SHORT REPORT
Legionella longbeachae detected in an industrial cooling tower linked to a legionellosis outbreak, New Zealand, 2015; possible waterborne transmission?

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SUMMARY

A legionellosis outbreak at an industrial site was investigated to identify and control the source. Cases were identified from disease notifications, workplace illness records, and from clinicians. Cases were interviewed for symptoms and risk factors and tested for legionellosis. Implicated environmental sources were sampled and tested for legionella. We identified six cases with Legionnaires' disease and seven with Pontiac fever; all had been exposed to aerosols from the cooling towers on the site. Nine cases had evidence of infection with either *Legionella pneumophila* serogroup (sg) 1 or *Legionella longbeachae* sg1; these organisms were also isolated from the cooling towers. There was 100% DNA sequence homology between cooling tower and clinical isolates of *L. pneumophila* sg1 using sequence-based typing analysis; no clinical *L. longbeachae* isolates were available to compare with environmental isolates. Routine monitoring of the towers prior to the outbreak failed to detect any legionella. Data from this outbreak indicate that *L. pneumophila* sg1 transmission occurred from the cooling towers; in addition, *L. longbeachae* transmission was suggested but remains unproven. *L. longbeachae* detection in cooling towers has not been previously reported in association with legionellosis outbreaks. Waterborne transmission should not be discounted in investigations for the source of *L. longbeachae* infection.

Key words: Legionnaires' disease, legionella, *Legionella longbeachae*, occupation-related infections, outbreaks, water-borne infections.

INTRODUCTION

Legionnaires' disease (LD) and Pontiac fever (PF) are the most common disease manifestations of legionella infection, collectively termed legionellosis. Internationally, the predominant cause of community-acquired

LD is *Legionella pneumophila* serogroup (sg) 1 [1], however in New Zealand infection with *Legionella longbeachae* has accounted for more than 50% of reported legionellosis cases annually since 2012 [2], and a review of New Zealand legionellosis epidemiology from 1979 to 2009 found that *L. longbeachae* occurred at the same frequency as *L. pneumophila* [3]. Some jurisdictions in Australia also report more *L. longbeachae* notifications than *L. pneumophila*, however this is not a consistent trend across

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Australia as a whole [4]. *L. longbeachae* infections where the source has been confirmed appear to be exclusively associated with soils, potting mixes and composts [5]; conversely, cases and outbreaks of LD due to other legionella species have often been linked to engineered systems that produce water aerosols [6], and *L. pneumophila* sg1 transmission from soil has also been described [7].

We describe a mixed species legionellosis outbreak associated with industrial cooling towers that were found to be heavily contaminated with multiple legionella species, including both *L. pneumophila* and *L. longbeachae*. An unusual feature of this outbreak was that *L. longbeachae* was detected both as a contaminant of cooling tower water and as an apparent cause of human infection among cases working at the industrial site; we are not aware that this has been reported previously.

Between 8 October and 4 November 2015, three patients diagnosed with LD due to *L. pneumophila* sg1 were notified in central North Island, New Zealand. The patients lived in three widely separated towns in two non-adjacent health districts, and recognition of a possible outbreak did not occur until concurrent investigations of the second and third patients identified that all three had worked at the same industrial site.

The industrial site, a dairy processing plant, was situated in a rural area distant from the residential addresses of all three initial cases. The plant had been extensively redeveloped to increase production capacity: this redevelopment had required substantial earthworks. Two new cooling towers had been built to accompany three existing towers on the site. The site workforce comprised approximately 120 workers, the majority employed as tanker drivers who transported milk to the plant for processing. All three initial cases had been ambulatory on the site during their incubation periods. We conducted an investigation to characterize cases, and identify and remedy the outbreak source.

METHODS

This work was undertaken as part of an urgent public health investigation therefore did not require institutional ethical review or approval. In addition to the first three reported cases, case finding was undertaken through review of disease notifications and workplace absenteeism and illness records, and requests to clinicians for notifications of suspected legionellosis.

