An Outbreak of Pontiac Fever with Respiratory Distress among Workers Performing High-Pressure Cleaning at a Sugar-Beet Processing Plant

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Background. In August 2000, the Minnesota Department of Health was notified of and investigated an outbreak of febrile respiratory illness among workers at a sugar-beet processing plant.

Methods. A case was defined as fever and respiratory symptoms occurring in a worker at the sugar-beet plant on or after 31 July 2000. Case patients were interviewed, medical and work records were reviewed, and clinical samples were obtained. The plant was inspected, and environmental samples were collected.

Results. Fourteen of 15 case patients performed high-pressure water cleaning in the confined space of an evaporator vessel. Symptoms included fever and chills (100%), chest tightness (93%), cough (80%), and shortness of breath (73%). In case patients, median temperature was 39.4°C, median oxygen saturation was 93%, and median white blood cell count was 8,500 cells/µL. Four (29%) of 14 case patients showed evidence of Legionella pneumophila exposure, according to serologic testing. Water sources contained up to 10^5 cfu/mL of L. pneumophila and 22,200 endotoxin units/mL.

Conclusions. Outbreak features were consistent with Pontiac fever. Respiratory symptoms, which are atypical for Pontiac fever, could be attributed to a high exposure dose of L. pneumophila from confined-space aerosolization or to endotoxin exposure. This outbreak demonstrates the potential occupational hazards for those performing high-pressure cleaning in confined spaces.

On 2 August 2000, the Minnesota Department of Health (MDH) was notified by hospital emergency-room personnel that 12 ill workers from a nearby sugar-beet processing plant had presented to the emergency room that day with symptoms of fever, chills, and respiratory difficulty. Of the 12 workers, 11 had been admitted to the hospital. Over the course of the next 24 h, 3 additional plant workers presented to the same emergency room with similar symptoms. MDH and other agencies launched a clinical, epidemiological, laboratory, and environmental investigation of the outbreak.

**PATIENTS AND METHODS**

A case was defined as fever and respiratory symptoms occurring in a worker at the sugar-beet plant on or after 31 July 2000. Respiratory symptoms included at least 1 of the following: cough, wheezing, chest tightness, or shortness of breath. To optimize case findings,
we (1) summarized the outbreak syndrome for distribution at the sugar-beet plant, to encourage reporting; (2) requested the reporting hospital to notify us of additional suspected cases; and, (3) on August 2 and 18, queried the hotel at which the contract workers had stayed about any illness among their guests or employees. Informed consent was obtained from all case patients; this was in compliance with MDH guidelines.

Case patients were interviewed during their illnesses. Follow-up interviews by MDH were conducted 1 and 4–6 weeks after illness onset. We reviewed medical records of all case patients and, when available, their work records.

We collected sputum and blood samples from case patients for routine bacterial and Legionella species cultures; urine samples were collected for Legionella pneumophila serogroup 1 antigen assays. We collected acute-phase serum samples from case patients for the determination of fungal titers against Aspergillus fumigatus, Aspergillus niger, Microsporidium faeni, and Thermoactinomyces vulgaris type 1. Case patient acute- and convalescent-phase serum samples were collected from case patients for serologic testing for L. pneumophila serogroups 1–6. Acute-phase serum samples were collected while the case patients were hospitalized; convalescent-phase serum samples were collected during 3 different postexposure periods: 6–12, 17–20, and 27–40 weeks. Acute- and convalescent-phase serum samples were tested in parallel by indirect immunofluorescence to detect L. pneumophila serogroups 1–6–specific IgG antibody. Acute-phase clinical testing was performed at the reporting hospital; otherwise, all testing was performed at the MDH Public Health Laboratory in Minneapolis.

A plant inspection on 3 August 2000 was conducted by MDH and other agencies, including the Minnesota Pollution Control Agency, the Minnesota Occupational Safety and Health Administration, and Renville County Public Health. The Minnesota Department of Agriculture was involved in additional plant inspections. Environmental samples from the plant were collected for microbiological, chemical, and endotoxin testing. A polymerase chain reaction method was developed and used to detect L. pneumophila DNA (see Appendix). Amplification and sequencing of the 16S ribosomal DNA was performed to identify, to the species level, organisms cultured from water. All microbiologic and chemical testing was performed at MDH. Endotoxin testing was performed at the University of Minnesota and the Centers for Disease Control and Prevention (CDC) by use of the Limulus lyase assay. This in vitro biological assay uses a crude lysate of the horseshoe crab (Limulus polyphemus) amebocyte to test for endotoxin [1]. Samples were tested as recommended by the various kits used: the University of Minnesota used assay kits obtained from Sigma Chemical Company, and CDC used kits obtained from Associates of Cape Cod.

