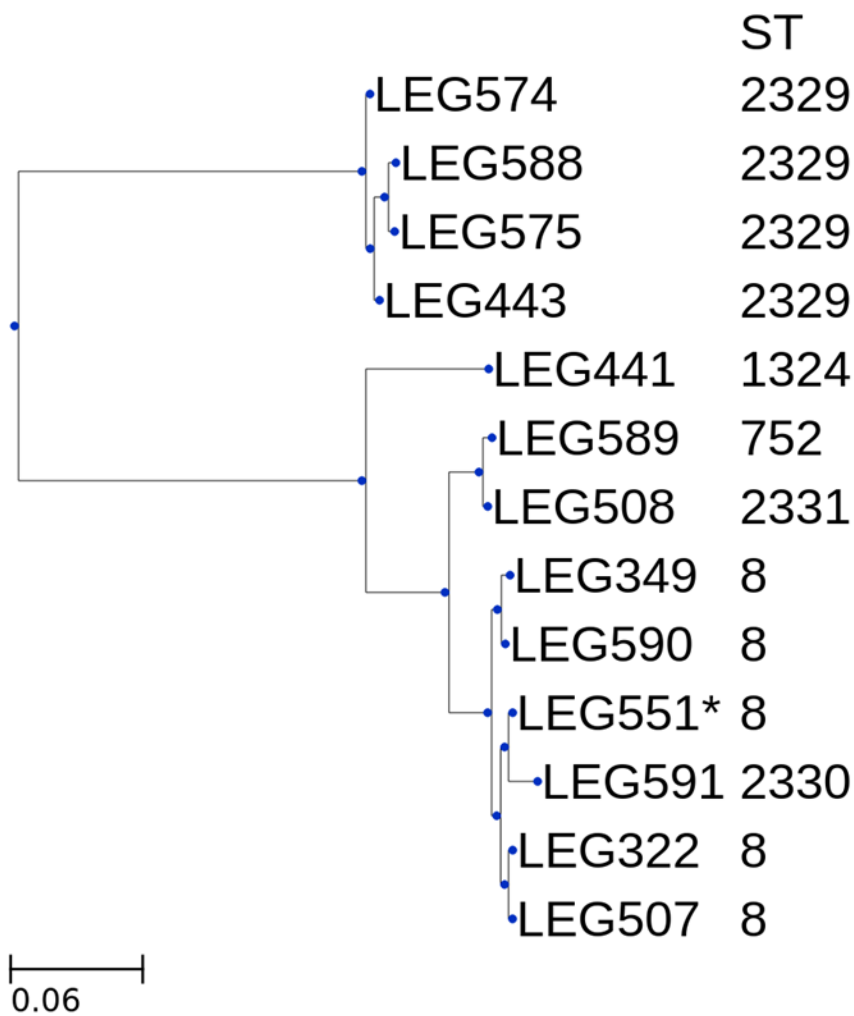

Figure 3.1. Phylogeny of 13 serogroup 1 L. pneumophila genomes based on aligned SNPs to reference assembly, LEG551.

Scale represents mean number of nucleotide substitutions per site. Sequence type (ST).

4.0 SIMULATION OF LEGIONNAIRES' DISEASE SPATIOTEMPORAL CLUSTER DETECTION, ALLEGHENY COUNTY, PA

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4.1 ABSTRACT

BACKGROUND: Legionnaires' disease (LD) outbreaks cause considerable morbidity and mortality. Health departments are tasked with detecting these outbreaks quickly to identify the source and prevent further transmission. The objective of this study is to determine the adaptability, utility and performance of an LD cluster detection system first used by the New York City Department of Health and Mental Hygiene through a prospective simulation in Allegheny County, PA.

METHODS: Three simulated LD outbreaks were generated based on data from actual outbreaks published in the literature. Simulated cases were imbedded in actual Allegheny County baseline 2014 – 2016 surveillance data using a simulated report date. SAS (v.9.4) and SaTScan (v.9.4.4) were used to mimic daily analyses using the prospective space-time permutation scan statistic. Analyses with 30-day and 180-day maximum temporal windows were conducted. The result of each daily analysis was categorized as either detecting a true positive cluster, a false positive cluster, a false negative cluster or a true negative cluster based on 20-day, 100-day and 365-day recurrence intervals. Validity statistics as well as time to detection were calculated.

RESULTS: Two large, simulated cooling tower-associated outbreaks were detected, whereas a small, simulated potable water-associated outbreak was not detected.

CONCLUSIONS: Health departments should consider adopting this cluster detection method for improved LD outbreak detection, faster investigation initiation and potential disease prevention.

4.2 INTRODUCTION

Legionnaires' disease (LD) is a pneumonia caused by *Legionella* bacteria that disproportionately affects elderly and immunocompromised persons and can lead to death [2]. LD is the second most common form of bacterial pneumonia in the US. Transmission occurs primarily through inhalation of aerosolized droplets from a contaminated water source. Known sources include large building water systems, cooling towers, soil, hot tubs and residential potable water systems [2].

In the US, only 4% of LD cases have been shown to be outbreak-associated [9]. Allegheny County, PA, which includes Pittsburgh and the surrounding suburbs, experiences LD rates four-times higher than the national rate [29]. The majority of these cases are sporadic or non-outbreak associated. At the Allegheny County Health Department (ACHD), as in many other health departments, staff routinely conducts patient interviews to identify risk factors and then review collected data to assess common exposures among cases. Detecting clusters without using statistical analyses relies on astute staff recognizing links between cases and can take days to weeks after cases begin to be reported.