Retrospective case finding extended back to 1 August 2015 because activation of the plant's cooling towers occurred around that date. We defined a case of confirmed LD as radiographically-confirmed pneumonia with laboratory evidence of legionella infection in a person with onset of illness between 1 August and 12 December 2015 and within 14 days of exposure to the site. Laboratory evidence of legionella infection comprised at least one of the following, as per the New Zealand surveillance definitions [8]: legionella isolated from a clinical specimen, *L. pneumophila* sg1 antigen detected in urine, or seroconversion indicated by a ⩾fourfold rise in immunofluorescent antibody titres against legionella species to ⩾256 between acute- and convalescent-phase sera tested in parallel. Probable LD was defined as above, with laboratory evidence of legionella infection of one or more antibody titres ⩾512 but no fourfold titre rise. Cases with confirmed or probable PF were defined as persons meeting laboratory criteria for confirmed or probable LD, respectively, without radiographic evidence of pneumonia but who had developed an illness with fever and at least one other symptom (of headache, cough, dyspnoea, myalgia, vomiting, diarrhoea) that had commenced within 3 days of exposure to the site in the 1 August–12 December 2015 period.

Data on demographic details, illness onset and symptoms, healthcare utilization, risk factors for legionellosis, and possible exposures to legionella sources in occupational and non-occupational environments were collected from standardized interviews with all persons identified. Medical examination and investigations were sought on all those with history of illness suggestive of legionellosis: for those with acute illness, investigations included sputum culture and isolation, urinary antigen testing, acute-phase serology, and chest radiographs if clinically indicated; convalescent-phase serum specimens were collected if symptoms had occurred 3 or more weeks prior to assessment, or 3 weeks after acute serum collection.

Retrospective detection of antibody levels to legionella species was undertaken using indirect immunofluorescent antibody testing (IFAT) on sera collected from suspected outbreak cases in order to ascertain both evidence of exposure and the identity of the causative Legionella species [9]. The IFAT used fluorescein isothiocyanate conjugated anti-human IgM, A and G antibody and heat-killed whole-cell antigens prepared from 32 type strains of legionella known to cause infection in New Zealand (based primarily on clinical culture isolation). These were grouped into five pools

(Supplementary material) and sera reactive against a pool were then retested against the individual pool components to identify the reactive legionella strain. Sera were tested at the Institute of Environmental Science and Research (ESR) Legionella Reference Laboratory (LRL) using a standard process irrespective of the patient's association with the outbreak. Urine specimens were tested for *L. pneumophila* sg1 antigens using the BinaxNOW kit (Alere Inc., Waltham, Massachusetts, USA). Sputum samples were cultured for legionella at the LRL; isolates were identified to species and serogroup level using direct fluorescent antibody staining (m-Tech, Milton, Georgia, USA) and by *mip* gene sequencing [10]. Sequence-based typing (SBT) was used to characterize DNA sequences of seven virulence genes in the order *fla*A, *pil*E, *asd*, *mip*, *momp*S, *pro*A, and *neu*A for the *L. pneumophila* isolates. The nucleotide sequences of these genes can be compared between clinical and environmental isolates, giving results that are considered highly discriminatory and epidemiologically concordant [11]. Each unique combination of the allelic profiles is assigned a unique Sequence Type (ST) using the on-line tool at http://bioinformatics.phe.org.uk/legionella/legionella_sbt/php/sbt_homepage.php [12].

An environmental investigation was conducted to identify possible legionella sources on the industrial site, in particular areas where water aerosols were created or where organic matter was being composted or handled. Possible exposure sources were also sought in case's domestic or non-occupational environments. Water, biofilm and soil samples were collected according to guidance methods [13], and tested at the LRL for legionella bacteria using the appropriate method depending on sample origin (either ISO 11731-2 for potable waters, AS/NZS 3896 for cooling tower waters, or AS/NZS 5024 for soils and composts). Isolates were analysed as described above. Water temperature and free available chlorine (FAC) levels were measured onsite using recently-calibrated instruments.

RESULTS

Including the initial three patients, 13 cases were identified with either LD or PF; six met criteria for LD (four confirmed, two probable), and seven met criteria for PF (all probable). The four cases with confirmed LD had *L. pneumophila* sg1 identified by culture (2 cases) or urinary antigen testing (1), or *L. longbeachae* sg1 identified by seroconversion (1). Other legionella species and serogroups were diagnosed by serology in cases with probable LD or PF (Table 1). A further 19 persons identified with respiratory illness following site exposure did not meet case criteria because they did not have clinical illness consistent with LD or PF (3), had negative tests (14) or had no testing done (2).

