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Editor's Choice

ORIGINAL ARTICLE

The sporadic nature of *Legionella pneumophila*, *Legionella pneumophila* Sg1 and *Mycobacterium avium* occurrence within residences and office buildings across 36 states in the United States

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Abstract

Aim: Premise plumbing may disseminate the bacteria *Legionella pneumophila* and *Mycobacterium avium*, the causative agents for legionellosis and pulmonary nontuberculous mycobacterium disease respectively.
Methods and Results: Using quantitative PCR, the occurrence and persistence of *L. pneumophila*, *L. pneumophila* serogroup (Sg)1 and *M. avium* were evaluated in drinking water samples from 108 cold water taps (residences: $n = 43$) and (office buildings: $n = 65$). *Mycobacterium avium*, *L. pneumophila* and *L. pneumophila* Sg1 were detected 45, 41 and 25% of all structures respectively. Two occurrence patterns were evaluated: *sporadic* (a single detection from the three samplings) and *persistent* (detections in two or more of the three samples).
Conclusions: The micro-organism's occurrence was largely sporadic. Office buildings were prone to microbial persistence independent of building age and square footage. Microbial persistence at residences was observed in those older than 40 years for *L. pneumophila* and was rarely observed for *M. avium*. The microbial occurrence was evenly distributed between structure types but there were differences in density and persistence.
Significance of and Impact of the Study: The study is important because residences are often suspected to be the source when a case of disease is reported. These data demonstrate that this may not be the case for a sporadic incidence.

Introduction

Respiratory diseases such as legionellosis (Mandell *et al.* 2007) and pulmonary nontuberculous mycobacterial (NTM) diseases (Griffith *et al.* 2007) can have a significant impact on disease prevalence and thereby on a country's healthcare system. In the United States, the incidence of legionellosis (Hicks *et al.* 2012) and the prevalence of pulmonary NTM disease (Adjemian *et al.* 2012) have increased over the past decade. In 2015, the US incidence for legionellosis was 1·9 cases per 100 000 persons and includes two manifestations: Pontiac fever and Legionnaires' disease (Adams *et al.* 2017). In Europe (United Kingdom, Germany, Spain, France, the Netherlands, Italy and Portugal), legionellosis incidences ranged from 0·6 to 5·6 cases per 100 000 persons in 2014 (ECDC, 2018). It is likely that these illness rates are underestimated because mild cases (Pontiac fever) rarely lead to medical consultation. The yearly healthcare costs related to the treatment of Legionnaires' disease in the United States are estimated to be about 250 million dollars (Collier *et al.* 2012).

Nontuberculous mycobacteria-related conditions, specifically pulmonary NTM diseases, are not nationally reportable/notifiable in either the United States or Europe. However, in the United States, the reported prevalence rate of 9·2–17·3 cases (patient) per 100 000 persons (Cassidy *et al.* 2009; Donohue and Wymer 2016) is higher than the 0·7–1·1 cases per 100 000 persons in some European

countries (Andrejak *et al.* 2010; Ringshausen *et al.* 2013; Rindi and Garzelli 2016). United States healthcare costs associated with the treatment of pulmonary NTM diseases range from 250 million to 1·7 billion dollars per year (Collier *et al.* 2012; Strollo *et al.* 2015). Both diseases generally occur in persons older than 60 years of age who have co-morbidity factors, such as chronic lung diseases and/or immunosuppression (Marston *et al.* 1994; Neil and Berkelman 2008) persistent.

Legionella pneumophila is the primary etiological agent responsible for causing most legionellosis cases (Fraser *et al.* 1977; McDade *et al.* 1977; Benin *et al.* 2002; Yu *et al.* 2002; Hicks *et al.* 2012). Of the 16 *L. pneumophila* serogroups, serogroup (Sg) 1 is responsible for all outbreaks (Garrison *et al.* 2016) and is a common serogroup for most community-acquired sporadic cases in the United States (Hicks *et al.* 2012). For pulmonary NTM diseases, *Mycobacterium avium*, *M. intracellulare* and *M. chimaera* species within the *M. avium* complex (MAC) are responsible for the vast majority of illnesses (Prevots *et al.* 2010; Henkle *et al.* 2015; Smith *et al.* 2016; Donohue 2018). Premise water is the exposure medium most likely to be aerosolized (e.g. showering, aquatic aerobics, humidifiers) in areas of human activity thereby providing a potential exposure routes that could lead to disease.

The built environment, specifically on-premise plumbing, is a potential source of *L. pneumophila* and *M. avium* exposure. Legionellosis and pulmonary NTM disease occur more often as sporadic community-acquired cases than as widespread 'outbreak' events (Griffith *et al.* 2007; Hicks *et al.* 2012). Building infrastructure features such as premise plumbing, air conditioning, cooling towers and indoor water features like decorative fountains at hotels, hospitals, commercial buildings and/or residences were determined to be the source of exposure in a number of case study investigations (Garrison *et al.* 2016). Recently, industry leaders (ASHRAE 2015) and governmental entities (VA 2014; EPA 2016; CDC 2017) published mitigation and prevention guidelines designed to reduce the occurrence of these micro-organisms in the hope of minimizing the transmission of their respective diseases.

