CAMBRIDGE
UNIVERSITY PRESS

Legionella pneumophila in residential water supplies: environmental surveillance with clinical assessment for Legionnaires' disease

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SUMMARY

Although cases of community-acquired Legionnaires' disease have been epidemiologically linked to residential water supplies, the risk of acquiring Legionnaires' disease from exposure to *Legionella pneumophila* in residential water systems is uncertain. The residential water supplies of 218 members of the American Legion in six different geographical areas in Pittsburgh were cultured for *L. pneumophila*. Residents of the homes provided a recent medical history and a blood sample for detection of antibodies to legionella. A urine sample for legionella urinary antigen testing was also requested from individuals residing in legionella-positive homes and individuals with a positive antibody test. Six percent (14/218) of the homes yielded *L. pneumophila* (range within six areas 0–22 %). Lower hot water tank temperature was significantly associated with legionella positivity ($P < 0.01$). Analysis of water samples for mineral content showed no association between legionella positivity and concentrations of calcium and magnesium. Water samples from the area where 22 % of the homes surveyed were positive for legionella had a higher iron content than water samples from the other areas tested. None of the individuals residing in legionella-positive homes showed elevated antibody titres to legionella or the presence of legionella antigen in urine. For the immunocompetent hosts, the risk of contracting Legionnaires' disease from exposure to contaminated household water supplies in the Pittsburgh area appears to be low.

INTRODUCTION

Legionnaires' disease has been linked to exposure to water sources that harbour *Legionella pneumophila*. The strongest epidemiological studies have linked exposure to contaminated hospital water distribution systems to acquisition of nosocomial Legionnaires' disease [1–3]. Community-acquired cases of Legionnaires' disease have also been linked to exposure to the contaminated water systems of hotels, office buildings, and industrial plants [4, 5]. Finally, at least seven cases of community-acquired Legionnaires' disease have been attributed to exposure to contaminated residential water distribution systems [6–9].

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We investigated the potential for in-home exposure by testing the water systems of residential homes for the presence of *L. pneumophila*. Our specific objectives were to: (1) determine the prevalence of *L. pneumophila* positive homes in a city where nosocomial Legionnaires' disease was known to be endemic, (2) determine whether environmental parameters correlated with positivity, and (3) determine whether individuals living in legionella-positive homes were more likely to have evidence of previous exposure/infection as determined by serology and urinary antigen testing.

METHODS

Selection of homes

Members of the American Legion in the city of Pittsburgh agreed to participate in this study. Zip-codes of six geographical areas surrounding the University of Pittsburgh Health Center were submitted to the American Legion State organization. The State office contacted each member in these areas by mail and requested their participation in: (1) supplying water samples from their home for legionella culture, and (2) supplying sera for legionella antibody testing.

Data collection

Written informed consent was obtained from all participants for legionella testing of their water system and for venepuncture. Clinical information about the resident was obtained by face-to-face interview at the time of specimen collection including age, smoking habits, hospitalizations, and medical history. Characteristics of the water system were surveyed including: source of water (commercial supplier), presence of a water softener, filter units, aerators, and the type of water pipes (copper or polyvinyl chloride). Information about the hot water tank was recorded by one of the study investigators including type of heater, age, volume, and maintenance record.

Specimen collection and processing

Environmental specimens. At least three of five sites were sampled from each residential water system: (1) hot water tank, (2) kitchen tap, (3) bathroom tap, (4) showerhead or (5) bath tub outlet. 200 ml of water was collected from the drain valve at the bottom of the hot water tank. Water temperature was monitored by thermometer measurement of a 10 ml aliquot. The distal outlets were sampled after allowing a small amount of water to run through the opening. A swab was inserted into the opening of the fixture and rotated several times to dislodge the sediment. The water temperature from one distal outlet was also recorded.

The environmental specimens were processed as described previously [10]. Briefly, 0·1 ml of a water sample was inoculated onto an agar plate. Swab samples were pre-treated in 2 ml of a HCl-KCl acid buffer (pH 2·2) for 2 min before 0·1 ml of the suspension was plated. All specimens were inoculated onto a selective-differential agar medium containing glycine, vancomycin, polymyxin B, and the dyes, bromocresol purple and bromothymol blue. Cultures were incubated for 5–7 days at 37 °C in a humidified atmosphere. Colonies consistent with legionella morphology were subcultured to blood agar and buffered charcoal yeast extract (BCYE) agar plates. Definitive identification was performed by direct fluorescent

antibody testing. Monoclonal antibody subtyping was performed on all *L. pneumophila* (SG) 1 isolates (courtesy of Dr Jean Joly, Quebec, Canada).