RESULTS

Case patient ascertainment. Fifteen case patients were identified. Fourteen were part of a 15-person contract crew hired to assist with yearly plant maintenance cleaning. The fifteenth case patient was a plant worker who was not part of the contract crew.

Exposure investigation. On the morning of 31 July, the contract crew began high-pressure water cleaning within the confined space of an evaporator vessel. This activity continued over 2 days until the outbreak illnesses occurred; cleaning activities were suspended on 3 August. The crew supervisor, who never entered the evaporator vessel to perform high-pressure cleaning, did not become ill. The fifteenth case patient, who was not part of the contract crew, performed high-pressure water cleaning at a location distinct from the evaporator vessels; he was not in a confined-space environment during this work. The number of plant workers who were present at the plant or who performed high-pressure water cleaning at the plant was unavailable. However, because the plant was closed for yearly maintenance, this number was probably small.

The 15 case patients had no common work history, apart from their work at the sugar-beet plant. None reported a recent illness or illness in household contacts that was consistent with the outbreak illness. No illnesses were reported among employees or other guests staying at the hotel occupied by the contract workers. Nine (64%) of 14 contract workers used the hot tub at their hotel. It was unknown how many of the hotel’s other guests used the hot tub.

Descriptive epidemiology. All 15 case patients were men; they were 22–53 years old (median, 30 years old). Eleven (73%) were current or former smokers; 2 had a history of asthma, with 1 using multiple inhalant medications; and 1 had a history of intubation and ventilation for pneumonia following influenza.

Figure 1 shows a comparison of the time of symptom onset versus the time of the start of the first shift during which a worker performed high-pressure cleaning. Monday morning (31 July) was the first shift during which the contract workers entered the evaporator vessel to perform high-pressure cleaning. The median incubation period calculated from the start of a worker’s first high-pressure cleaning shift was 32 h (range, 16–57 h).

Clinical findings and measures. Symptoms reported by case patients are described in table 1. Fevers ranged from 38.3 to 40.6°C (median, 39.4°C). The differential diagnoses during evaluations of case patients included hypersensitivity pneumonitis and heat stress caused by elevated environmental temperatures. All case patients were documented to have dehydration and received intravenous (iv) fluids. Because of clinical findings of bronchoconstriction and respiratory compromise, nebulizer treatments were given to 13 case patients, and oxygen was administered to 12. All case patients received antibiotics (levofloxcacin) and steroids. Thirteen
Figure 1. Case patient symptom onset, by first shift during which high-pressure cleaning was performed

(87%) of 15 case patients were hospitalized, with 1 admitted to the intensive-care unit (ICU). Two other case patients received medical care in the emergency room but were not hospitalized. The duration of hospitalization was 1–3 days (median, 1 day). The case patient admitted to the ICU was hospitalized for 3 days.

Twelve (80%) of 15 case patients had oxygen saturations of 82%–97% (median, 93%); 2 of these had levels of 82%–83%. Five (42%) of 12 case patients had arterial blood-gas measurements performed: PO₂ values ranged from 47 to 74 mmHg and from 40 to 50 mmHg for 2 case patients (normal level, >75 mmHg). Thirteen (87%) of 15 case patients had normal chest x-rays. The other 2 had pulmonary infiltrates; 1 was diagnosed with pneumonia and was admitted to the ICU.

White blood cell counts were 9–21 × 10³ cells/µL (median, 12 × 10³ cells/µL; normal level, ≤10 × 10³ cells/µL); no leftward shift was noted. Electrolyte, renal, and liver-function tests were noncontributory. Urine toxicology screens were negative.

Diagnostic results. Fourteen acute-phase blood and 3 sputum samples were collected from 14 case patients for routine bacterial cultures; all were negative for growth. Thirteen blood samples and 3 sputum cultures from 13 case patients were cultured for Legionella, and all were negative for growth. Acute-phase urine antigen assays for L. pneumophila serogroup 1 from 14 case patients were negative. Serum fungal titers for 12 case patients were negative.

Four (29%) of 14 case patients demonstrated seroconversion to L. pneumophila by a 4-fold increase in titer (table 2); seroconversions were evident at 6–12 weeks after exposure. On follow-up testing, the 4 positive titers had decreased to levels of ≤1:64. One case patient was lost to follow-up and had none of these tests performed.