Through both prospective and retrospective methods, statistical cluster detection has been shown to successfully identify LD outbreaks quickly and accurately. Sansom et al. fit a Poisson distribution-based model which estimates the strength of association between cases based on location and timing of infection [91]. A cluster size of three provided the best combination of higher sensitivity and lower false alarm rate. This method was validated using UK historical outbreak and non-outbreak associated cases and relied on the availability of population data to fit the model which is often difficult to ascertain [91].

Another cluster detection method demonstrated in the Netherlands utilized the software SaTScan^{TM} [27]. The investigators conducted simulated prospective weekly and daily analyses using the space-time permutation scan statistic [26] to detect lower respiratory tract outbreaks in seven years of Dutch lower respiratory tract infection syndromic surveillance data [92]. An advantage of using the scan statistic method is that it does not require population-at-risk data for the analysis. The scan statistic uses cylinders to analyze the data in space and time, with the circle representing space and the height representing time.

This method was validated using data from two large Dutch community-acquired LD outbreaks. The first outbreak involved 188 cases and was associated with a hot tub at a Dutch flower show [93]. The second outbreak involved 30 cases and was associated with a cooling tower [94]. These outbreaks were detected by this cluster detection program 2 and 3 days before public health practitioners noticed an increase in cases. This suggests that spatiotemporal analyses for syndromic surveillance are useful if epidemiologic and microbiological data are available as a supplement [92].

A prospective spatiotemporal analysis is performed daily by the New York City Department of Health and Mental Hygiene (DOHMH) to detect clusters of reportable infectious disease conditions. DOHMH used the space-time permutation scan statistic in SaTScan^{TM} to conduct daily analyses of 35 reportable infectious disease conditions, including LD [28]. The maximum geographical cluster size is set at half of all cases and the maximum temporal window is set at 30 days for most conditions. A signal is created when the recurrence interval (RI) for an identified cluster exceeds a pre-specified RI threshold. The RI is defined as the number of days of analyses required in order to expect the number of clusters at least as unlikely as the observed cluster to be equal to 1 by chance alone. Several LD outbreaks have been detected using the

DOHMHM method since its inception in 2014, including the nation's second largest community-acquired outbreak which was caused by a South Bronx hotel cooling tower [28]. In this instance, the DOHMH SaTScan^{TM} cluster detection method detected a significant cluster before any individual noticed an increase in cases.

Only one community-acquired LD outbreak occurring in 2008 has been identified in Allegheny County in the past decade. Traditional patient interview-based surveillance methods have not identified common exposures amongst community-acquired Allegheny County LD cases. Clusters of seemingly sporadic cases could go undetected as sources such as cooling towers are difficult to identify with surveillance methods that rely on human review and descriptive epidemiology. More timely detection would lead to faster outbreak investigation, source mitigation and disease prevention. The objective of this study is to determine the adaptability, utility and performance of DOHMH's SaTScan cluster detection method for LD outbreak detection through a prospective simulation in Allegheny County.

4.3 METHODS

Data on legionellosis cases reported in Allegheny County in 2014 - 2016 were obtained through Pennsylvania's National Electronic Disease Surveillance System (PA-NEDSS). Date of report and latitude and longitude coordinates of residence were used to represent cases. Legionellosis is comprised of two conditions caused by *Legionella* bacteria: LD and Pontiac fever, which is a milder febrile illness and therefore less commonly diagnosed and reported. A confirmed case of legionellosis is defined by the Council of State and Territorial Epidemiologists as a clinically compatible illness confirmed by laboratory culture, urine antigen or antibody seroconversion [95].

Three simulated outbreaks were created based on data published on community-acquired LD outbreak investigations [96-98]. These three simulated outbreaks were created because they represent three distinct LD community-acquired outbreak types that could potentially be detected by this cluster detection method: 1) a fast-growing cooling tower-associated LD outbreak, 2) a moderate-growing cooling tower-associated LD outbreak, and 3) a slow-growing potable water distribution system-associated LD outbreak. The outbreaks varied by environmental source, number of cases, duration, growth of epidemic curve, radius of affected area, and season (Table 1). The specific published outbreak investigations used to create the simulations were chosen in part because the population size of the affected area was relatively similar to Allegheny County's 1.2 million people (Table 1) [99]. The cases from each individual outbreak were inserted into Allegheny County baseline data based on a simulated report date to mimic the published epidemic curve. An epidemic curve for simulated outbreak 3 was not available in the published manuscript [98]; however, this outbreak investigation was the only published community-acquired potable water LD outbreak identified through a PubMed search on October 24, 2017. Information from the manuscript was used to estimate an epidemic curve.

Each published outbreak used for a simulation included a point map of case spatial distributions. For each simulated outbreak we mimicked the published outbreak spatial distribution by calculating the distribution of published outbreak cases within circular bands of increasing radius centered on the outbreak source, and then assigning locations to simulated outbreak cases to achieve the same distribution relative to the simulated outbreak area. We also recreated the visual density of cases immediately surrounding the simulated outbreak source.

The published manuscript used as the basis for simulated outbreak 2 also included the spatial distribution of cases during two time periods. We used this information to further refine the case spatial distribution of simulated outbreak 2. Only home addresses were simulated and included in this analysis.