The 13 cases had a median age of 51 years (range 29–72 years), 12 were male. Nine were current or previous smokers; three had a history of asthma, and one was taking anti-rejection immunosuppressant medication following a kidney transplant. The cases' illnesses began between 15 August and 20 November 2015 (Fig. 1). Six (all with LD) had been hospitalized during their illness, one requiring intensive care.

Of the 13 cases, 12 had been working on the site during their incubation periods: three had worked on the site for the entirety of each working day, and the remaining nine were tanker drivers who visited the site regularly during their working shifts. The remaining case lived on a neighbouring property 500 m from the site and passed close to the site perimeter on each working day. Site investigation identified the five water-based cooling towers as possible sources of legionella exposure; all cases who were site workers had been ambulatory in open air close to the cooling towers. Other possible sources on the site included shower facilities and dust from earthworks. Potential non-site exposures for cases included potting mix (1 case), roof-collected untreated water (3), spa pool (1) and decorative fountain (1).

The cooling towers were situated at ground level, with air intake sites covered by ventilation grills and otherwise open to circulating air. Each of the cooling towers contained sediment and obvious algae growth, with soil residues visible on the ventilation grills on cooling tower A. Although a hypochlorite-based biocide was being used in the cooling towers, FAC levels were undetectable in the two newly-installed cooling towers (A and B), and ranged between 0·07 and 0·14 mg/L in the three pre-existing towers (C, D and E). Legionella bacteria were cultured from samples from all five cooling towers (Table 2). Seven different legionella species were identified from the cooling towers; *L. pneumophila* sg1 and *L. longbeachae* sg1 were found in the highest concentrations. The SBT allelic profile for *L. pneumophila* sg1 isolates from each cooling tower was 12,8, 11, 21, 40, 12, 9; matching the unique profile ST 1694. A 100% DNA sequence homology was established between the *L. pneumophila* sg1 isolates from cooling towers and from the clinical isolates of the two cases diagnosed on the basis of legionella culture. No legionellae

Table 1. *Basis of case classification and infecting organism*

Case classification and basis for classification	*Legionella* species	Number of cases	Serological titres (first sample/second sample)
Legionnaires' Disease – Confirmed			
Sputum culture positive	*L. pneumophila* sg1	2	Not collected
Urinary antigen positive; two elevated convalescent titres	*L. pneumophila* sg1	1	512/1024
Fourfold rise in serological titres	*L. longbeachae* sg1	1	1024/4096
Legionnaires' Disease – Probable			
Two elevated serological titres	*L. pneumophila* sg5	1	512/512
Single elevated serological titre	*L. longbeachae* sg1	1	2048/not collected
Pontiac Fever – Probable			
Two elevated serological titres	*L. longbeachae* sg1	3	512/512 512/1024 512/1024
	L. longbeachae sg2	1	512/1024
	L. pneumophila sg1	1	512/512
	L. sainthelensi	1	512/512
Single elevated serological titre*	*L. sainthelensi*	1	512
Total cases		13	