Plant inspection. The high-pressure cleaning by the contract workers was targeted at 5–10-foot vertical tubes in the evaporator vessel, which contained sugar-beet residue and precipitate [2]. Two leased portable power washers were transported by the contract company to the plant. Workers stood on the platform created by the ends of the vertical tubes and placed the high-pressure hoses into the tube ends to facilitate cleaning. High-pressure cleaning, performed at 20,000 psi, created substantial amounts of aerosolized and droplet material within the confined space of the evaporator vessel.

The contract workers used standard personal protective equipment, including hard hats, raincoats, goggles, face shields, Tyvek suits, cotton work gloves covered by nonpermeable gloves, and rubber boots. No respiratory protection was used.

As part of standard operating procedures for confined spaces, the contract crew used a 4-gas analyzer hourly and before any entry into the evaporator vessel. The analyzer checked for the presence of hydrogen sulfide, carbon monoxide, oxygen, and lower-level limits of explosive gases and vapors. Analyzer readings were negative, and analyzer calibrations were performed successfully.

The contract workers reported a malfunction of the ventilation fans for the evaporator vessel on Monday (31 July) evening and Tuesday (1 August) evening. Ambient temperatures in the evaporator vessel rose to as high as 51°C.

The fifteenth worker (noncontract) performed high-pressure cleaning at a location distinct from the evaporator vessels and was not in a confined-space environment. The high-pressure
Table 1. Symptoms seen in case patients (n = 15).

<table>
<thead>
<tr>
<th>Symptom</th>
<th>No. (%) of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>15 (100)</td>
</tr>
<tr>
<td>Chills</td>
<td>15 (100)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>15 (100)</td>
</tr>
<tr>
<td>Chest tightness</td>
<td>14 (93)</td>
</tr>
<tr>
<td>Headache</td>
<td>14 (93)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>14 (93)</td>
</tr>
<tr>
<td>Light-headedness</td>
<td>12 (80)</td>
</tr>
<tr>
<td>Nausea</td>
<td>12 (80)</td>
</tr>
<tr>
<td>Cough</td>
<td>12 (80)</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>11 (73)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>6 (40)</td>
</tr>
<tr>
<td>Wheezing</td>
<td>4 (27)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4 (27)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>4 (27)</td>
</tr>
<tr>
<td>Painful/red eyes</td>
<td>3 (20)</td>
</tr>
<tr>
<td>Sore throat</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Fainting</td>
<td>1 (7)</td>
</tr>
</tbody>
</table>

cleaning equipment that he used was different from that used by the contract workers. The water source, however, was the same.

The source of water for the cleaning was the plant’s system of lagoons, which served as reservoirs for recycled water from plant processing (figure 2). Lagoon water entered a wastewater treatment facility, where it underwent initial treatment steps. It exited as wastewater effluent and entered a treatment plant, where it was stored in a reactivator tank before undergoing dual-media filtration and reverse osmosis. Treated water was stored in a clear well tank and was then piped to a 500,000-gallon treated water tank outside the main plant building. A pump house drew water from this tank and stored it in a house hot-water tank. The house hot-water tank supplied the water for the high-pressure portable power washers.

Plant inspection revealed a bypass mechanism between the reactivator tank and the clear well tank. When the reactivator tank became full, water would overflow, bypass the steps of dual-media filtration and reverse osmosis, and go straight to the clear well tank. This was assessed to be a source of potential contamination by compromising the water treatment processing.

**Environmental testing.** Figure 2 indicates the location of environmental sampling during the plant inspection on 3 August. The samples included water from the pump house, which was representative of water quality in the treated water tank; residual water from the portable power washers 1 and 2; and a swab of the evaporator vessel tubes.

Test results of the environmental samples are shown in table 3. Testing of the pump house and power-washer samples revealed total bacterial counts (heterotrophic plate count) of $1.6 \times 10^7$, $3.3 \times 10^7$, $1.8 \times 10^7$ cfu/mL, respectively; this concentration would be comparable to untreated surface water [3]. Total coliform counts from the pump house and the power washers were >24,000 organisms/100 mL; this is >24,000 times the amount allowable for drinking water [4]. *Escherichia coli* counts were 620–1100 (most probable number) cells/100 mL; this was 4–8 times the level of 130 *E. coli* organisms/100 mL that is considered by the Environmental Protection Agency to be the maximum acceptable level for swimming beaches [5, 6].