We analyzed the simulated study data for Allegheny County, which included baseline or routine public health surveillance data spiked with simulated outbreak cases, using a SAS program created by DOHMH [28], modified for use by ACHD. The original DOHMH SAS program was easily modified by an ACHD epidemiologist with intermediate SAS skills. Minor modifications included editing portions of the original code to conform to PA-NEDSS-specific nuances, removing code related to secondary addresses, and editing portions of the code that reference NYC-specific boroughs and United Hospital Fund (UHF) neighborhoods [28]. The DOHMH standard maximum spatial cluster size of 50% of all cases reported during the study period was used for this analysis. The maximum temporal cluster size chosen for each simulated outbreak analysis was determined by the time span of each simulated outbreak epidemic curve (Table 1). A 30 day and 180 day maximum temporal cluster size has been used to detect rapidly and slowly accelerating LD outbreaks, respectively [28]. Simulated outbreak 2 was analyzed with both 30 and 180 day maximum temporal windows as the epidemic curve was not clearly either fast or slow-growing. The maximum temporal cluster sizes of 30 and 180 days require 365 and 730 days of historical baseline data, respectively. A test statistic and p-value was calculated for each cylinder to determine whether an observed cluster in the cylinder during the specified time period was due to chance. The RI for a given cluster was calculated by taking the reciprocal of the p-value for the associated cylinder.

We assessed 2014 – 2016 Allegheny County legionellosis case reports for previously unidentified true clusters through a retrospective analysis using the space-time permutation scan statistic in SaTScan™. These years were analyzed given they represented baseline years for prospective analyses. For each of the three LD outbreak types, we spiked the 2016 baseline data with a simulated outbreak. The 2016 data with each of the three outbreaks were analyzed separately one time. We mimicked daily prospective analyses for the entire year of 2016 using three RI thresholds: 20, 100, and 365 days. Analysis days were restricted to days in which a baseline or simulated case was reported given the potential for cluster signaling. Analysis days with at least one cluster exceeding the RI threshold were classified as positive, while analysis days with no clusters exceeding the RI threshold were classified as negative. The results of a daily analysis were considered to be true positive if 1) RI $\geq$ threshold assigned and 2) $\geq$ 3 simulated cases included in cluster detected. The results of a daily analysis were considered to be false positive if 1) RI $\geq$ threshold assigned and 2) $<$ 3 simulated cases included in cluster detected. The results of a daily analysis were considered to be true negative if 1) RI $<$ threshold assigned and 2) $<$ 3 simulated cases were reported in maximum temporal window. Finally, the results of a daily analysis were considered to be false negative if 1) RI $<$ threshold assigned, 2) $\geq$ 3 simulated cases were reported in maximum temporal window and 3) $\geq$ 1 simulated case was reported that day.

These daily analysis assignments, based on the three RI thresholds, were used to calculate the validity statistics of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for each simulated outbreak. Sensitivity was defined as the proportion of true positive daily analyses amongst all daily analyses that should have signaled a cluster. Specificity was defined as the proportion of true negative daily analyses amongst all daily analyses that should not have signaled a cluster. PPV was defined as the proportion of true positive daily analyses amongst all signaled daily analyses. NPV was defined as the proportion of the true negative daily analyses amongst all non-signaled daily analyses.

Time to outbreak detection was calculated for each simulated outbreak by subtracting the earliest outbreak detection date from the report date of the third simulated outbreak-associated case. All analyses were performed using SAS (v.9.4) and SaTScan™ (v.9.4.4).

4.4 RESULTS

During 2006–2016, the observed number of LD cases reported per year in Allegheny County ranged between 54 and 118, and 90 cases were reported in 2016 (Figure 1). When retrospectively analyzing these 2014 – 2016 Allegheny County LD surveillance data, no clusters were detected.

A total of 144 outbreak cases were added as part of three separate outbreak simulations (Figure 2, 3). The shortest time to detection was 1 day for outbreak 1 and 22 days for outbreak 2; however, simulated outbreak 3 was not detected (Table 2). Time to detection was shortened by using a lower RI threshold for all three simulated outbreaks. Time to detection was shortened for simulated outbreak 2 when a 30 day maximum temporal window was used (Table 2).

Using a 30 day maximum temporal window and an RI signaling threshold ≥20 days or ≥100 days, all validity statistics for simulated outbreak 1 detection were ≥90%, thus few false negative and false positive days were produced (Table 3). Using a 30 day maximum temporal window, the sensitivity of simulated outbreak 2 detection was low using a RI ≥20 days threshold and very low using a RI ≥100 day threshold, whereas all other validity statistics were ≥64%

(Table 4). When using a 180 day maximum temporal window and either RI signaling threshold, sensitivity of simulated outbreak 2 detection was ≥43%. The other validity statistics were ≥76% (Table 4). Using a 180 day maximum temporal window and either RI signaling threshold, outbreak 3 was not detected, so sensitivity was 0% and specificity was 100% (Table 5).

4.5 DISCUSSION

In this study, the DOHMH SaTScan cluster detection method successfully identified two larger, more explosive simulated outbreaks and failed to detect one smaller, slow-growing simulated outbreak. The simulated outbreaks analyzed were based on outbreaks that occurred in locations with comparable population sizes to Allegheny County; however, Allegheny County's underlying LD burden is most likely elevated in comparison. One might expect outbreak clusters to be more difficult to detect in areas with a high LD incidence; however, large outbreaks like simulated outbreaks 1 and 2 would be difficult to miss given the large number of cases. Both outbreaks were detected relatively quickly. The improved validity statistics of the second simulation demonstrate the utility of using the 180 day maximum temporal window, while the 30 day maximum temporal window was more appropriate for the first simulation. Both simulated outbreaks 1 and 2 were based on cooling tower outbreaks. Cooling tower outbreaks can be difficult to quickly detect through human review given patients are often unaware of cooling tower exposures.