Previously, (Donohue *et al.* 2014, 2015) reported the occurrence of *L. pneumophila* Sg1 and NTM in potable water samples at points of use. This publication expands the data from the earlier publications and investigates the relationship between structure type and the presence of both micro-organisms at taps within locations of human occupancy. Filling this data gap can provide insights on the structure type that has a greater occurrence of the micro-organisms. This information can help public health officials pinpoint locations of transmission, inform decisions on detection/recovery of the causative agent and improve public health protection.

This study examined the occurrence pattern (sporadic *vs* persistent) of *L. pneumophila, L. pneumophila* Sg1 and *M. avium* in plumbed water systems of residences (single-family home or apartment complex) and office buildings (business). A goal of this report was to identify which structure type most likely harbours the micro-organisms in its on-premise water supply. Concentrations of each micro-organism were examined at the points of sample collection to determine if differences by structure type and by occurrence pattern existed. Lastly, the detection frequency and concentrations were compared to the age and size of each structure type to inform environmental engineers and building managers when identifying points of exposure where prevention measures or mitigation efforts (e.g. pipe material, aerator removal, low-flow fixture adjustments or installation of *in situ* treatment devices) could reduce the exposure risk for inhabitants.

Materials and methods

Study design

Cold water from 108 taps was monitored between January 2009 and November 2014. Of the 108 taps, 65 were in offices and 43 in residences ($n = 43$) that are actively occupied year-round. The offices and residences were dispersed across 31 states, one federal territory and one federal district within the United States.

The office and residential water samples were collected from the same tap at three independent time points distributed over an approximately 1-year time period. On average, there was a 3-month time gap between sampling events. The taps sampled were kitchen sinks, bathroom sinks, utility sinks, drinking water fountains and refrigerator-door dispensers. At all taps, cold water was collected in three, 1-l high-density polypropylene bottles, 15 s after the water started flowing. The 15-s flush ensured that water collected came from behind the cold and hot water interface. For the results to be more reflective of cells than exogenous DNA, the inherent disinfectant residual was maintained throughout shipping. This is important because Thomson *et al.* (2008) demonstrated that the use of a quencher will reduce the recovery of *M. avium*. Samples were packed with ice and shipped for next day delivery. Samples were vacuum filtered using a sterile glass filter holder for 47-mm-disc filters. The study generated 324 residence and office building samples. This number does not include method blanks or positive/negative controls used for data quality control.

Definitions

Two *occurrence* terms are used in this report: *sporadic* and *persistent*. The *sporadic* term is applied when only

one of the three sampling events was positive for a specific pathogen. *Persistent* occurrence refers to the repeated detection of a specific pathogen at more than one water sampling event taken over the course of a year. A residence is defined as a structure where activities related to home life (e.g. showering, sleeping, gardening, cooking), and was either a single-family home (39 taps) or an apartment complex (four taps) (hotels were not sampled) (International Code Council 2000) (File S2). The term office building is defined by International Build Code (IBC) as a place of business where office/professional or service transactions are performed (International Code Council 2000).

Structure characteristic

The year the structure was built, and its square footage were obtained from publicly available property information. Square footage was used as a surrogate for building complexity and as an inference for a more complex plumbing system, for example, dead ends, pipe bends and underutilized taps. Structure age was determined by subtracting the year of the last sample collection from the year of building construction.

Samples and DNA extraction used for quantitative PCR analysis

Upon sample arrival, 3 l of water was vacuum filtered through a sterilized Whatman® Nucleopore™ Track-etched membrane, 47 mm, 0·4-μm polycarbonate membrane (Whatman Inc., Piscataway, NJ). The filters were stored at −80°C in sterile 2·0 ml O-ring screw cap microcentrifuge tubes containing 0·30 ± 0·05 g 0·1 mm of sterile glass beads (BioSpec Products, Bartlesville, OK) until extraction.

Details of the DNA extraction from filters have been published previously (Beumer *et al.* 2010). Briefly, each polycarbonate membrane from the filtration step was minibead-beaten in a bead-beater (BioSpec Products) with 500 μl of tissue and cell lysis solution (Lucigen Corporation, Middleton, WI). The sample lysate was transferred into a 2-ml microcentrifuge tube and 2 μl of Proteinase K (50 μg μl^{-1}) (Lucigen Corporation) was added followed by incubation at 65°C in a water bath for 15 min. Next, 2 μl of RNase A (5 μg μl^{-1}) (Epicentre Biotechnologies, Philadelphia, PA) was added to the mixture and incubated at 37°C for 30 min. Subsequently, 350 μl of MPC Protein Precipitation Reagent (Epicentre Biotechnologies) was added to precipitate the cellular proteins. The resulting supernatant was transferred to a microcentrifuge tube with an equal volume of ice cold (~−4°C) isopropanol. The sample tubes were inverted manually up to 40 times and centrifuged at 10 000 ***g*** for 10 min. The isopropanol was poured off and the resulting DNA pellet washed with 500 μl of ice-cold (~−4°C) 70% ethanol. Sample tubes were centrifuged and the ethanol removed. The pellets were resuspended in 150 μl of nuclease-free sterile water and stored at −80°C until analysed.

Quantitative polymerase chain reaction

Preparation of qPCR standard/positive control

A previously published method was used for the preparation of the DNA standards for the quantitative polymerase chain reaction (qPCR) method described below (Beumer *et al.* 2010; Donohue *et al.* 2014).