sg, serogroup; *titre below cut-off value on second sample.
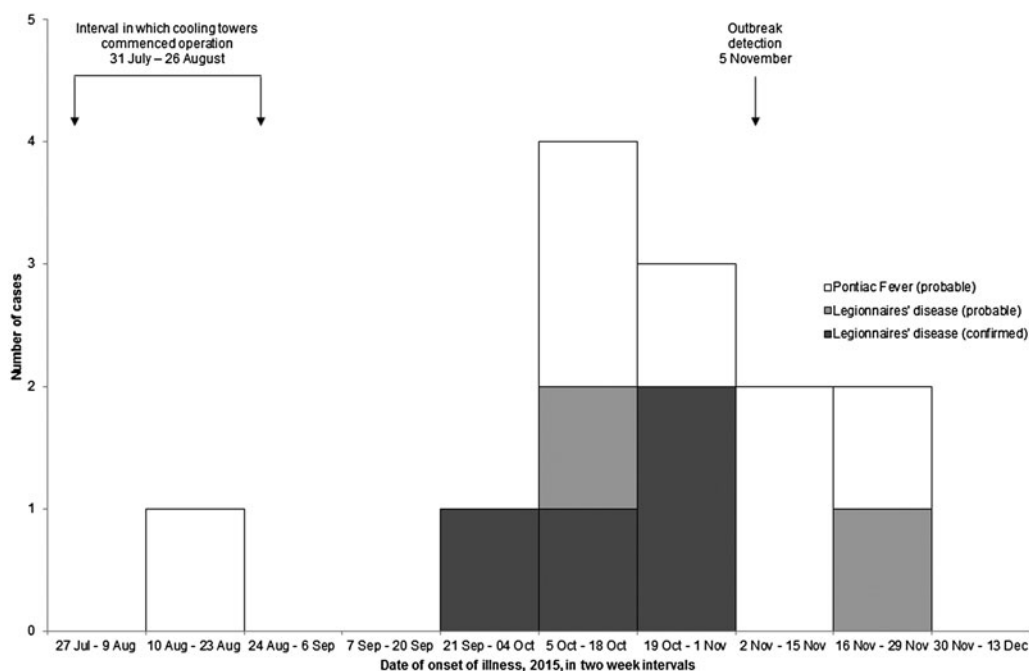

Fig. 1. Epidemic curve for 13 patients with legionellosis, August–November 2015, New Zealand.

were detected in environmental specimens collected from other locations within the industrial site, including uncovered soil adjacent to cooling towers. *L. pneumophila* sg1 was isolated from home compost used by one outbreak case, however the SBT allelic profile for the compost isolate was distinct from that of the case and the cooling tower isolates. No legionellae were detected in other non-site implicated sources – these included both domestic hot water systems and compost used by cases during their incubation period.

An investigation was undertaken to find factors contributing to the outbreak. It was established that cooling towers A and B had been newly commissioned and had commenced operation on 7 August 2015 and

Table 2. *Legionella testing results on samples collected from cooling towers*

Cooling tower	Sample type	Water temperature	FAC (mg/L)	Legionella species identified*	Enumeration (CFU/mL)
A	Water	29 °C	0	*L. pneumophila* sg 1**	10–100
B	Water	24 °C	0	*L. pneumophila* sg 1**	560–640
				L. longbeachae sg 1	170–180
				L pneumophila strain 91–033	10
				L. taurinensis	10–130
	Biofilm	n/a	n/a	*L. pneumophila* sg 1**	n/a
				L. longbeachae sg 1	n/a
C	Water	18 °C	0·07	*L. rubrilucens*	10
D	Water	18 °C	0·14	*L. pneumophila* sg 1**	160–220
				L quinlivanii sg 1	10–40
				L rubrilucens	40–50
	Biofilm	n/a	n/a	*L. pneumophila* sg 1	n/a
				L. quinlivanii sg 1	n/a
E (not in operation)	Water		0·12	*L. birminghamensis*	10

FAC, free available chlorine; CFU, colony-forming units.

* Species identification carried out using *mip* gene sequence analysis [10].

** SBT allelic profiling of these *L. pneumophila* sg 1 isolates indicated 100% homology to ST 1694 strain.

31 July 2015 respectively; pre-existing towers C and D were re-started on 26 August and 6 August, respectively. Tower E remained offline and non-operational throughout, although the basin contained water. No documentation was available to verify biocide management of the towers before starting operation; following start-up, towers A and B were managed by manual 'slug' dosing with biocides once per month, and towers C and D were automatically dosed with two biocide products. Water samples had been collected from all four operational towers on a monthly basis to test for the presence of legionella by a private service provider, and all test results had been negative. However, the samples had been collected using containers that did not include a biocide neutralising agent, were chilled in transport and were often subject to a delay in excess of 48 h between collection and testing.

Following recognition of the outbreak and detection of legionella contamination of the cooling towers, all cooling towers on the site were cleaned and hyperchlorinated to eliminate bacterial growth. Decontamination and clearance testing was undertaken in accordance with AS/NZS3666·3 [14]. The cooling towers were considered under control after two consecutive samples tested at the LRL were legionella culture-negative. Clearance specimens collected from all cooling towers 3 days after hyperchlorination were culture-negative, however repeat samples collected from tower B on 26 November 2015 were found to be positive for *L. pneumophila* sg1 (enumerated at 60 CFU/mL) and

clearance of this tower was not achieved until samples collected on 15 December 2015 were found to be negative. Clearance samples collected from each of the other four towers remained negative.