In 2015, the DOHMH SaTScan cluster detection method detected a cluster with an RI of 500 days which included eight cases centered on the South Bronx. This cluster was identified three days before BCD staff independently noted an increase in LD cases, and four days before staff from a South Bronx hospital notified DOHMH of an increase in LD among emergency department patients [28]. Resource-intensive methods such as patient interviews, multi-focused cluster tests for cooling tower sampling prioritization, and extensive environmental sampling were employed to identify the cooling tower source [19]. The DOHMH SaTScan cluster detection method significantly contributed to the timeliness of the outbreak investigation and mitigation and was useful for tracking the scope of the outbreak after initial detection, as additional cases were reported.

The scan statistic is advantageous for prospective infectious disease cluster detection because it scans across all possible spatial and temporal boundaries within specifications, does not require population-at-risk, and accounts for the problem of multiple testing when analyzing closely overlapping spatial areas and time windows [26]. This method most successfully detects outbreaks that are highly focal and are circular in shape, such as LD cooling tower-related outbreaks, given the scanning window cylinder is circular. Nevertheless, non-circular shaped outbreaks have been successfully detected by this method [100]. Previously, the effectiveness of the scan statistic for cluster detection was demonstrated through analysis of West Nile dead bird surveillance, hospital emergency department syndromic surveillance, ambulance dispatch call surveillance, pharmacy sales data, shigellosis surveillance, and campylobacteriosis surveillance [26, 100-105].

Simulated outbreak 3 was not detected, as it occurred over a longer time period and included few cases. The frequency of this type of local potable water LD outbreak is unknown. If these types of outbreaks do occur regularly, they most likely go undetected. This type of outbreak may not be detected through other surveillance methods, although the ACHD public health nurses who conduct patient interviews might notice the proximity of residential addresses.

Additional analyses could be automated to identify multiple cases within a defined period sharing a common potable water source [106].

Health departments should consider adopting this SaTScan™ method for LD cluster detection. For optimized sensitivity and PPV, daily analyses should be run simultaneously using both a 30 and 180 day maximum temporal window and a high RI threshold, such as 100 days. This cluster detection method should be considered by health departments especially for detection of cooling tower-associated LD outbreaks. This method could also reinforce spatiotemporal trends observed by public health investigators and provide additional evidence to support the need for further investigation. Each simulated outbreak was detected more quickly using an RI threshold of 20 days; however, more false positives were produced with this threshold that could overextend limited public health resources. Detecting a significant cluster using this method should initiate an investigation of a potential source including enhanced patient interviews and environmental sampling. Adopting this cluster detection method for LD outbreak detection is also advantageous for health departments given additional conditions could be analyzed using this method.

This simulation method has several limitations. First, PA-NEDSS case report date was the only date simulated to mimic the published epidemic curve, rather than onset or diagnosis date, which are more meaningful epidemiologically, but more difficult to simulate based on published data available. In actuality, report dates might not have the same temporal pattern as onset or diagnosis dates. Report dates and can also be delayed because of batch electronic reporting, increasing the time to outbreak detection.

Second, residential address was the only address simulated for each case. DOHMH also analyzes work address when available which improves sensitivity for detecting clusters where a patient's exposure occurred near his or her worksite but not residence. At this time, PA-NEDSS does not include data on work address in a systematic way, thus this analysis reflects the current limitations of PA-NEDSS. Most likely, other health departments are similarly limited in case address availability. Each publication utilized to simulate outbreaks described case spatial distributions based solely on residential address.

Third, daily analyses were simulated only over one year. Results may vary if this daily analysis simulation were repeated over several years given fluctuation in baseline Allegheny County LD case counts. Only three simulated outbreaks were generated for this analysis. These three types were chosen because they represent three distinct types of community-acquired LD outbreaks. Many simulations of one outbreak type could have been generated with parameter specifications; however, we chose to simulate one outbreak of each type as accurately as possible based on information available through the publication including spatial distribution and epidemic curve. Information about the epidemic curve of simulated outbreak 3 was limited.

The method used to simulate the spatial distribution of each outbreak is novel and has limitations. The outbreaks used for simulation occurred outside of Allegheny County in jurisdictions that differ from Allegheny County in many ways. Creation of these simulations required making assumptions about the spatial distribution of cases that in actuality may take a different form because of differences in Allegheny County population density and distribution, topography, and wind patterns. Also, we did not take into account area-based poverty when considering the spatial distribution of simulated cases. This may have affected our ability to detect increases in case counts relative to baseline LD.

Finally, these findings might not be fully generalizable to jurisdictions with low LD incidence. It may be more difficult to detect clusters in Allegheny County than in other locations with lower baseline case counts. Detection of smaller outbreaks like simulated outbreak 3 is certainly difficult in Allegheny County and may perhaps be easier in locations with lower baseline case counts.