Assays and conditions for qPCR

Three primer–probe sets were used to detect and quantify *L. pneumophila* (species Lp16S), *L. pneumophila* Sg1 (LpSg1) and *M. avium* (MA) in water (Merault *et al.* 2011; Donohue *et al.* 2014; Chern *et al.* 2015). All DNA extracts were analysed using the Lp16S and MA primer–probe sets (Table S1). Any extract that was positive for *L. pneumophila* Lp16S was also analysed for the presence of *L. pneumophila* Sg1 using LpSg1 primer–probe set. All three primer–probe sets and qPCR conditions have been previously published (Donohue *et al.* 2014; Chern *et al.* 2015). Details of the assays, the qPCR conditions and controls are in the supplemental file associated with this paper (File S1). The specifics on limit of detection, limit of quantification and sensitivity for each assay, these have been previously published (Donohue *et al.* 2014).

Interpretation of qPCR

An extract was considered positive for both the *L. pneumophila* and *M. avium* assay if two or more of the triplicates had a quantification cycle (C_q) value <39. If an extract was determined to be positive for *L. pneumophila,* the extract was analysed for the presence of *L. pneumophila* Sg1. For the *L. pneumophila* Sg1 assay, both replicates were required to have a C_q value <39 to be considered positive. Each qPCR reaction for *L. pneumophila* and *L. pneumophila* Sg1 represents 100 ml of the original sample volume. For the *M. avium* assay each qPCR reaction represents 200 ml of the original sample volume.

Statistical analysis

The C_q values were initially transformed to genomic target numbers using the standard curve. The average of the genomic target number per replicate was calculated for each sample that had a C_q <39. For taps that had a

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positive detection, a median and an average value was calculated. Statistical significance between sporadic/persistent, age and square footage was evaluated using the Mann–Whitney *t*-test. Significance between detection frequencies was established by either the Fisher's exact test, or Chi-squared analysis in Sigma Plot 13.0 (Systat, San Jose, CA).

Results

Occurrence rate and persistent status

Water samples taken at point of use taps ($n = 108$) were collected throughout the United States. *Mycobacterium avium*, *L. pneumophila* and *L. pneumophila* Sg1 were detected, regardless of structure type, at 42% (45/108) 38% (41/108) and 23% (25/108) of the taps respectively (Table 1 and File S2). The occurrence for *L. pneumophila* and *L. pneumophila* Sg1 was not significantly different by structure type (residence; $n = 43$ or office building; $n = 65$). *Mycobacterium avium* occurrence showed a strong statistically significant trend (Chi-square $P = 0{\cdot}03$) favouring office buildings (Table 1) when compared to residences. These detection frequencies demonstrated that both structure types had the potential to expose humans to the respective disease-causing micro-organisms over the span of a year.

Detection frequency and concentration by structure type

Figure 1 depicts the occurrence type: *sporadic*—one positive detection or *persistent*—two to three positive detections for each micro-organism by structure type. The occurrence pattern was largely sporadic for all three micro-organisms (Tables S2 and S3). *Legionella pneumophila* persisted equally within residences 21% (9/43) and office buildings 21% (14/65), Fig. 1(a,b). The data also revealed that the majority of *L. pneumophila*-positive detections were not Sg1, the serogroup most responsible for causing disease. *Mycobacterium avium* detections were largely sporadic, representing 63% (20/32) to 92% (12/13) of the positive detections at office buildings and residences respectively. *Mycobacterium avium* persisted more often in office buildings, 12% (8/65), compared to residences 2% (1/43) (Chi-square $P = 0{\cdot}03$) (Fig. 1e,f).

The concentrations measured for each of the three micro-organisms in the cold water samples spanned a dynamic concentration range of 10^1–10^4 genomic targets or cell equivalences (CE) per litre, Tables S2 and S3. Within residences, concentrations *L. pneumophila* and *M. avium* were equivalent. The differences in sporadic and persistent concentrations of *L. pneumophila* Sg1 were close to achieving statistical significance in both residences (*t*-test; $P = 0{\cdot}05$) and office buildings (*t*-test; $P = 0{\cdot}08$). At office buildings, the concentrations of persistent *L. pneumophila* were significantly higher than the sporadic concentrations (*t*-test; $P = 0{\cdot}007$) (Fig. 2a). *Mycobacterium avium* was found to persist mainly in office buildings. No statistically significant differences in concentrations were observed between sporadic and persistence concentrations of *M. avium* at either location.

