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Strengths and limitations of molecular subtyping in a community outbreak of Legionnaires' disease

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SUMMARY

An epidemiological and microbiological investigation of a cluster of eight cases of Legionnaires' disease in Los Angeles County in November 1997 yielded conflicting results. The epidemiological part of the investigation implicated one of several mobile cooling towers used by a film studio in the centre of the outbreak area. However, water sampled from these cooling towers contained *L. pneumophila* serogroup 1 of another subtype than the strain that was recovered from case-patients in the outbreak. Samples from two cooling towers located downwind from all of the case-patients contained a *Legionella* strain that was indistinguishable from the outbreak strain by four subtyping techniques (AP-PCR, PFGE, MAb, and MLEE). It is unlikely that these cooling towers were the source of infection for all the case-patients, and they were not associated with risk of disease in the case-control study. The outbreak strain also was not distinguishable, by three subtyping techniques (AP-PCR, PFGE, and MAb), from a *L. pneumophila* strain that had caused an outbreak in Providence, RI, in 1993. Laboratory cross-contamination was unlikely because the initial subtyping was done in different laboratories.

In this investigation, microbiology was helpful for distinguishing the outbreak cluster from unrelated cases of Legionnaires' disease occurring elsewhere. However, multiple subtyping techniques failed to distinguish environmental sources that were probably not associated with the outbreak. Persons investigating Legionnaires' disease outbreaks should be aware that microbiological subtyping does not always identify a source with absolute certainty.

INTRODUCTION

Legionnaires' disease is a severe form of pneumonia caused by infection with bacteria of the genus *Legionella*. These bacteria are commonly present in man-made water systems, and can grow to high concentrations when the water temperature is between 25° and 42 °C. They cause disease when a susceptible person inhales a contaminated aerosol or aspirates contaminated water. Showers, faucets, cooling towers, and many other devices can produce these aerosols. During outbreaks of Legionnaires' disease, attack

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rates are low and generally < 5% of persons exposed to a contaminated aerosol become ill [1]. The majority of cases are sporadic, i.e. not part of a recognized outbreak [2] and person-to-person transmission has not been reported. The disease is probably underdiagnosed and underreported [3] and so detection of a small cluster of legionellosis cases often indicates a larger problem.

In the first week of December 1997, the Acute Communicable Disease Control Unit of the Los Angeles County (LAC) Department of Health Services received reports of six cases of Legionnaires' disease, three in persons residing or working in Culver City with an onset of disease between 17 and 20 November. In 1996, 12 cases of Legionnaires' disease had been reported in all of LAC (population ~ 9·8 million, for an incidence density rate of 0·12 reported cases per 100000 persons per year). The population of Culver City is approximately 40000. Based on the background LAC incidence rate, the Poisson probability of three unrelated cases occurring by chance in Culver City within one week is < 0·000000005.

An investigation was initiated in collaboration with the Centers for Disease Control and Prevention (CDC) to determine the extent of the outbreak and to identify a source of transmission.

METHODS

Case finding

Pulmonologists, infectious disease physicians, internists and infection control practitioners of all LAC hospitals were faxed information about the outbreak and requested to test patients with pneumonia for legionella and to report immediately all unreported or newly diagnosed legionellosis cases to the LAC Health Department. Local clinical laboratories in LAC and reference laboratories that performed legionella tests were contacted to request lists of positive legionella test results in LAC residents. At the main hospital serving Culver City we reviewed medical records of all patients who had pneumonia with onset during the preceding 3 months to try and identify patients with unreported legionellosis or with pneumonia in which the aetiologic agent was not identified but who could still be tested for legionella antigen in urine.

To determine the extent of the outbreak we compared isolates from case-patients within the outbreak cluster with isolates from patients in other parts of LAC by monoclonal antibody testing (MAb) [4], pulsed-field gel electrophoresis (PFGE), and arbitrarily primed polymerase chain reaction (AP-PCR) [5].

We defined the outbreak area for this investigation as consisting of a large part of Culver City and a southern strip of the adjacent city of Palms (Fig. 1). A case of Legionnaires' disease was defined as illness occurring in a person living or working within the outbreak area with a clinical presentation of pneumonia, confirmed by chest radiograph, with onset in November or December 1997, plus at least one of the following: isolation of legionella from lung tissue or respiratory secretions; a fourfold or greater rise in paired acute- and convalescent-phase antibody against *L. pneumophila* serogroup 1 to a titre of 128 or higher; a positive direct fluorescent antibody test for *L. pneumophila* serogroup 1 from lung tissue or respiratory secretions; detection of *L. pneumophila* serogroup 1 antigen in urine.

All case-patients or their surrogates were interviewed using an open-ended questionnaire to describe their daily activities and if they had done anything unusual in the 2 weeks prior to their illness. They were also asked if they knew anyone else who had become ill. The case-control study questionnaire was based on the results of these interviews.