The mineral content of the hot water tank samples was determined by standard methods using atomic absorption spectroscopy [11]. All samples were tested for calcium and magnesium concentration. Additional testing for iron, copper, zinc, and lead was performed on a subset of samples.

Blood and urine

Blood samples were collected from each consenting adult member of the household by a certified phlebotomist, centrifuged in the laboratory on the day of collection and the serum stored at -20 °C. Serum diluted 1 : 80 to 1 : 640 was tested for the presence of antibodies to *L. pneumophila* SG 1–6 and *L. micdadei* by the enzyme-linked immunosorbent assay (ELISA) method [12]. Test results were reported as the reciprocal of the dilution (titre). A positive test result was defined as a titre of 320 or greater.

Urine samples were collected for detecting the presence of *L. pneumophila* SG 1 urinary antigen. They were obtained only from individuals who were demonstrated to have a positive antibody titre (320) and those residing in homes from which legionella had been isolated. The urine and a second blood sample were obtained 8–10 weeks after the initial blood sample. The legionella urinary antigen test for *L. pneumophila* serogroup 1 was performed according to the manufacturer's instructions (Binax, Inc., South Portland, ME).

Data analysis

Results from the environmental culture survey and legionella tests on the residents were stored in a computer data bank (Prophet, Division of Research Resources, NIH). Statistical correlations were assessed by Fisher's exact test or T-test on unpaired data (equal variance). A stepwise logistic regression analysis was used for multivariate analysis (BMDP, University of California). The dependent variable was legionella positivity.

RESULTS

Environmental

Of those contacted, 218/816 (27 %) agreed to participate in the survey of home water distribution systems. *L. pneumophila* was isolated from the water distribution systems of 14/218 (6·4 %) of the homes. The rate of positivity in the six geographical areas ranged from 0 to 22 % (Table 1). *L. pneumophila* was isolated from 30 specimens: 6 hot water tank samples, 6 kitchen taps, 7 bathroom taps, 3 showerheads, and 8 bath tub outlets. The majority, 12/14 (85 %), of isolates recovered from residential water systems were *L. pneumophila* SG 1. Multiple serogroups of *L. pneumophila* (1 and 6) were recovered from only one water system. *L. pneumophila* SG 3 and 4 were isolated from water systems in areas 6 and 3 respectively. The concentration of *L. pneumophila* isolated from hot water tank samples ranged from 1×10^4 to 6×10^5 colony forming units (c.f.u.)/l. The concentration of *L. pneumophila* recovered from swab samples ranged from 1×10^4 to 3×10^6 c.f.u./l. Other species of *Legionella* were not isolated.

Table 1. *Distribution and percentage of homes found to be colonized with*
Legionella pneumophila

Area	No. contacted	No. sampled	No. positive	Percent positive
1	257	73	5	6·8
2	85	41	2	4·9
3	142	36	1	2·7
4	137	33	1	3·0
5	102	18	4	22·2
6	68	10	1	10·0
Other*	25	7	0	0·0
Total	816	218	14	6·4

* Seven homes were sampled in four other areas of the city.

Monoclonal antibody subtyping was performed on 12 isolates of *L. pneumophila* SG 1. Eight isolates were identified as subtype OLDA, 2 as subtype Bellingham and 2 as subtype Oxford 4032E.

Lower hot water tank temperature was significantly associated with positivity ($P < 0.01$, T-test). The mean hot water temperature in legionella-negative homes was 47·4 °C, while in positive homes it was 41·0 °C. The mean temperature for distal sites was also significantly associated with legionella-positive homes ($P < 0.01$, T-test). The mean distal site temperatures for negative and positive homes were 54·0 and 48·0 °C, respectively.

No significant difference between positive and negative homes was observed for calcium or magnesium concentration in hot water tank samples, hot water tank capacity, source of water, presence of a water softener, filter units, aerators or type of water pipes. 207/218 (95%) of homes had gas water heaters and 11/218 (5%) had electric heaters. The average age of legionella-negative homes was 65 years and of legionella-positive homes 70 years.

Eleven hot water tank samples from area 5 and 29 hot water tank samples from areas 1, 2, 3, 4, and 6 underwent a complete atomic absorption analysis for minerals which included calcium, magnesium, iron, lead, copper, and zinc. Water samples from area 5 had a significantly higher concentration of iron ($P < 0.01$, Fisher's exact test). The mean iron concentration for the area 5 samples was 265 μg/l, whereas the means for the other areas tested ranged from 130 to 188 μg/l.