Cultures of water from the pump house and the power washers revealed 2 species of *Legionella*: *L. pneumophila* and *L. londiniensis*. Bacterial counts were in the range of $10^4$–$10^5$ organisms/mL and included *L. pneumophila* serogroups 1, 3, 4, and 6. In addition, a swab of 1 of the evaporator tubes was positive for *L. pneumophila*.

Water samples from the pump house and power washers were tested for endotoxin at the University of Minnesota; initial levels were >10 mg/mL. After centrifugation and reincubation, the free levels of endotoxin were measured at 100 µg/mL in all submitted samples. The endotoxin controls included *Salmonella typhimurium* wild type, measured at 10 ng/mL (the end point for reactivity), and Minneapolis tap water, measured at <10 ng/mL.

Confirmatory endotoxin testing by the CDC of the same samples revealed endotoxin levels of 22,200 endotoxin units (EU)/mL from the pump house and 20,400 EU/mL from the power washers. The endotoxin control of *E. coli* was measured at 15.5 EU/mL. After the conversion of EU to metric units (conversion factor, 1 EU = 0.1 ng = 0.0001 µg), this was consistent with a level of 2.2 µg/mL [1].

Table 2. Specimen collection testing dates and results.

<table>
<thead>
<tr>
<th>Case patient</th>
<th>Convalescent-phase</th>
<th>Legionella antibody titer (date of collection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative (29 September)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Positive (15 September); 1:128</td>
<td></td>
</tr>
<tr>
<td>3\textsuperscript{b}</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Negative (20 September)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Negative (30 October)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Negative (15 September)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Negative (6 October)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Negative (15 September)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Negative (18 September)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Negative (12 September)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Negative (8 September)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Positive (12 September); 1:128</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Negative (19 September)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Positive (12 September); 1:256</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Positive (18 September); 1:128</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{*} All acute-phase serum samples were negative.
\textsuperscript{b} No testing performed.
DISCUSSION

We believe that the outbreak investigation findings support *L. pneumophila* as the causative agent for the illness seen in this outbreak, with illness manifested as Pontiac fever. Features of the outbreak that are consistent with an outbreak of Pontiac fever include the high attack rate, incubation period, lack of evidence of pneumonia in all but 1 case patient, lack of secondary transmission, and short course of illness for acute symptoms. A total of 4 (29%) of 14 case patients demonstrated seroconversion to *L. pneumophila*, which indicates an immune response to this agent and implicates it as a causative factor in their illness.

*Legionella* species are gram-negative bacteria that primarily reside in aqueous environments. Bioaerosols of *Legionella* species have been implicated in illnesses; settings have included contaminated cooling towers, whirlpool spas, fountains, air conditioning systems, and sewage plants [7–11].

Two distinct clinical and epidemiological syndromes are associated with *Legionella* species—Legionnaires’ disease and Pontiac fever. Although both present with fever, chills, fatigue, myalgia, and headache, there are major differences between the 2 illnesses. The incubation period for Legionnaires’ disease is typically 2–10 days, versus only 5–66 h for Pontiac fever. Legionnaires’ disease is an infection, whereas Pontiac fever may be considered to be an “exposure event” or an “allergen reaction” and is thought to be a nonpneumonic form of legionellosis [12]. It is possible to have both Pontiac fever and Legionnaires’ disease in the same outbreak [13].

Pontiac fever has been theorized to result from exposure to nonviable *Legionella* organisms [14]. This may explain the failure to detect a viable infectious agent or to observe a typical infectious process with the illness, as well as the high attack rates with no secondary transmission [12, 15]. Exposure to amebal pathogens that serve as natural hosts for *Legionella* species may also be a possible etiologic factor for Pontiac fever [16].

The low percentage of case patients with *Legionella* seroconversion seen in our cohort of case patients is not atypical and has been observed in previous investigations of Pontiac fever.
outbreak [8]. In addition, acute cultures and urine antigen assays for Legionella species are commonly negative in Pontiac fever outbreaks. It is also possible that the early administration of high-dose steroids and iv antibiotics to the case patients in this outbreak may have blunted individuals’ immune responses.