Case-control study

Case-patients were assigned a disease category 1–4, based on their underlying disease status: (1) healthy non-smoker; (2) healthy smoker; (3) persons with chronic non-immunocompromising disease; (4) persons taking immunosuppressive medication or persons with immunocompromising disease.

For each case-patient, two controls were selected matched by gender and age (not more than 5 years age difference with the case) within the same underlying disease category from patient lists and records that were supplied to us by physicians in Culver City. Controls were contacted by phone to verify the underlying disease and smoking status. Selection for a matched set was complete when two eligible controls agreed to co-operate.

Interviewers visited each case-patient and each control in person to administer a standard questionnaire about their activities and whereabouts in the 2 weeks preceding the disease onset in the case-patient. The interviewees were shown a calendar to facilitate recall of specific activities. Questions in-

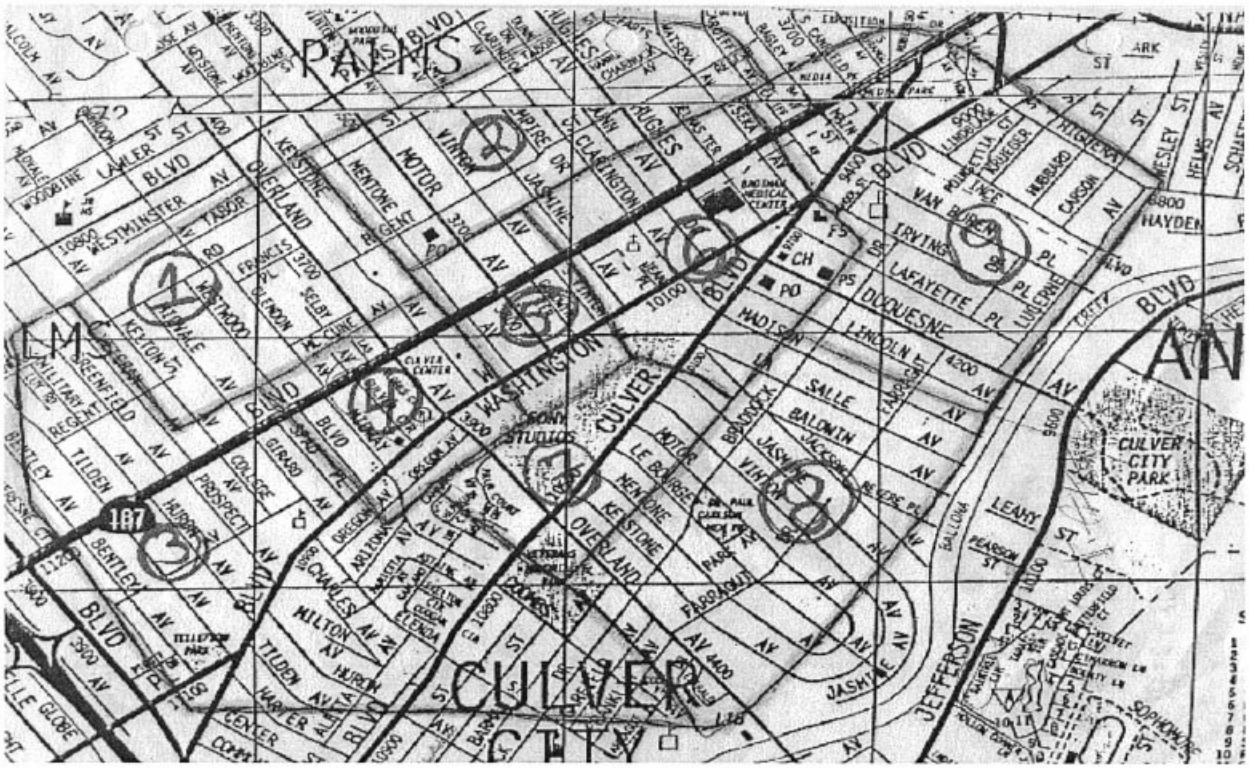

Fig. 1. Structured map used for the interview of study participants, showing sub-areas. Frequently visiting or living in sub-areas 4 and 5 was associated with increased risk of disease.

cluded occupation and workplace; where they did their shopping; if they visited banks, post offices, churches, schools; where they ate and if they visited any restaurants; if they had been near to the car wash; if they visited a hospital; where they usually showered or bathed; if they had used a whirlpool spa or other public bathing facility; what mode of transportation they had used, which bus stops they had waited at, which routes they walked frequently; if they had been near to any decorative fountains; if they drank from any public drinking fountains; if they had travelled outside of the Culver City area; if anyone among their relatives, friends, or neighbours had been ill with respiratory disease.