Structure's age and square footage

Occurrence patterns based on a structure's age and size were evaluated to determine if either parameter was helpful in predicting persistence. At residences <20 years of age, neither *L. pneumophila* nor *L. pneumophila* Sg1 were detected in tap water samples. Persistence was observed in residences greater than ≥40 years of age for *L. pneumophila* and >100 years for *L. pneumophila* Sg1 respectively. There was a statistically difference between the newer residences and those older than 20 years (Fisher exact test, $P = 0{\cdot}04$) (Fig. 3a). The 20-year mark used to distinguish between 'newer' and 'older' residences was defined in the 1992 Energy Policy Act (EPACT92)

Table 1 Frequency of detection and concentration range for *Legionella pneumophila*, *L. pneumophila* Sg1 and *Mycobacterium avium* by residence and office building

Micro-organism	Structure type	Number of taps	Number of positive detections *n* (%)	Minimum genomic target (CE) per litre	Median genomic target (CE) per litre	Average genomic target (CE) per litre	Maximum genomic target (CE) per litre
L. pneumophila	Total	108	41 (38)	27	472	2537	29 328
	Residence	43	18 (42)	102	1196	3188	29 328
	Office building	65	23 (35)	27	472	2028	15 792
L. pneumophila Sg1	Total	108	25 (23)	38	1000	5659	48 888
	Residence	43	9 (21)	70	690	7616	48 888
	Office building	65	16 (25)	38	1046	4558	19 252
M. avium	Total	108	45 (42)	30	90	3207	54 532
	Residence	43	13 (30)	31	106	2006	23 157
	Office building	65	32 (49)	30	80	3695	54 532

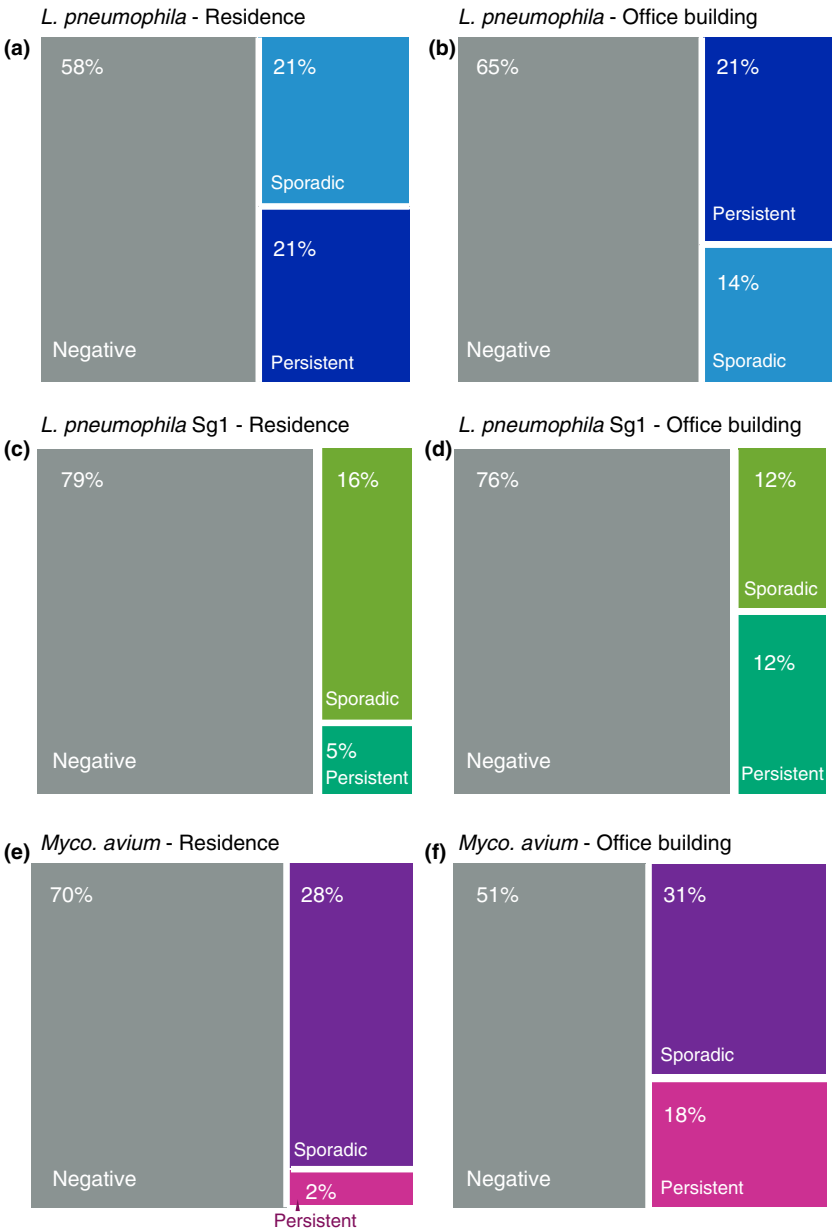

Figure 1 Occurrence and persistence of *Legionella pneumophila* a and b, *L. pneumophila* Sg1 c and d and *Mycobacterium avium* e and f at tap by location. Sporadic (detection 1X), persistent (detection 2X of 3X). [Colour figure can be viewed at wileyonlinelibrary.com]

(Congress 1992). In the case of *M. avium*, the rate of detection was about the same (Fig. 3e) for newer and older residences (Chi-square $P = 0{\cdot}35$). The size of a residence did not appear to significantly affect the detection for any of the microbes studied (Fig. 3b,d, and f). In smaller residences, 1000 and 1999 sq. feet, *L. pneumophila* persisted in 19% (4/21 residences). That was not the case for either *L. pneumophila* Sg1 or *M. avium*. Persistence was only observed within structures with sizes ≥4000–4999 sq. ft. (≥371·6–464·4 square metres) for both microorganisms.