The case patients’ respiratory distress during their clinical presentation necessitated nebulizer treatments, oxygen supplementation, and the administration of steroids. In addition, some case patients displayed oxygen desaturation and abnormal arterial blood-gas levels. Such pronounced respiratory findings are not typical for Pontiac fever. However, respiratory symptoms of cough, chest tightness, and shortness of breath have been reported [9, 11, 17, 18]. In previous outbreaks, a dry cough was seen in 30%–60% of cases; chest pain or tightness was seen in 40% of cases [15]. To our knowledge, respiratory distress with compromise has not been reported in previous outbreaks of Pontiac fever. In the outbreak described here, the dose of Legionella species to which the workers were exposed might have been substantially higher than that in previous outbreaks. This could have been caused by the use of high-pressure cleaning within a confined-space environment.

The water used for the cleaning was also heavily contaminated with endotoxin. Endotoxin is the lipopolysaccharide (LPS) compound in the outer membrane of gram-negative bacteria that is found in many agricultural and occupational environments [1, 19]. Endotoxin may be released into the environment on bacterial cell death or during active cell growth. Increased endotoxin levels suggest the presence of large numbers of gram-negative bacteria [19]. Endotoxin may exist as part of the cell wall of gram-negative bacteria, it may be free in suspension, or it may be present as particulate matter (micelles). The concentrations of all of these forms of endotoxin together represent "total" endotoxin levels. Occupational illness caused by endotoxin has been identified at sewage treatment facilities, in industrial settings with contaminated wash-water aerosols, and in settings with exposure to humidifier mists [20–22].

Exposure to high levels of endotoxin result in a profound influenza-like illness with symptoms of fever, chills, and myalgia. In addition, exposure may cause respiratory symptoms, such as cough, chest tightness, shortness of breath, and abnormal pulmonary-function test results [21, 23–27].

Measured environmental endotoxin levels are notoriously variable. In light of this, the disparate endotoxin test results seen here are not surprising. Because of the lack of standardized threshold limits for endotoxin-related illness, we examined published exposure levels measured from water samples in previous experimental and observational studies that have been found to cause illness; these were 14,400 EU/mL and 34–46 µg/mL [8, 21]. The endotoxin levels in water samples in the present outbreak investigation were 22,200 EU/mL and 100 µg/mL, from CDC and the University of Minnesota, respectively. Both levels are above the thresholds in water samples that had previously been found to cause illness.

Because there is some overlap between the clinical and epidemiologic features of Pontiac fever and endotoxin-related illness, it is not surprising that the role of endotoxin was examined in a previous Pontiac fever outbreak. An investigation of Pontiac fever caused by Legionella micdadei in a whirlpool spa found the highest concentration of endotoxin from samples in which L. micdadei was recovered [8]. Those researchers suggested that endotoxin might play a role in the pathogenesis of Pontiac fever by originating from nonviable legionellae or from other aquatic bacteria existing in the same water source. Symptoms reported by the case patients in that study included headache, fever, chills, myalgia, shortness of breath, and fatigue. The only respiratory symptom reported was shortness of breath, in 33% of case patients. Information was obtained through a standardized questionnaire, and no medical evaluation of case patients’ respiratory symptoms was mentioned. In contrast, the dramatic clinical presentation of the case patients from the sugar-beet plant outbreak necessitated immediate medical evaluation. Case patients’ clinical findings of respiratory distress and compromise help to support the notion of a contributory role of endotoxin in Pontiac fever. To our knowledge, this is the first Pontiac fever outbreak reported in which the role of endotoxin is supported by the illness features of respiratory distress.

The role of endotoxin in Pontiac fever outbreaks may be underestimated and underevaluated. Pontiac fever outbreak in-

### Table 3. Environmental laboratory results.

<table>
<thead>
<tr>
<th>Sampling location</th>
<th>Total plate count, cfu/mL&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total coliform, MPN/100 mL</th>
<th>Escherichia coli, MPN/100 mL</th>
<th>Legionella species</th>
<th>Endotoxin, EU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water from pump house</td>
<td>$1.6 \times 10^7$</td>
<td>$&gt;24,000$</td>
<td>620</td>
<td>$1 \times 10^7$/mL L. pneumophila</td>
<td>22,200</td>
</tr>
<tr>
<td>Water from power washer 1</td>
<td>$3.3 \times 10^7$</td>
<td>$&gt;24,000$</td>
<td>1100</td>
<td>$1.5 \times 10^7$/mL L. londiniensis</td>
<td>20,400</td>
</tr>
<tr>
<td>Water from power washer 2</td>
<td>$1.8 \times 10^7$</td>
<td>$&gt;24,000$</td>
<td>890</td>
<td>$9 \times 10^7$/mL L. pneumophila</td>
<td>20,400</td>
</tr>
<tr>
<td>Swab of evaporator vessel tubes</td>
<td>$&gt;4$</td>
<td>...</td>
<td>...</td>
<td>Positive for L. pneumophila</td>
<td>...</td>
</tr>
</tbody>
</table>

**NOTE.** … no testing performed; cfu, colony-forming units; EU, endotoxin units; MPN, most probable number.

<sup>a</sup> Heterotrophic plate count.