At the end of each interview a map of Culver City and vicinity was shown on which lines were drawn that divided the area into nine sub-areas (Fig. 1). Interviewees were asked to rank these sub-areas according to the amount of time they had spent in each during the 2-week period. After two cooling towers in the east of Culver City were found to contain *Legionella* strains identical to the outbreak strain (see Results section), all case-patients and controls were re-interviewed and asked specifically if they had been in the vicinity of these two locations.

Matched odds ratios were calculated with the McNemar test (stratified Mantel–Haenszel) using Epi-Info version 6. Confidence intervals were estimated using exact methods.

Environmental investigation

Starting one week after the onset of the last case-patient we surveyed the outbreak area on foot and from high buildings for potential sources of aerosol such as cooling towers. Aerial photographs of Culver City and vicinity were studied for the same purpose. We interviewed about 50 persons working and living in the area about possible temporary or hidden sources of aerosol and about locations of public drinking fountains. After the epidemiological analysis showed that visiting or living in sub-areas 4 and 5 (Fig. 1) was associated with risk of contracting Legionnaires' disease, these sub-areas and sub-area 3 (which was upwind from sub-areas 4 and 5) were systematically searched for any missed sources of aerosol. In sub-areas 3, 4, and 5 operators of all apartment complexes, offices, factories, and other buildings large enough to potentially use a cooling

tower were contacted and asked if a cooling tower or evaporative condenser was present. If the answer was positive or questionable, these buildings were inspected. We sampled all identified aerosol sources. Hot and cold water was sampled in the home of one case-patient and at several other sites within both municipal water distribution systems.

Weather reports of the Los Angeles Times newspaper were reviewed and the national weather service at LA International Airport was contacted to obtain information about the wind direction on each day in November.

Laboratory methods

Two swab samples and two 1 l bottles of water were collected from each site. These were cultured for legionella and serogrouped both by the LAC Public Health Laboratory and by the CDC Respiratory Diseases Laboratory in Atlanta. If overgrowth of media occurred, samples were acid treated as described previously [5].

At the CDC all *L. pneumophila* serogroup 1 isolates were subtyped using MAb reagents [4] by dot immunoblot [6]; isolates of the same MAb subtype as the clinical isolates were also compared with these isolates by AP-PCR [5], multilocus enzyme electrophoresis (MLEE), and, at the LAC Public Health Laboratory, by PFGE.

AP-PCR was performed with 1 μM primer (M13 Forward, 21 bp; 5′ TTA TGT AAA ACG ACG GCC AGT 3′), 5 μl boiled cell lysate, and 0·25 U of *Taq* DNA polymerase (Perkin–Elmer Cetus). Amplification was performed in a DNA thermal cycler (Perkin–Elmer Cetus) programmed for 45 cycles of 1 min at 94 °C, 1 min at 36 °C, and 2 min at 72 °C. Amplicons were analysed by agarose gel electrophoresis and visualised by ethidium bromide staining.

MLEE was performed according to published methods [7].

For PFGE, bacteria were grown on BCYE agar at 35 °C in 2–3% CO_2 for 72 h. Bacterial cells were washed and suspended in TES buffer (200 mM NaCl, 10 mM Tris, 100 mM EDTA), and the turbidity was adjusted to an optical density of 1·2 at 600 nm. Genomic DNA was obtained as previously described [8]. Restriction digestion of genomic DNA was performed with 100 U of *Sfi*I (Boehringer–Mannheim) for 18 h at 50 °C. A 1 × 5 mm plug slice was electrophoresed using a 1% PFGE certified agarose gel and 0·5 × Tris-Borate-EDTA buffer in a contour-clamped homogenous electric field system (CHEF Mapper, BioRad) at 14 °C and 200 V with increasing switch times of 10–35 sec for 22 h. A lambda DNA ladder (Boehringer–Mannheim) was used as the molecular weight standard. The gel was stained with ethidium bromide, destained, and imaged via the Alpha Innotech image acquisition system (Alpha Innotech).

RESULTS

Epidemiological investigation

Eight cases of Legionnaires' disease were identified, with onset between 1 November and 8 December, who lived or worked in the outbreak area (Figs 2, 3). Seven more legionellosis cases occurred within the same time period in other parts of LAC, with residential addresses at distances of 5–17 miles from Culver City. None of these case-patients worked in the vicinity of Culver City, nor had they visited the area in the 2 weeks preceding the onset of their illness. Three *L. pneumophila* serogroup 1 isolates from these case-patients were available for testing and these were different from each other and from the outbreak strain by MAb, AP-PCR, and PFGE (Fig. 4). These case-patients were therefore considered to be unrelated to the outbreak.

Five of the outbreak case-patients had *L. pneumophila* serogroup 1 antigen in their urine and three were diagnosed through isolation of the organism from respiratory secretions. All three clinical isolates were of MAb subtype (1,2,5,6) and were indistinguishable by AP-PCR, PFGE, and MLEE.