Conclusion

This cluster detection method was easily adapted for use in Allegheny County and will continue to be used for prospective LD cluster detection going forward. This method quickly detected simulated cooling tower-related outbreaks that otherwise might have required more time to detect by surveillance methods that rely on human review of descriptive case epidemiology, given cases are unaware of exposures. A smaller, more slow-growing potable water outbreak was not detected using this method. Nevertheless, health departments should consider utilizing this cluster detection method for improved LD outbreak detection and potential disease prevention.

4.6 TABLES AND FIGURES

Table 4.1. Legionnaires' disease simulated outbreak characteristics based on published outbreak investigations.

Outbreak Simulation	Outbreak Location	Population size	Case Count	Duration	Rapid/Slow Growth	Maximum Temporal Cluster Size	Environmental Source	Outbreak radius	Season
# 1 [96]	Edinburgh, Scotland	495,360	50	38 days	Rapid	30 day	Cooling tower	6 miles	Early summer
# 2 [97]	Pas-de-Calais, France	1,452,590	84	82 days	Moderate	30 day, 180 day	Cooling tower	3.75 miles	Fall
# 3[98]	New Jersey, exact location not disclosed	n/a	10	163	Slow	180 day	Potable water	1 mile	Summer

Table 4.2. Days from third outbreak-associated case report to outbreak detection for three simulated Legionnaires' disease outbreaks, Allegheny County, 2016.

	30 day max temporal window			***180 day max temporal window***		
	RI ≥20	**RI ≥100**	**RI ≥365**	**RI ≥20**	**RI ≥100**	**RI ≥365**
Outbreak simulation 1	1 day	5 day	5 days	n/a	n/a	n/a
Outbreak simulation 2	22 days	38 days	Not detected	33 days	33 days	36 days
Outbreak simulation 3	n/a	n/a	n/a	Not detected	Not detected	Not detected

Table 4.3. Simulated outbreak 1 daily analyses validity statistics (n = 105 days).

	30 day max temporal window		
	RI ≥20	**RI ≥100**	**RI ≥365**
Sensitivity	100%	95.2%	90.4%
Specificity	98.8%	100%	100%
Positive Predictive Value	95.4%	100%	100%
Negative Predictive Value	100%	98.8%	97.7%

Represents proportions of total days in which no baseline and/or simulated case(s) were reported were not analyzed

Table 4.4. Simulated outbreak 2 daily analyses validity statistics (n = 125 days).

	30 day max temporal window			**180 day max temporal window**		
	RI ≥20	**RI ≥100**	**RI ≥365**	**RI ≥20**	**RI ≥100**	**RI ≥365**
Sensitivity	29.5%	6.8%	0%	50.0%	50.0%	43..2%
Specificity	98.8%	100%	100%	98.8%	98.8%	98.8%
Positive Predictive Value	92.9%	100%	Undefined	95.7%	95.7%	95.0%
Negative Predictive Value	72.1%	66.4%	64.8%	78.5%	78.5%	76.2%

Represents proportions of total days in which no baseline and/or simulated case(s) were reported were not analyzed

Table 4.5. Simulated outbreak 3 daily analyses validity statistics (n = 94 days).

	180 day max temporal window		
	RI ≥20	**RI ≥100**	**RI ≥365**
Sensitivity	0%	0%	0%
Specificity	97.7%	100%	100%
Positive Predictive Value	0%	Undefined	Undefined
Negative Predictive Value	91.3%	91.5%	91.5%

Represents proportions of total days in which no baseline and/or simulated case(s) were reported were not analyzed