In office buildings, *L. pneumophila*, *L. pneumophila* Sg1, and *M. avium* were detected and found to persist at nearly the same frequencies in both newer (≤20 years) and older (≥20 years) structures (Chi-square values, $P = 0{\cdot}52$, $P = 0{\cdot}39$ and $P = 0{\cdot}80$ respectively) (Fig. 4a,c, and e). As was the case for residences, there was no clear relationship between a building size and persistence of the microorganism (Fig. 4b,d, and f). Persistence was detected within an office building regardless of its dimensions.

Discussion

Legionella pneumophila and *M. avium* infections occur when aerosols contaminated with the pathogens are inhaled by vulnerable individuals. The pathogens can be

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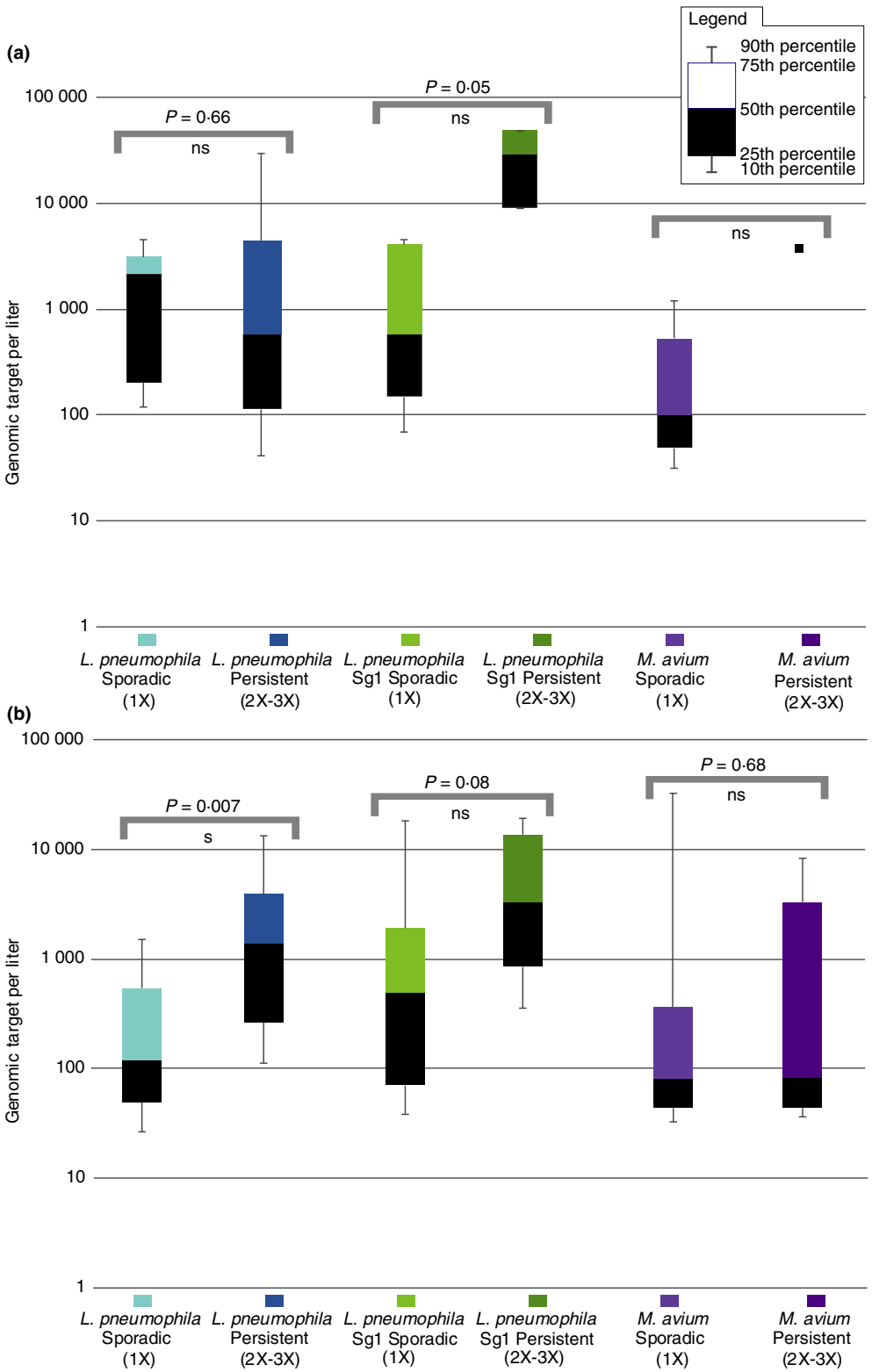

Figure 2 The average distribution of concentrations detected at taps (sporadic *vs* persistent). (a) Residences and (b) office building. See Table S2 and Table S3 for values. S = statistically significant (*t*-test) and ns = not statistically significant. [Colour figure can be viewed at wileyonlinelibrary.com]

present in the on-premise plumbing of both residences and businesses. In this study, *L. pneumophila* Sg1 was detected at a quarter of the taps, while, *L. pneumophila* and *M. avium* were detected in more than a third of the monitored taps. These results demonstrate that opportunities for exposure are occasional but other factors (aerosols and host) are necessary for disease transmission.