Seven case-patients had onset of disease in the week of 17–24 November, the eighth, who was confirmed by the urine antigen test, fell ill on 8 December (Fig. 2). The ages of the patients ranged from 32–74 years (median age 48 years) and six were male. One patient was a healthy non-smoker, two were smokers without chronic medical conditions, three patients had chronic non-immunocompromising disease, and two had symptomatic HIV infection. Seven of the case-patients lived within a 1·5 × 1·5-mile area and one patient lived elsewhere but worked in the office of a film studio in Culver City (Fig. 3). This patient drove to work by car and rarely visited any locations outside of the film studio but he frequently went outside to smoke.

The main findings of the case-control study are

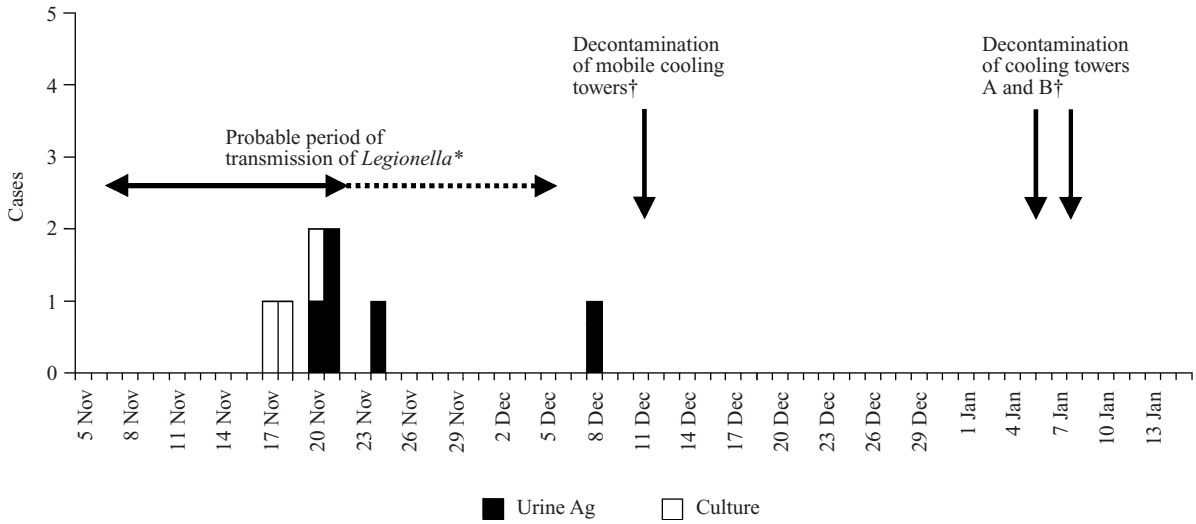

Fig. 2. Cases of Legionnaires' disease by date of onset, Culver City, November 1997 to January 1998. A horizontal arrow shows the possible dates of exposure, based on the usual spread of incubation periods for Legionnaires' disease. The right side of the arrow is dotted because the last case-patient was diagnosed by the urinary antigen test. Vertical arrows indicate approximate dates of control measures. * Based on an incubation period of 2–10 days; † dates are approximate.