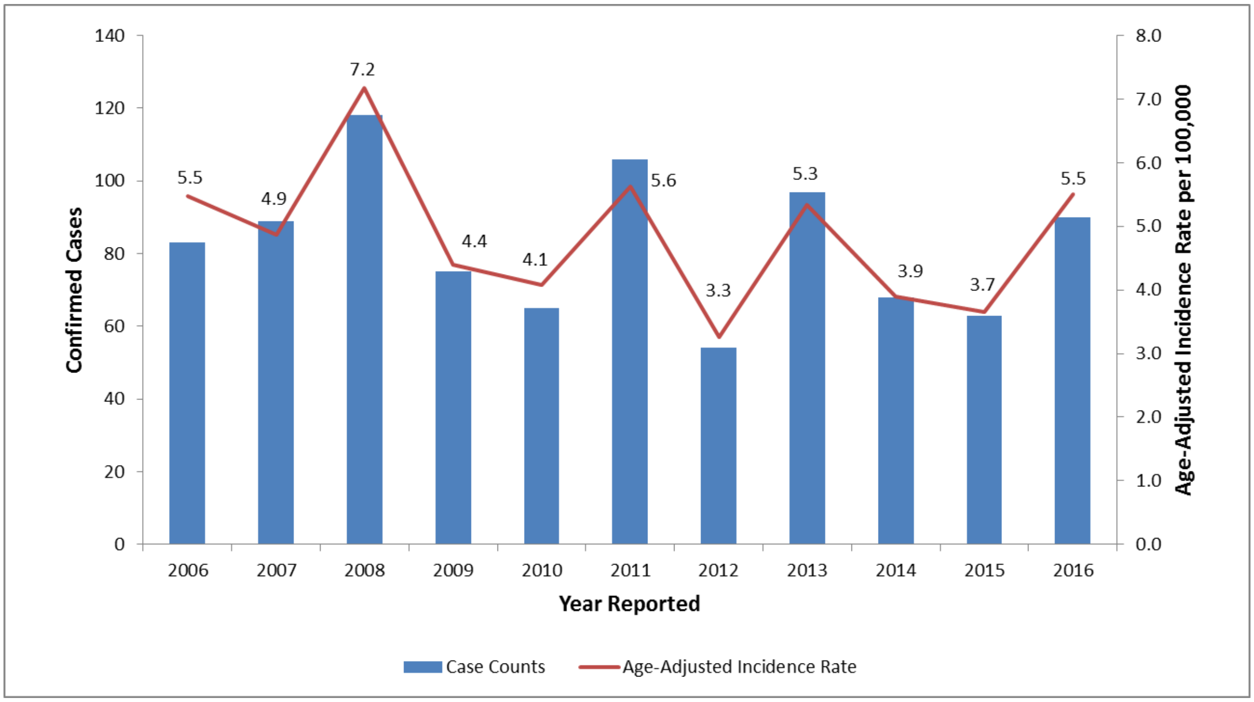

Figure 4.1. Confirmed legionellosis cases and age-adjusted legionellosis incidence rates, Allegheny County, PA, 2006-2016.

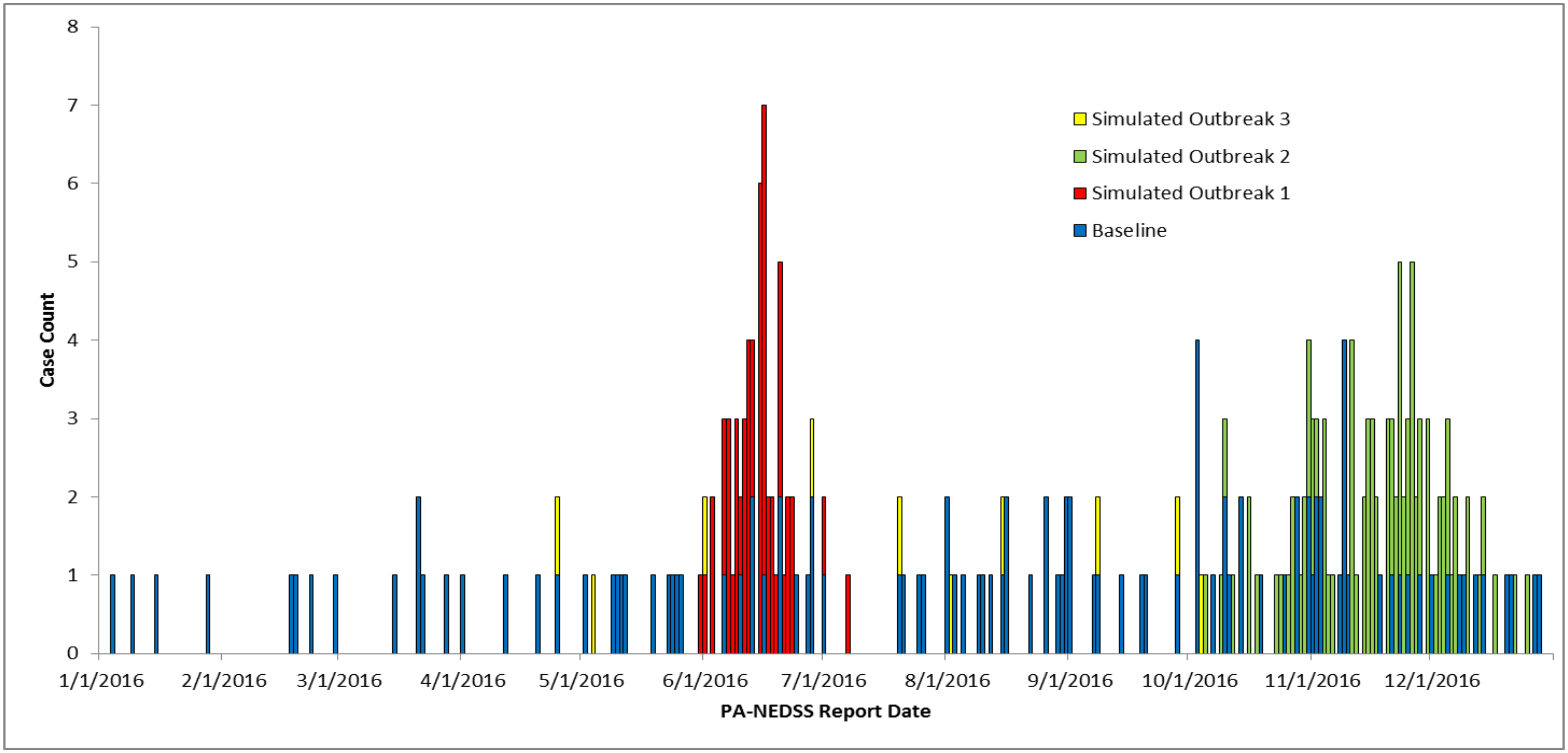

Figure 4.2. Observed baseline and simulated outbreak-associated legionellosis cases, Allegheny County, PA, 2016.