Multivariate analysis showed that both tank temperature and Area 5 location were significantly associated with legionella positivity.

Clinical

Recent medical history was obtained from 352 adults. The average age was 62 years (range 29–97); 106/352 (30%) were cigarette smokers; 10/352 (2·8%) were immunosuppressed as a result of malignancy or medication; 25/352 (7·1%) were diabetic; and 17/352 (4·8%) had underlying lung disease. Only one respondent had been hospitalized for pneumonia during the preceding 12 months, whereas 63/352 (18%) reported having an upper respiratory infection.

Adults from 149/218 (68%) of the homes surveyed agreed to submit a blood

sample for legionella serology. Serum was obtained from 173 individuals representing 140 legionella-negative homes and from 11 individuals representing 9 legionella-positive homes. Only two of the individuals residing in legionella-positive homes, were considered immunosuppressed and none had a positive antibody titre to *L. pneumophila* SG 1–6. Two individuals had an antibody titre of 320 to *L. micdadei*. Six individuals residing in legionella-negative homes had a positive antibody titre to *L. pneumophila* (3/6 to SG 1), and six had an elevated level of antibody to *L. micdadei*. Only one of the six individuals with antibody to *L. micdadei* was also positive to *L. pneumophila*. None of these individuals was immunocompromised.

A second blood sample and a urine sample were requested from individuals who had a positive antibody titre to *L. pneumophila* or resided in legionella-positive home. Urine samples were collected from 11 individuals living in six legionella-positive homes but none was positive. Blood and urine samples were obtained from five of six individuals whose serum had been positive despite residing in legionella-negative homes. None of the urine samples was positive; however, two of five individuals still had a positive antibody titre.

DISCUSSION

Legionnaires' disease can be acquired by exposure to contaminated residential water systems [6–9]. The potential risk of legionella infection for individuals living in residences with colonized water supplies is uncertain.

Several studies have surveyed residential water systems for the presence of legionella but have reported differing results and the majority have suffered from the problem of inadequate sample size. In these studies, *L. pneumophila* was isolated from 2/92 (2%) of residences in Vermont, 6/55 (11%) in Pittsburgh, 69/211 (32%) in Quebec City, and 30/95 (32%) in Chicago [13–16]. In the study from Chicago, the majority of the residences were apartment buildings rather than single-family dwellings [16]. Many large buildings such as apartment buildings, hospitals, hotels, and office buildings are known to be colonized with legionella and represent an environmental niche that is different from that of single-family dwellings [17–19].

Our sample population was not randomly selected. Members of the American Legion were asked to participate because of their historic role in the discovery of Legionnaires' disease [20] and might, therefore, be more willing than the general public to allow their private homes to be surveyed and to submit a blood sample for legionella serology.

Our study is the largest sampling of residential homes reported. We sampled residences in a city where legionella was already known to colonize and cause disease in virtually every hospital within the University Medical Center (Fig. 1) [21–24]. Furthermore, community-acquired Legionnaires' disease occurs with consistent frequency in this same geographical area [25, 26]. Interestingly, even in a city with a high incidence of nosocomial Legionnaires' disease, only 14/218 (6%) of the residential water systems were colonized with *L. pneumophila* (Table 1).

One plausible explanation for the lower prevalence of legionella in residential water systems is that the structural and functional differences of water tanks that
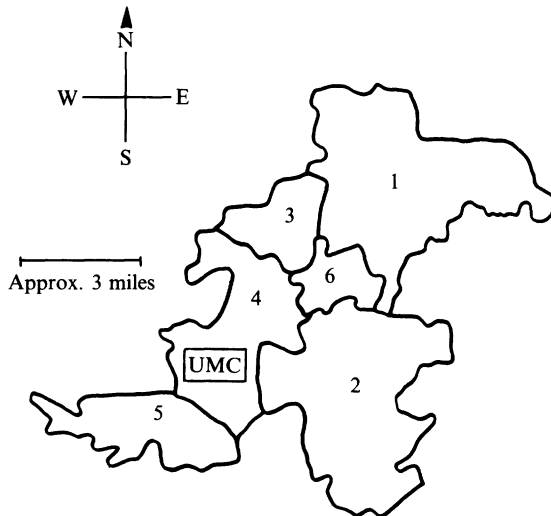
Fig. 1. Geographical location of residential areas relative to the University Medical Center (UMC). The number of homes surveyed from each area is presented in Table 1.

service large building *vs.* those in single-family homes are factors in legionella colonization. The large-volume hot water tanks that are found in hospitals, hotels, and apartment buildings typically hold 2000–4000 litres and are part of a recirculation system designed to deliver heated water rapidly. The presence of legionella in these large-volume hot water tanks has been attributed both to temperature stratification and sediment accumulation within the tank [17]. In addition, recirculating hot water systems would provide continuous seeding of distal fixtures with the organism.