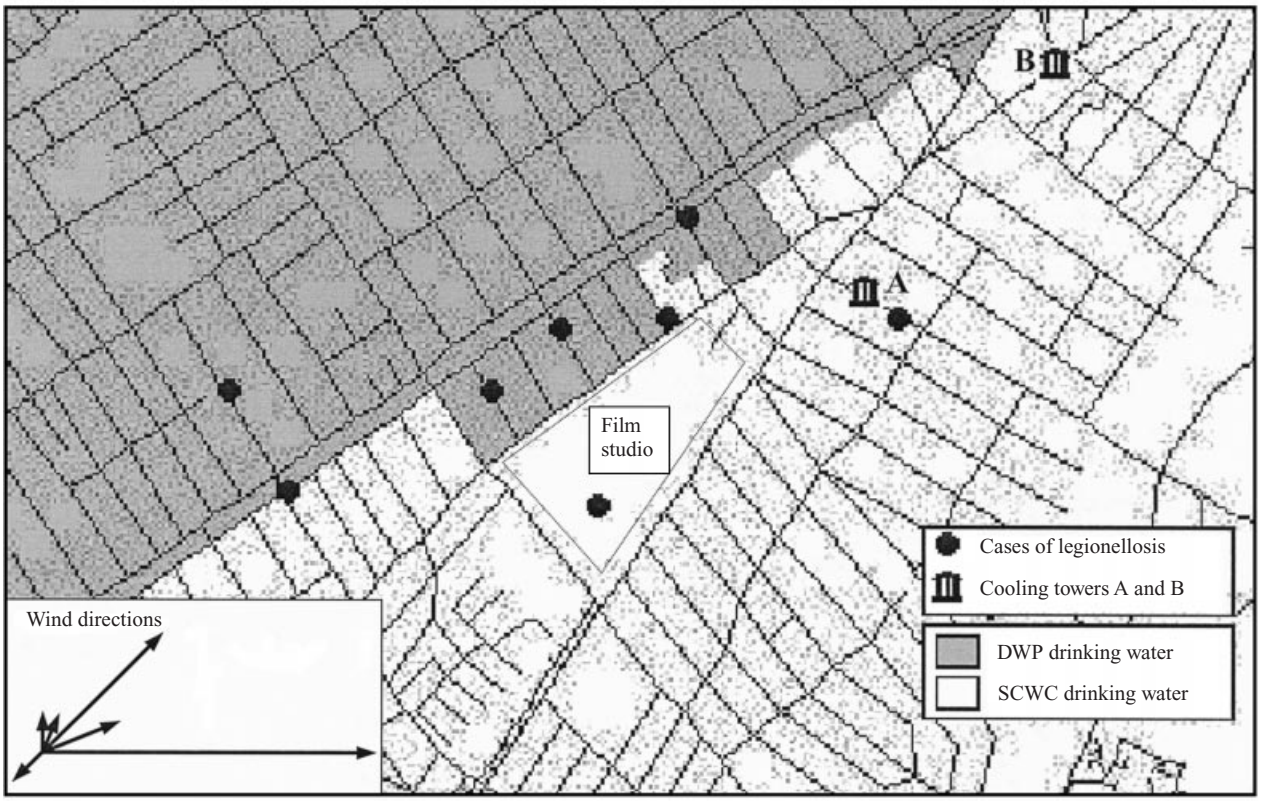

Fig. 3. Map with the location of residence or work place of case-patients, cooling towers A and B, and drinking water distribution areas of the two water providers. Wind direction is shown in the lower left corner; the length of each arrow represents the number of days that the wind blew in that direction during the period 6–22 November. DWP, Los Angeles City Department of Water and Power; SCWC, Southern California Water Company.

given in Table 1. Visiting or living in sub-area 4 and 5 in the west of Culver City (Fig. 1) was associated with risk of disease: case-patients were more likely than controls to indicate that sub-areas 4 and 5 were among the two most frequently visited locations (matched odds ratio [MOR] for sub-area no. 4, 9·0;

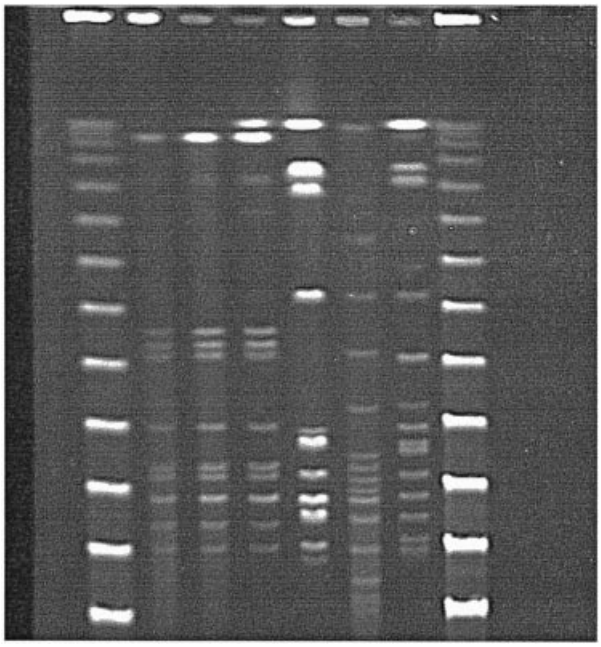

Fig. 4. Pulsed-field gel electrophoresis comparing legionella isolated from outbreak case-patients to strains from unrelated cases of Legionnaires' disease that occurred around the same time in Los Angeles County. Lanes 1 and 8, lambda ladder; 2–4, patients in outbreak; 5–7, non-outbreak case-patients.

95% confidence interval [CI], 1·2–227; MOR for no. 5 was undefined but greater than 1 and $P = 0{\cdot}01$). Answering 'yes' to the question 'Do you drive your own car?' was highly protective (MOR was undefined; $P = 0{\cdot}0005$). Patients that did not drive their own car usually went on foot for their daily activities, and took the bus or were driven by family or friends whenever they needed to travel outside of the Culver City area. Use of specific bus stops was not associated with risk of disease and other locations or activities also were not statistically associated with risk of disease.