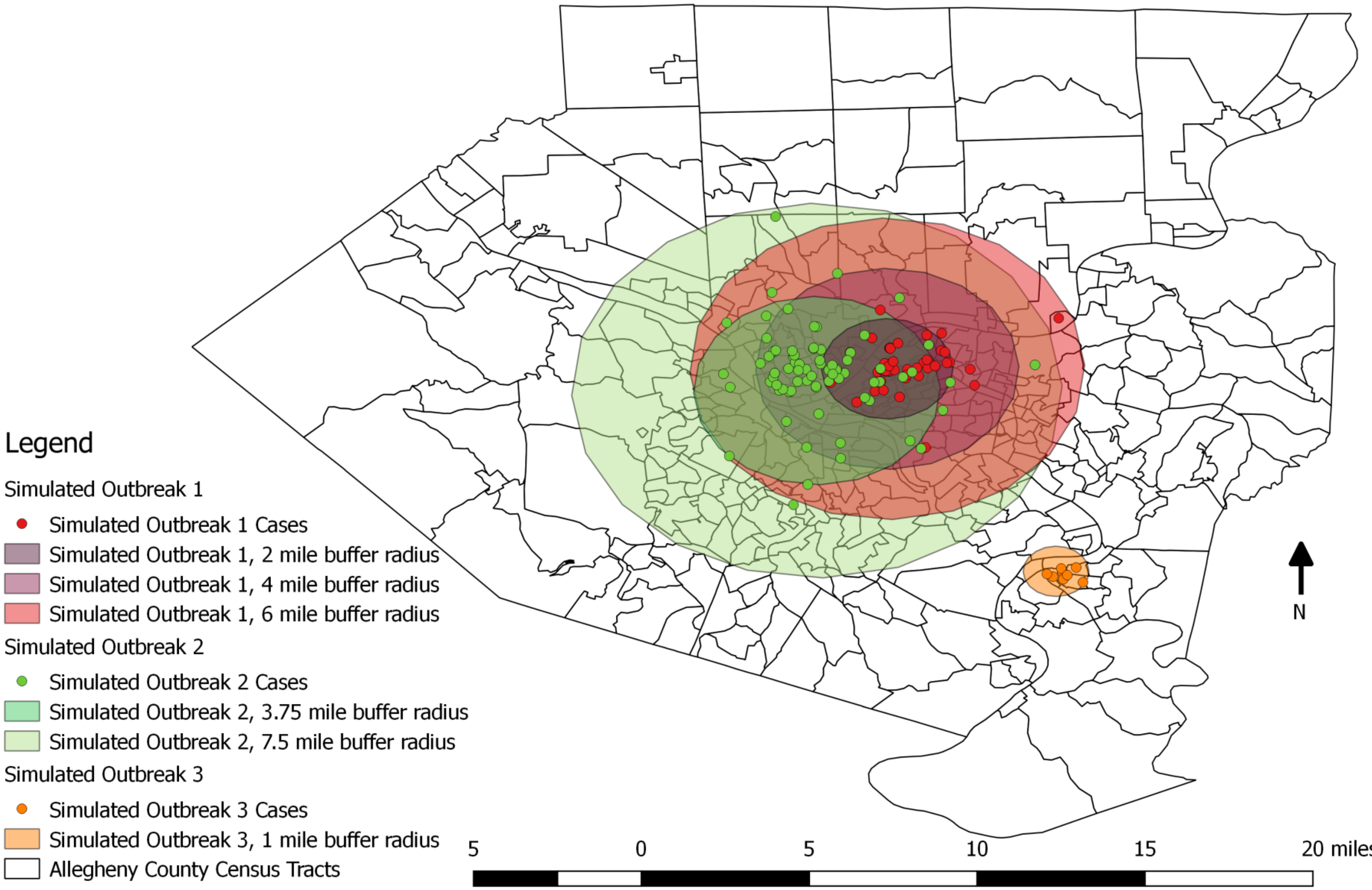

Figure 4.3. Simulated outbreak- associated Legionnaires' disease cases and simulated outbreak buffers, Allegheny County, PA.

5.0 DISSERTATION DISCUSSION

5.1 MAJOR FINDINGS

Sporadic LD is a disease of relatively high morbidity and mortality, yet epidemiologic research on the subject is lacking. This dissertation aimed to improve our understanding of sporadic LD epidemiology and suggests means for LD incidence reduction through three complementary studies. The first explores the sources of sporadic LD and suggests directions of future research. The second assesses cooling towers as a source of sporadic and outbreak-associated LD and provides recommendations to prevent *Legionella* contamination and thus LD transmission. Finally, the third assesses the adaptability, performance and utility of a cluster detection program that could aid in identifying relatedness among seemingly sporadic LD cases, leading to identification environmental sources and prompt remediation to prevent LD transmission.

The literature review presented in chapter two of this dissertation highlighted gaps in our understanding of the significant environmental sources of sporadic LD. Based on the limited literature available, significant sources included residential potable water, motor vehicle travel, and large building water systems. Source significance was determined through evidence of environmental source and case linkage as well as *Legionella* bacteria found in the environmental source to which vulnerable populations could be exposed. Cooling towers may also be a significant source of sporadic LD, but linkage between cooling towers and sporadic LD is difficult. Ultimately, we found that the source of the majority of sporadic LD is not determined given the many limitations of environmental source investigations.

The results of the cooling tower *Legionella* prevalence study are presented in chapter three of this dissertation. Almost half of Allegheny County cooling towers sampled were positive for *L. pneumophila*. *L. pneumophila* concentration level was positively associated with cooling tower age, larger tower capacity, year round usage and hospital status. Cooling tower age was the most important predictor of *L. pneumophila* concentration level. ACHD issued recommendations to building managers in response to these results including a recommendation to develop a water management plan and to conduct annual basin emptying, quarterly cleaning, quarterly *Legionella* testing and diligent inspection of older towers.