The lack of persistence at a tap is noteworthy. Only 25% of the taps had persistent detections for *L. pneumophila* and <12% had persistent detections for *M. avium* and *L. pneumophila* Sg1. The lack of consistent detections reduces the potential to cause an outbreak among a family or worker cluster. It also suggests that the sporadic occurrence could be more frequent than the 25–33% indicated, based on the fact that only three samples were collected across a 1 year period.

Sporadic detections pose a monitoring challenge in assessing the relationship between occurrence and illness. The Cohn *et al.* (2015) publication on community legionellosis outbreaks in New Jersey illustrates a situation where the sporadic community-acquired legionellosis cases exceeded the case rate of two outbreak episodes in a single geographical area over a 5-year period. During one of the outbreak investigations, no *Legionella* sp. was cultured from a cold or hot water tap where the patients were likely to have been exposed. However, water was still suspected to be the source because a few of the patients had never left the facility constraining the pathogen exposure location. This case study demonstrates both the difficulty in identifying the source and the struggle of linking sporadic community-acquired cases to the suspected

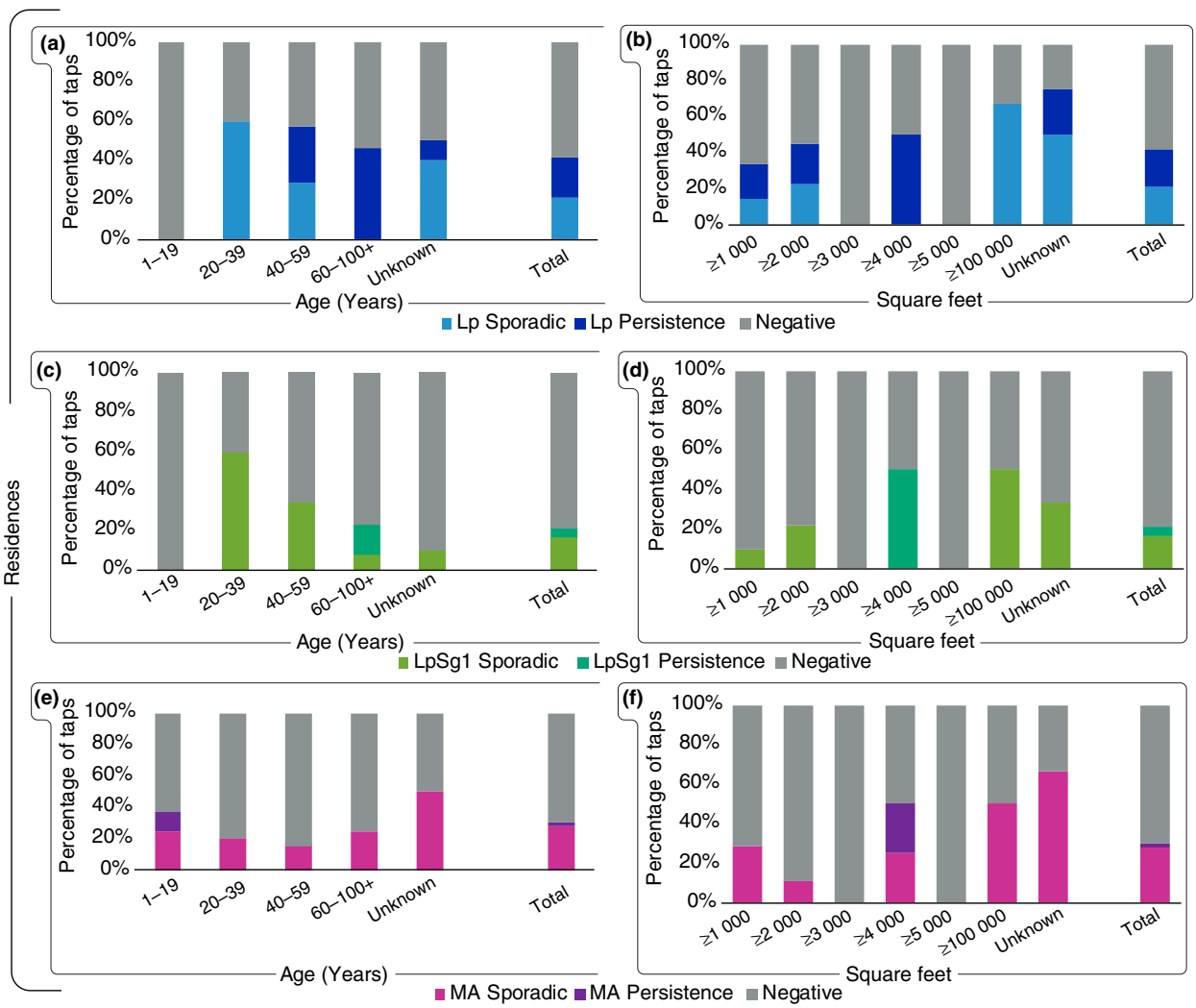

Figure 3 Occurrence and persistence of *Legionella pneumophila* (Lp) a and b, *L. pneumophila* Sg1 (LpSg1) c and d, and *Mycobacterium avium* (MA) e and f at residences by age (years) and square footage. [Colour figure can be viewed at wileyonlinelibrary.com]