DISCUSSION

An outbreak of legionellosis with six confirmed and probable LD cases and seven probable PF cases occurred in an industrial site between August and November 2015. Several findings from the investigation indicated that one or more cooling towers on the site were the source of infection: the industrial site was the sole exposure common to all cases, and the cooling towers were the only identified feature containing legionella bacteria; all cases who worked on site were exposed to the cooling tower aerosols, and the one case who did not work on the site regularly passed the site and lived within the radius of possible legionella cooling tower transmission [15]; the epidemic curve for the outbreak reflects evolution of the outbreak over several months, which is similar to other legionellosis outbreaks linked to cooling towers [16, 17]; and finally, clinical *L. pneumophila* sg1 isolates from two cases matched isolates from each of the site cooling towers using SBT. The ST 1694 strain identified by SBT during this outbreak is the first time this *L. pneumophila* sg1 strain has been detected in New Zealand, although a detailed understanding of circulating environmental strains is unknown. SBT testing of other *L. pneumophila*

isolates referred to the LRL prior to, during and after this outbreak have not identified other ST 1694, suggesting that the strain is rare and supports identification of the cooling towers as the outbreak source.

L. pneumophila sg1 contamination of cooling towers has been previously linked to legionellosis outbreaks in New Zealand [16]; *L. pneumophila* in water are released in cooling tower aerosols [18], and many outbreaks have been temporally linked to cooling tower restarts following inactivity [19]. Operation of cooling systems after periods of shutdown has been shown to rapidly increase the legionella concentration in water, considered due to sloughing of biofilm and disturbance of sediments by water flow [20]. The conditions in the cooling towers in this outbreak suggest abundant biofilm was available to act as a legionella reservoir, and biocide levels were inadequate to effect control. The effectiveness of biocide would have been further reduced by sediment present in the cooling tower water basins; this may have resulted from the earthworks undertaken during the site redevelopment.

An unusual feature of this outbreak was detection of *L. longbeachae* sg1 in the water of one cooling tower, and serological evidence of *L. longbeachae* sg1 infection in one confirmed LD case and four probable LD and PF cases. *L. longbeachae* is rarely identified in water [21], and the few reported outbreaks of *L. longbeachae* legionellosis have typically implicated horticultural growing media [22]. One *L. longbeachae* outbreak has been linked to a ventilation system, although the ultimate source was unclear; *L. longbeachae* was not detected in environmental specimens, and the ventilation system did not involve water reservoirs [23].

None of the cases in the current outbreak with serology indicating *L. longbeachae* sg1 infection had clinical isolates that would enable comparison with environmental *L. longbeachae* isolates, so it is impossible to confirm the cooling tower as the source of their infections. The *L. pneumophila* sg1 findings provide circumstantial evidence suggesting transmission of legionellae from these cooling towers was feasible, and no alternative sources for *L. longbeachae* infection were identified. In a previously reported outbreak, the strain of *L. pneumophila* bacteria found in soil matched that of isolates from an adjacent spa pool and of clinical isolates from exposed cases [24]; similar links can only be implied in this outbreak because legionella was not detected in soil from the site, although it is feasible that legionella-containing airborne soil may have been the source of the cooling tower contamination. Waterborne transmission should not be discounted in investigations for the source of *L. longbeachae* infection.