After cooling towers A and B (Fig. 3) were found to contain legionella that could not be distinguished from the outbreak strain, the case-patients and controls were re-interviewed. One patient and his controls were excluded because he had died during the investigation and his surrogates knew little about his previous habits and whereabouts. The individual shops and locations around cooling towers A and B as well as a summary variable, which included all shops and locations around cooling towers A and B, were not significantly associated with risk of disease. Although 5 of the 7 re-interviewed case-patients came within 3 blocks of cooling tower A on at least one occasion in the 2 weeks before onset of disease, they were no more likely to do so than their controls (MOR, 0·4; 95% CI, 0·1–1·8). Only 2 case-patients came within 3 blocks of cooling tower B (MOR not calculated). Two case-patients indicated that they had not been in the vicinity of either cooling tower.

Environmental investigation

We sampled 66 different potential sources and *L. pneumophila* serogroup 1 was identified in 20 (30%) of these. Sampled sites included 38 cooling towers, 17 (45%) of which were positive for *L. pneumophila* (Table 2). Isolates from two different cooling towers in the east of the city where the outbreak occurred (marked with 'A' and 'B' in Fig. 3) were indistinguishable from the outbreak strain by all subtyping techniques (Fig. 5). The PFGE profile was similar to a published profile from isolates of an outbreak that had occurred in Providence, Rhode Island, in 1993 [5, 9]. Subsequent re-testing by one laboratory of all isolates confirmed that strains isolated from the Rhode Island outbreak were indeed indistinguishable, by each subtyping method, from the strain that caused this outbreak (Fig. 6).

One of the sources containing *L. pneumophila* serogroup 1 (MAb subtypes 1, 6 and 1, 6, 7, different from patient isolates) was a hospital cooling tower. Two of the cooling towers that harboured legionella were mobile units at a film studio. These were used intermittently as required for stage productions. There had been several stage productions in November during which mobile cooling towers were used but it was not possible to ascertain which were used and at what times, because no record was kept. The film studio's fixed cooling towers were routinely disinfected according to a schedule, and biocide concentrations were monitored and logged. No routine disinfection had been performed between the time of the outbreak and the time of the environmental sampling. The mobile cooling towers, however, were not routinely treated and may not have been disinfected for several years prior to the outbreak.

At the time of the investigation 5 of the 6 mobile cooling towers were drained and not in operation. When appraised of the outbreak, the film studio decontaminated all mobile cooling towers with sodium hypochlorite. Although the mobile cooling towers were sampled after they had already been decontaminated two of them yielded *L. pneumophila*

Table 1. *Main findings of the case-control study: locations visited and activities engaged in during two weeks preceding disease onset of the case-patient*

Risk factor	Matched odds ratio	95% CI or *P*
Sub-area no. 4 was among the two most-frequently visited of 9 sub-areas*	9·0	1·2–227
Sub-area no. 5 was among the two most-frequently visited of 9 sub-areas*	Undefined (risk)	$P = 0·01$
Driving one's own car	Undefined (protective)	$P = 0·0005$
Passing within three blocks of cooling tower A	0·4	0·1–1·8
Visiting the main shopping centre	3·65	0·7–29
Passing within one block of car-wash	Undefined	$P = 0·7$
Passing near any decorative fountain	5·3	0·5–154
Visiting the hospital	3·0	0·4–28
Using a bus	6·6	0·5–197
Shopping at any grocery store in CC	2·6	0·3–85
Visiting any church or synagogue	Undefined	$P = 0·36$
Passing plaza in front of film studio	2·1	0·3–13
Using the laundromat in CC	Undefined	$P = 1·0$
Visiting park A in CC	0·9	0·0–8·5

* Including the sub-area where the home and/or the workplace was located.
CI, confidence interval; CC, Culver City.

Table 2. *Summary of sites sampled for legionella*

Type of sampling site	Number of different sites	Number from which legionella was recovered	Serogroup (MAb subtype)
Fixed cooling towers	32	15	Serogroup 1: (1, 6, 7), (1, 6), (1, 2, 5, 6), (1, 4, 7), serogroup 4; serogroup 10; non-1 unknown serogroup; unidentified *Legionella*-like organism.
Mobile cooling towers*	6	2	Serogroup 1 (1, 6, 7), unidentified *Legionella* sp.
Decorative fountains	10	0	
Hot water in a patient's private home	2	0	
Car wash recycled water	1	0	
Film studio water tower	3	0	
Film studio saucer tank (used for underwater filming)	1	1	Serogroup 4
Water blasters (connected to DWP drinking water)	1	0	
Water truck that had supplied a water blaster	1	1	Serogroup 1 (1, 4, 7)
Fire hydrants that supplied water blasters	8	0	
Dry cleaner evaporative cooling device	1	1	Serogroup 1 (1, 6, 7)
Total	66	20	

* Most of the mobile cooling towers had been treated with sodium hypochlorite prior to sampling, including those that yielded legionella.

serogroup 1 of MAb subtype (1, 6, 7), which was different from the outbreak strain.