Finally, the results of the LD cluster detection simulation are presented in chapter four of this dissertation. In general, the cluster detection method was relatively adaptable for a local health department with moderate informatics capabilities. Larger, cooling tower-associated outbreaks were quickly detected, whereas a smaller, potable water-associated outbreak was not detected. Health departments should consider adopting this method for improved LD outbreak detection, faster investigation initiation and potential disease prevention.

5.2 PUBLIC HEALTH SIGNIFICANCE

Health departments are tasked with protecting the public health of their citizens. Often health department financial and personnel resources are limited must be used efficiently. LD is a devastating and costly disease that is difficult to control given the exact source is often hard to pinpoint. Nevertheless, it is also a disease of public interest and concern, especially during an outbreak. Health department resources should be allocated to LD prevention measures to preempt outbreaks and increase cost-efficiency as well as reduce overall LD incidence.

The results of three complementary studies described in this dissertation inform locally-focused intervention strategies for LD prevention. The environmental sources of sporadic LD as described in chapter two should be considered potential targets for preemptive interventions. An example of such an intervention is provided by the Allegheny County cooling tower *Legionella* prevalence study as described in chapter three. Finally, chapter four describes a spatiotemporal method for efficient outbreak detection that should be considered for local implementation. This method could relate seemingly sporadic cases to an environmental source and contribute to faster outbreak detection and disease prevention.

LD prevention is especially important in Allegheny County given high LD incidence. The topic of this dissertation was selected based on the needs of ACHD. Resources for LD prevention in Allegheny County are limited and have historically been directed towards healthcare-associated LD prevention. In recent years, healthcare-associated LD has been a focus of public attention in Allegheny County given the high profile outbreak investigation at the VA Pittsburgh Health System hospital [107]. Nevertheless, the vast majority of LD in Allegheny County is sporadic, community-acquired LD and the environmental source is unknown.

Chapters three and four of this dissertation are of particular public health significance for sporadic and outbreak-associated LD prevention in Allegheny County. In chapter three, we identified important characteristics of Allegheny County cooling towers that were associated with higher *Legionella* concentration such as cooling tower age. As a result, ACHD developed and broadly distributed recommendations in Allegheny County to influence maintenance practices and reduce the risk of *Legionella* contamination in these structures. Although many of the cooling towers we sampled were related to healthcare facilities, this particular project has the potential to influence both cooling tower-associated community-acquired and healthcare-associated LD. We also established important relationships with Allegheny County water treatment professionals who were imperative to the success of the study. Collaboration with these professionals provided invaluable insight and established contacts for potential future consultation. Additionally, the spatiotemporal cluster detection method described in chapter four established the use of this cluster detection method for LD in Allegheny County and enabled ACHD to use this method to analyze other infectious conditions. The collaboration between ACHD and DOHMH epidemiologists was critical to this simulation study and underscores the importance of health department collaborations to advance public health practice.

5.3 FUTURE DIRECTIONS

Additional, large-scale epidemiologic research needs to be conducted to further assess the environmental sources of sporadic, community-acquired LD and their respective relative importance. The narrative literature review presented in chapter two highlights the lack of research in this area and suggests environmental sources to target for additional research such as cooling towers. Ideally, a case-control study should be conducted that would assess the risk factors and environmental exposures of sporadic, community-acquired LD cases compared to controls potentially matched on age and gender. If conducted in Allegheny County, this type of study could more definitively identify sources of sporadic disease and thus targets for intervention in Allegheny County.

Furthermore, this case-control study could be expanded to included cases and controls from multiple geographic regions in addition to Allegheny County. This expansion would allow for the specifics of geographic location (i.e. weather, topography) to be compared. For example, in Allegheny County, potable water is obtained from surface water sources. Examining LD cases in other geographic locations could help to explore the role of ground water-sourced potable water.

The Allegheny County cooling tower *Legionella* prevalence study, as presented in chapter three, highlighted the importance of preemptively assessing cooling towers to potentially prevent sporadic and outbreak-associated LD. A repeat survey should also be considered by the Allegheny County Health Department to determine if dissemination of recommendations led to decreased contamination. Cooling tower age was the most important predictor of *L. pneumophila* concentration level. Future research should further investigate the characteristics and maintenance practices associated with lower *Legionella* concentration levels specifically in older towers. This research could identify prevention strategies effective for older towers and may also suggest an appropriate replacement age if remediation efforts are unsuccessful.

The Allegheny County cooling tower *Legionella* prevalence study also helped to identify a proportion of cooling towers in Allegheny County; however, it is likely that the majority of cooling towers have yet to be identified. Identification of all Allegheny County cooling towers through a cooling tower registration could aid ACHD in future cluster investigations. The impact of cooling tower regulation in NYC is being studied by the NYC Department of Health and Mental Hygiene (DOHMH). Based on the results of this DOHMH analysis, other jurisdictions including Allegheny County may need to consider the utility of cooling tower registration and/or regulation.

The spatiotemporal cluster detection method is now being utilized by ACHD for prospective LD cluster detection. The real-time utility of this method in Allegheny County remains to be seen. Ultimately, this system will need to be sensitive in detecting outbreaks and yet have a high positive predictive value to minimize the number of false alarms. The results of the simulations as presented in chapter four are promising especially for identification of large cooling tower-associated LD outbreaks. In the future, ACHD should evaluate the application of this spatiotemporal cluster detection method for other infectious conditions such as sexually transmitted infections and non-infectious outcomes such as opioid overdose deaths.

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