Multiple strain outbreaks of legionellosis have been reported, usually demonstrated as multiple clones of the same species and serogroup [25, 26]. The majority of cases in this outbreak appeared to have been infected with either *L. pneumophila* sg1 or *L. longbeachae* sg1, however other cases had serological evidence of probable infection with either *L. pneumophila* sg5, *L. longbeachae* sg2 or *Legionella sainthelensi*, none of which were detected in environmental specimens. Our laboratory investigation of retrospective cases relied on serologic testing methods since many were identified after the sampling period for appropriate acute-phase serum, sputum or urine collection had elapsed. To mitigate the possibility of antibody cross-reactivity in the IFAT we tested paired serum specimens collected at least 2 weeks apart and in parallel for each patient, and titres $\geqslant 512$ were required to meet the serological case definition in the absence of seroconversion, however misclassification cannot be excluded. The legionella strains *L. longbeachae* sg1, *L. longbeachae* sg2 and *L. sainthelensi* are all closely related both antigenically and genetically, and have many shared epitopes. This contributes to the observed antibody cross-reaction patterns seen in the IFAT between these strains, and likewise similar cross-reaction patterns are observed between the different *L. pneumophila* strains. The IFAT relies on visual determination of the fluorescent endpoint and its subjective nature coupled with the antibody cross-reactions contribute to the low specificity of the test, and results require interpretation with caution. The antibodies detected to *L. longbeachae* sg2 and *L. sainthelensi* could potentially be the result of a *L. longbeachae* sg1 infection for these cases, and by the same argument, the antibodies detected to *L. pneumophila* sg5 may be the result of an infection to *L. pneumophila* sg1. In general, the sensitivity and specificity parameters of serological testing to diagnose non-*L. pneumophila* sg1 disease are less well established than for *L. pneumophila* sg1 infection [27].

There were limitations to this investigation. Full case ascertainment may not have occurred because we neither attempted a systematic investigation of the entire workforce, and nor did we proactively approach residents in the immediate vicinity of the site due to concerns that the presence of concurrent influenza-like illness in the region may have complicated case assessment. We instead raised awareness

of the outbreak through media and directly approached medical practitioners to solicit notifications of suspect cases that had sought medical attention or had been hospitalized. We did not undertake a systematic investigation of the entire cohort of workers on the site and therefore were unable to test measures of association between illness and variables such as work roles and risk factors; however, limited comparisons between cases and non-cases included in the investigation did not yield hypotheses that would have warranted a larger epidemiological study.

Primary factors that appeared to have contributed to the cause of legionella growth in the cooling towers implicated in this outbreak were inadequate biocide control and inadequate removal of organic matter that accumulated in the towers during earthworks in the vicinity. Additionally, tests on the cooling tower water prior to the outbreak detection appeared to have given false-negative results and therefore did not provide an alert that problems were developing; cooling tower operators should be cognisant of the impact of all relevant test results, both chemical and microbiological, on water quality in cooling towers. The findings from this outbreak have implications for prevention and control of legionellosis transmission from cooling towers, particularly with regard to routine microbiological monitoring of cooling towers. In New Zealand, the standard for managing the microbiological control of cooling towers is the joint Australia/New Zealand four-part Standard AS/NZS 3666 (Standards New Zealand, Wellington, New Zealand). Cooling tower water samples collected and processed in accordance with the Standard, were not found to contain legionella, however our testing undertaken using ESR guidance [13] as part of the outbreak investigation revealed legionella in abundant quantities. The sampling and processing techniques differed between our testing and that conducted for routine monitoring: we collected samples in containers with biocide neutralizer, kept specimens at ambient temperatures (>10 °C, but <20 °C) while in transit and tested samples within 6 h of collection; in contrast, samples collected for routine monitoring used containers without biocide neutralizer, were chilled while in transit and the delay to testing often exceeded 48 h after collection. False-negative legionella testing results have occurred when water is sampled using containers not containing biocide neutralising agent [28], and it is possible that this may explain the negative results from tests performed before the outbreak was detected.

CONCLUSIONS

Information from this outbreak investigation suggests that legionellosis outbreaks may be heterogeneous, both in the infecting organisms and in the flora of the presumed outbreak source. Testing patients with community-acquired pneumonia using methods that enable identification of legionella species, other than solely relying on urinary antigen tests that only detect *L. pneumophila* sg1 antigen, and consideration of atypical transmission mechanisms would help improve understanding of legionellosis outbreaks. Contamination of wet cooling towers with legionella may be unavoidable; it is therefore critical to have good maintenance systems in place, and to follow effective monitoring practices that enable prompt detection and mitigation of legionella contamination to avoid outbreaks.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at https://doi.org/10.1017/S0950268817001170

CONFLICT OF INTEREST

None.

ETHICAL STANDARDS

This work was undertaken as part of an urgent public health investigation therefore did not require institutional ethical review or approval.

DISCLAIMERS

Nil.

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