The predominant wind direction during the entire month of November was from the west and south-west (Fig. 3). Only once, on 22 November, was the wind predominantly from the east. Therefore, cooling towers A and B were situated downwind from the residences and the work place of all the case-patients and they were also downwind from sub-areas 4 and 5 (Figs 1, 3).

The residences and the work place of the case-patients did not have a common water provider (Fig.

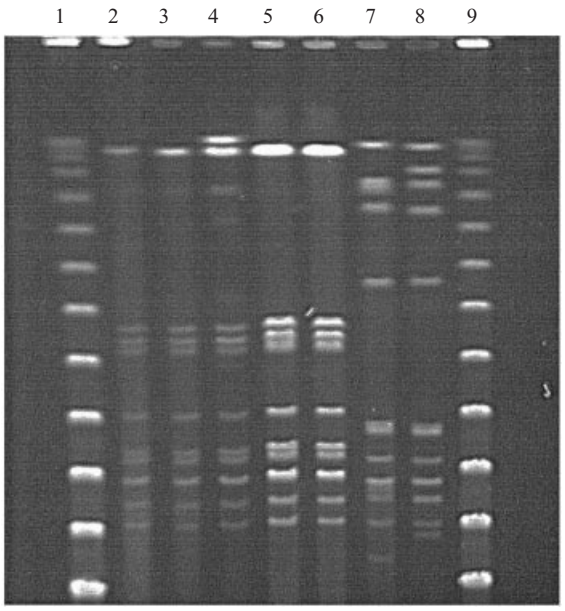

Fig. 5. Pulsed-field gel electrophoresis comparing *L. pneumophila* serogroup 1 isolates from outbreak case-patients and environmental samples from Culver City. Lanes 1 and 9, lambda ladder; 2, 3, and 4, patients that were part of the outbreak; 5 and 6, cooling towers A and B; 7, mobile cooling tower at the film studio; 8, other cooling tower.

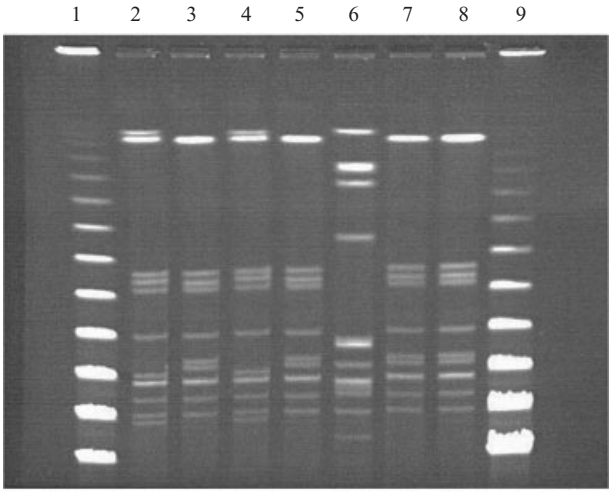

Fig. 6. Pulsed-field gel electrophoresis of isolates collected during four outbreaks. Lanes 1 and 9, lambda ladder; 2, Fall River 1993 environmental isolate; 3, Providence 1993 environmental isolate; 4, Fall River 1993 clinical isolate; 5, Providence 1993 clinical isolate; 6, unrelated control strain of *L. pneumophila* serogroup 1; 7, Culver City cooling tower A; 8, Culver City cooling tower B.

3). No water piping construction or pipe network dead ends were identified that may have been related to the outbreak and water authorities confirmed that there had been no water system-related events such as leaks or pressure drops in October or November. Water samples collected from the hot water system of one case-patient's home were negative for legionella.

One of the public utility companies was operating a sewer line excavation site in the area where water blasters were used to remove concrete from old underground sewer lines. A water blaster emits water at very high pressure, resulting in aerosol. This aerosol may have escaped through manholes that were used for ventilation along the sewer line. With the help of water utility staff the locations of these manholes were noted and added to the case-control questionnaire. Water used by the water blasters came from the drinking water distribution system through fire hydrants. Water and swabs were sampled from these hydrants and from water blasters but yielded no legionella. A sample from a water truck that had supplied water to one of the water blasters yielded *L. pneumophila* serogroup 1, MAb subtype (1, 4, 7). However, more detailed information from its log showed that this water truck had not been used in Culver City in November. Another potential source of aerosol was a car wash, in which cold water was used and recycled car wash water samples failed to yield legionella. Furthermore, a water tower at the film studio was noted to leak large amounts of water at the time of the investigation, producing sprays of water possibly resulting in some aerosol. Water and swab samples from the leaking pipe and from the inside of the water tank were negative. No heat source was identified that could have increased the temperature of the water in this tower.