<sup>b</sup> Results from the Centers for Disease Control and Prevention.
vestigations are often retrospective, when endotoxin sampling and testing may no longer be feasible or optimal. In addition, because there is a dose response with endotoxin-related illness, low levels of exposure may not cause appreciable respiratory distress; thus, endotoxin levels may remain unevaluated [23, 24].

It is intriguing to postulate that Pontiac fever and endotoxin-related illness might share the same etiologic process. However, L. pneumophila LPS has been found to have low endotoxic potential [28]. In general, gram-negative bacteria vary in their endotoxicity [29]. Future investigations of Pontiac fever outbreaks should consider endotoxin measurements of environmental samples.

Several environmental factors likely contributed to the outbreak occurrence at the sugar-beet processing plant. These factors include (1) a contaminated water source, as defined by high levels of bacteria, Legionella species, and endotoxin; (2) the creation of bioaerosols from the contaminated water through the use of high-pressure cleaning equipment; (3) the lack of respiratory protection for workers performing high-pressure cleaning; and (4) the high-pressure cleaning being performed within a confined-space environment, which intensified worker exposure to bioaerosols.

In conclusion, this outbreak of Pontiac fever was characterized by unusually pronounced symptoms of respiratory distress. This may be attributed to a high exposure dose of L. pneumophila and/or to endotoxin exposure. The outbreak also demonstrates the potential hazards of occupational illness among workers performing high-pressure cleaning. To minimize such hazards, we recommend microbiological assessment of water sources used for any high-pressure cleaning within a confined-space environment. If that is not feasible, respiratory protection should be provided by the industry to workers performing high-pressure cleaning within these environments.

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APPENDIX

Polymerase chain reaction (PCR) was used to confirm the identification of Legionella cultures. Primers were designed to specifically amplify the 16S rDNA of Legionella species in a nested PCR reaction. Template DNA for the primary PCR was prepared by use of spin columns (DNeasy Kit; Qiagen). PCR reactions contained 10 mmol/L Tris HCl, 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 1 μmol/L forward and reverse primers (LEG225, 5′-AAGATTAGCCCTGCGTCCGAT-3′, and LEG858, 5′-GTCAACTTATCGGTGTTGCT-3′), and 2 μL of template DNA in a total volume of 50 μL. The cycling reaction was performed in a Perkin Elmer 9600 (Applied Biosystems) thermal cycler for 40 cycles of 1 min at 95°C, 1 min at 60°C, and 1 min at 72°C. Reaction conditions were the same for the nested PCR reactions, except that the primers used were LEG450 (5′-GGACGTACCCGACAGAAGAA-3′) and LEG635 (5′-CGGAAATTCCACTACCATC-3′). Amplification products were resolved by electrophoresis on a 2% Tris-borate-EDTA agarose gel, and imaging was done by use of a Gel Doc 2000 (BioRad) imager. This test was performed on cultures obtained from environmental samples.

DNA sequencing was done to confirm the results of the PCR and to identify the nature of the agent. The 185-bp PCR product generated with primers LEG450 and LEG635 was purified by use of a QiaQuick Spin Column (Qiagen). Sequencing of the purified PCR product was done by use of a Beckman CEQ 2000 automated DNA sequencer with Beckman dye terminator cycle sequencing reagents (Beckman Coulter). Sequencing was performed in both directions by use of PCR primers LEG450 and LEG635 as sequencing primers. Sequence data were edited by use of Sequencher (version 4.0.5; Gene Codes), and species identification was done by conducting a BLAST analysis against the GenBank database.

The analytical sensitivity of this PCR method has not been determined. Specificity tests have not revealed any cross-reactivity of the primers with bacterial respiratory pathogens, including Bordetella pertussis, Bordetella parapertussis, Mycoplasma pneumoniae, and Chlamydia pneumoniae. To monitor for false-positive and false-negative results, we ran positive (L. pneumophila and L. micdadei) and negative controls with each assay.

References