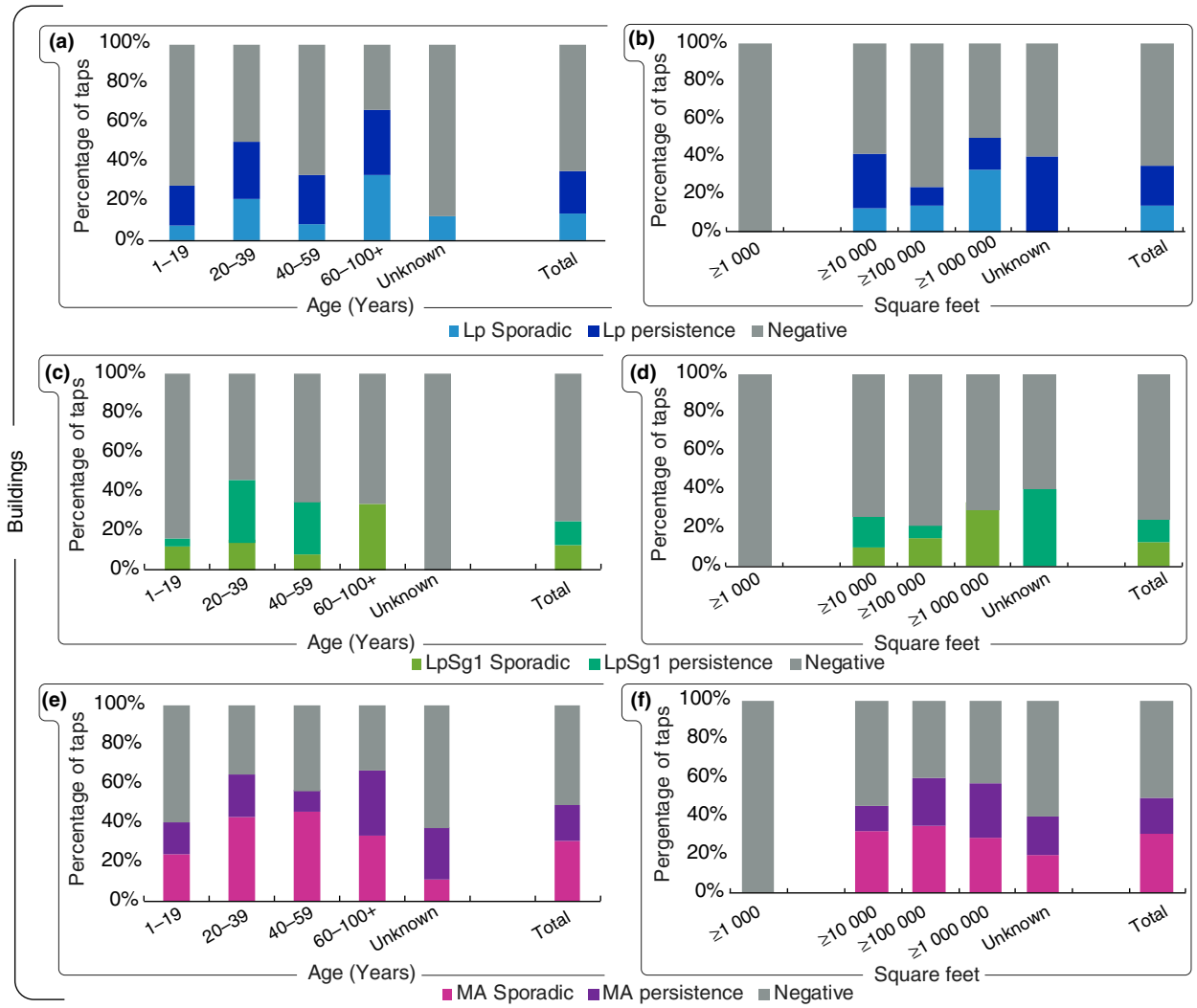

Figure 4 Occurrence and persistence of *Legionella pneumophila* (Lp) a and b, *L. pneumophila* Sg1 (LpSg1) c and d, and *Mycobacterium avium* (MA) e and f at office building by age (years) and square footage. [Colour figure can be viewed at wileyonlinelibrary.com]

source due *Legionella's* sporadic occurrence within premise plumbing.

Only a small fraction of reported cases for either disease is observed as an outbreak event. It is estimated that 97% of legionellosis cases in the United States are sporadic community-acquired illness (Hicks *et al.* 2012). As for pulmonary NTM disease, most cases are viewed as sporadic community acquired, due to a lack of a unifying location and time of infection among the patients afflicted (Griffith *et al.* 2007).

In this study, a comparison of the detection frequency for *L. pneumophila* compared with *L. pneumophila* Sg1 at residences shows that the *L. pneumophila* serogroups other than Sg1 are more likely to persist (21% *vs* 5%). *Mycobacterium avium* did not persist in the cold water lines in the residences studied. This observation could indicate that residence plumbing systems (cold water line) typically do not have water quality environments that promote the growth of the *L. pneumophila* Sg1 and *M. avium* species or water usage is sufficient to minimize colonization. However, due to the small positive sample size, more work is necessary to support this observation.