DISCUSSION

A cluster of Legionnaires' disease cases caused by a single strain of *L. pneumophila* occurred in November 1997. Epidemiological investigation suggested an environmental common source but microbiology failed to identify the outbreak strain in the implicated locale. If a single source caused the outbreak, it may have been missed during the environmental survey and epidemiological investigation, or may have been decontaminated prior to sampling. Alternatively, the source may have disseminated legionella for only a short duration. The latter possibility is corroborated by transmission primarily occurring during a one-week period in November, ending well before any decontamination of potential sources was carried out (Fig. 2). The patient with onset on 8 December was diagnosed by the urine antigen test and so no isolate was available to compare with the outbreak strain. It

is possible that the source of the outbreak had already stopped disseminating legionella by late November and that the 8 December case was not related to the outbreak.

The mobile cooling towers from the film studio were shown to harbour *L. pneumophila* serogroup 1. The routinely short periods of use of these devices would fit with the apparent short duration of the outbreak. Moreover, these were located in the area implicated by the epidemiological analysis in combination with the wind direction. Since some of these devices were drained and disinfected at the time of the investigation, the results from sampling may have been unreliable.

The finding that driving one's own car was protective may indicate air-borne spread on the street or another common source in a public place, as opposed to, for example, drinking water in patients' homes. This latter possibility was made further unlikely by the fact that two different water authorities supplied the patients' residences with water from two different origins and that no legionella was recovered from drinking water from a patient's home and from eight fire hydrants, in both water distribution systems.

The matched case-control study showed that visiting sub-area 4 or 5 in the west of the city was associated with risk of disease. However, no environmental source in or near these areas was identified that yielded *Legionella* strains identical to the patients' isolates. The two cooling towers (cooling tower A and B, Fig. 3), whose legionella isolates were indistinguishable from the three available patient isolates by four subtyping techniques, are located in the east of the city where the outbreak occurred. The finding that a seemingly clonal strain of *Legionella* was isolated from two geographically dispersed environmental sources suggests that this particular strain was common in that area. The epidemiology and the information about wind direction argue against either cooling tower being the sole source of the outbreak. In other words, it is unlikely that either cooling tower A or B could have been the source of infection for all case-patients. Moreover, had either cooling tower been the source of this outbreak, we could have expected to find at least some case-patients downwind from it. Also, since the cooling towers were still contaminated at the time of sampling, persons should have continued to be infected up to the time of decontamination; however, the outbreak appeared to have stopped at least one month before any decontamination of cooling towers A and B was done (Fig. 2). Cooling towers A and B also were not statistically associated with risk of disease in the case-control study. Because of the low statistical power of the case-control study due to the small number of subjects we could not completely rule out that cooling towers A and B were associated with the outbreak, or that they contributed to infection of some of the cases, but in view of the data discussed above it seems unlikely that either cooling tower caused the outbreak. The probability of unrelated cases of Legionnaires' disease occurring in such a small population within such a short period of time is extremely low and it seems unlikely that two independent environmental sources would have caused an outbreak at the same time.

The operators of all cooling towers that harboured legionella were informed of the study findings and advised to follow published guidelines for maintenance and treatment of cooling towers [10]. In addition to improving routine maintenance, the owners of the two laboratory-implicated cooling towers A and B and the hospital were advised to decontaminate their cooling towers. No new cases of Legionnaires' disease have been reported from the area where the outbreak occurred in the two following years.

Molecular subtyping was useful in our outbreak investigation because it distinguished clinical outbreak isolates from clinical isolates of patients in other areas of the County. However, multiple DNA fingerprinting techniques could not distinguish isolates of two cooling towers from each other, from the outbreak strain, and even from an unrelated Legionnaires' disease outbreak that occurred in 1993 in Rhode Island [5, 9]. This implies that this subtype may be common in the United States or that the subtyping techniques were insufficiently discriminating for this strain. Other investigators have made similar observations. A study in Paris, France, found that 25 of 75 epidemiologically unrelated clinical legionella isolates obtained over a 10-year period and 16 of 64 randomly selected environmental isolates were indistinguishable by AP-PCR and PFGE [11]. Likewise, a comparison of legionella isolates from seven epidemic investigations found identical PFGE- and AP-PCR patterns in several epidemiologically unrelated strains [5]. Other studies have employed more subtyping techniques [12] but, to our knowledge, this is the first report that as many as four different subtyping techniques failed to distinguish between epidemiologically unrelated *Legionella* strains from geographi-

cally distant locations. Because the strains were initially tested in two different laboratories, cross-contamination does not appear to be a possibility.

The results of this study support and confirm the importance of epidemiological observation in the investigation of legionellosis outbreaks. Microbiological investigations are invaluable for investigating outbreaks of Legionnaires' disease but even extensive subtyping may not accurately identify the source of an outbreak in the absence of epidemiological data. Environmental strains indistinguishable from clinical strains are not necessarily the cause of an outbreak of Legionnaires' disease.

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