Virtually every study on legionella in water distribution systems has found an association between the presence of *L. pneumophila* and water temperature [14, 17, 27, 28]; temperatures below 20 °C or above 60 °C will prevent the growth and survival of legionella [29–31]. In a survey of hospitals, hot water tank temperature below 60 °C was significantly associated with the presence of *L. pneumophila* [17].

We also confirm this association within water supplies of single-family dwellings. Lower hot water temperature was significantly associated with legionella positivity ($P < 0.01$). We and others have shown that domestic electric heaters are more likely to harbour legionella than gas-heated tanks [14, 15]. Alary and Joly [15] cultured water from 211 houses in the Quebec City area and found 84 % of the homes had electric water heaters and 39 % of these heaters harboured legionella. Since the water temperature at the bottom of electrically-heated tanks tends to be lower (due to the placement of the heating coils several inches above the bottom of the tank), this association may be an indirect one. It should be noted that 207/218 (95 %) of the homes in our study had gas-heated water tanks so we were unable to assess the question of gas *vs.* electric heaters. However, in view of the data from the Canadian study, one wonders if the low prevalence of legionella in Pittsburgh homes derives from the widespread use of gas-heated water tanks.

Although overall the rate of legionella-positive homes was only 6 %, the rate of positivity in Area 5 (22 %) was significantly higher when compared with the other

areas ($P < 0.05$). This difference in rate of positivity could not be explained by water temperature or any of the other parameters studied. We hypothesized that there may have been a difference in physicochemical quality of the water in Area 5 that was not reflected by differences in calcium and magnesium. In our initial evaluation of water quality, only calcium and magnesium were considered because a previous study showed that among numerous minerals studied (zinc, lead, iron, calcium, magnesium), only calcium and magnesium were significantly associated with legionella positivity [17]. Since atomic absorption spectroscopy for all hot water tank samples could not be repeated, analysis for other metals was performed in a limited subset of samples that represented each area. Water samples from Area 5 were found to contain a higher concentration of iron ($P < 0.01$). Although this finding is not definitive given the limited sample size, it is provocative given the documented growth-promoting properties of iron for *L. pneumophila* [32]. In fact, in a study of the effects of metals on the growth of *L. pneumophila* in drinking water systems, iron was the only parameter of 23 variables that was significantly associated with growth [33].

Isolated of *L. pneumophila* SG 1 that were recovered from residential water supplies were subtyped using monoclonal antibodies. Monoclonal antibody 2 (Mab-2) positive subtypes have been found to be more likely to cause disease and are presumably more virulent [34, 35]. Interestingly, the isolates that are most often recovered from the environment are Mab-2 negative strains [34]. In this study, only Mab-2 negative subtypes (OLDA, Bellingham 1, and Oxford 4032E) were recovered from legionella-positive homes. We caution, however, that these strains cannot be considered totally benign, since Mab-2 negative environmental strains have been linked to nosocomial Legionnaires' disease [35].

Given the presence of *L. pneumophila* in home water supplies, what is the risk of infection from in-home exposure? We obtained blood samples from 184 adults to determine whether individuals living in legionella-positive homes were more likely to have evidence of previous exposure or infection than individuals living in legionella-negative homes. Since legionella antigen in urine can be excreted for months after infection [36], we obtained a urine sample to test for legionella urinary antigen from individuals with an elevated antibody titre or those residing in legionella-positive homes. The results of serological and urinary antigen testing showed that none of the individuals residing in legionella-positive homes had any evidence of previous infection. Our findings are consistent with those from a smaller study performed by Arnow and colleagues [16] who also failed to find significantly higher antibody titres in residents of homes colonized with *L. pneumophila*.

What are the weaknesses of this study? First, this was not a random sampling of homes. Second, since the precise prevalence of community-acquired Legionnaires' disease is uncertain, the sample size of 218 residences (although the largest study ever reported) may be insufficient to address this issue adequately. Finally, our findings cannot necessarily be extrapolated to other populations in different geographical areas.

In conclusion, we have shown that the prevalence of *L. pneumophila* in residential dwellings was low in an area known to have endemic nosocomial Legionnaires' disease. More importantly, immunocompetent adults do not appear

to be at high risk of acquiring Legionnaires' disease even if residing in homes with contaminated water supplies. These results remain to be confirmed in larger-scale prospective studies in other geographical areas.

ACKNOWLEDGEMENTS

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