In this study, two structure types were investigated for possible human exposure and to determine where preventive measures might be effective in preventing exposures that lead to disease. Both *L. pneumophila* and *L. pneumophila* Sg1 were detected in both residences and office environments at approximately equal rates: 42% (18/43) *vs* 35% (23/65) and 21% (9/43) *vs* 25% (16/65) respectively (Table 1). This indicates that both structure types have

similar potential for disseminating the bacteria. Subtle differences between structure types exist. For instance, water samples from office buildings had a higher persistence rate for *L. pneumophila* Sg1 than residential samples (Fig. 1d). This observation is supported by CDC outbreak data where 78% of Legionnaires disease outbreaks (21/27) occurred at large complex structures such as hotels/resorts, long-term care facilities and hospitals (Garrison *et al.* 2016).

Mycobacterium avium also had a higher detection frequency and persisted more often in office buildings. Pulmonary infections or diseases related to *M. avium* are not acute illnesses. Therefore, identifying the exact exposure source is difficult to identify due to the large time lapse that exists between receiving an infective dose and the onset of symptoms. However, water has always been strongly suspected in the dissemination of *M. avium*-related illnesses (Falkinham *et al.* 2008) (Hilborn *et al.* 2008; Thomson *et al.* 2013).

The presence of disease-causing micro-organisms in the water supply of a structure does not by itself lead to disease. Infection requires a sufficient dose to a susceptible recipient under appropriate exposure conditions, with only a small proportion of the infections potentially resulting in illness. Berendt *et al.* (1980) showed that as little as 10–120 colony-forming units (CFUs) of aerosolized *L. pneumophila* caused fever in guinea pigs. In the guinea pig dose–response model for infection, increased temperature was accepted as a biological sign for an immune system response initiated by bacteria. Comparable dose–response data for humans could not be identified.

In this survey, positive samples contained 10–10 000 cells per litre of water based on qPCR results which are not a measure of viability or infectivity. Thus, it is not possible to directly correlate the findings to the risk for infection from *L. pneumophila*, *L pneumophila* Sg1 and/or *M. avium*. However, 21 646 cases of legionellosis were reported to the CDC between 2009–2014 (CDC 2011, 2012, 2013, 2014) and there are an estimated 86 230 cases of pulmonary NTM infections in the United States per year (Strollo *et al.* 2015). Thus, data on the presence of micro-organisms in water at residences and office buildings is worth considering as a surrogate for exposure risk.

In this study, no differences were observed in microbial detections at 'newer' (≤20 years) residence/offices and 'older' (≥20 years) residence/offices. However, lower rates of persistent detections were seen in newer residences compared to newer office buildings. The size of the residence or office building did not affect persistent detections. Water stagnation (increased water age, lack of movement and lack of disinfection residual) within these structures could create niches within the distribution systems where the waterborne micro-organisms could flourish and later be transported to taps where exposures occur. Biofilm formation within a structure is another factor that should be considered as it relates to persistence, especially in large office buildings or vacation-only homes with opportunities for water stagnation where taps are occasionally not used for extended periods of time. Both mycobacteria (Schulze-Robbecke *et al.* 1992) and legionella (Abdel-Nour *et al.* 2013) have been shown to thrive in biofilm.

In the United States and across Europe, the incidences of legionellosis and NTM diseases are increasing. Recent outbreak events have been reported in Flint, MI (*Legionella*) (June, 2014–November, 2015) (MDHHS, 2015), the Bronx, NY (*Legionella*) (Raphael *et al.* 2016) and Munich, Germany (MAC) (Haller *et al.* 2016). The results from this study and others have shown that the causative micro-organisms can be present in water at large and small, newer and older residences (Stout *et al.* 1992; Feazel *et al.* 2009; Donohue *et al.* 2014; Schwake *et al.* 2016) and office buildings (Dutka *et al.* 1984; Flannery *et al.* 2006; Hilborn *et al.* 2006; Moore *et al.* 2006; Donohue *et al.* 2015).

The occurrence of the pathogens in water was found to largely be sporadic with only a small portion of on-premise plumbing taps sampled demonstrating persistence. The findings are consistent with the fact that legionellosis (Garrison *et al.* 2016) and pulmonary NTM diseases generally occur as sporadic outbreaks of community-acquired illness.

Societies such as Infectious Diseases Society of America and the American Thoracic Society (Griffith *et al.* 2007; Mandell *et al.* 2007) agree that water is a source of concern for pathogens and that exposure primarily occurs through inhalation of contaminated aerosols. Thus, attention should be given to understanding patterns of human activities as they relate to premise water, especially those activities that generate aerosols (e.g. showering, humidifiers, aquatic activities) when providing advice to the public on actions that can reduce exposure. Once the factors that relate to risk are identified, measures can be taken to inform the public of actions that can reduce risk of infection such as disinfecting aerators, cleaning showerheads and changing behaviours (e.g. filling-up a cup of water from a fountain rather than drinking from it directly) (Falkinham 2016). These actions may help reduce the risk especially for those that are the most vulnerable.

Conflict of Interest

Authors do not have any conflicts of interest to report.

Disclaimer

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subjected to the Agency's administrative review and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article:

File S1. Assays and Conditions for qPCR.

Table S1. Primer and Probe sequences.

Table S2. Frequency of detection and concentrations (sporadic vs persistent) taps at residences.

Table S3. Frequency of detection and concentrations (sporadic vs persistent) taps at office buildings.

File S2. Raw data.