Case report
Scand J Work Environ Health 1999;25(3):291-295
doi:10.5271/sjweh.437

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This article in PubMed: www.ncbi.nlm.nih.gov/pubmed/10450782
Case reports

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Pontiac fever at a sewage treatment plant in the food industry

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Gregersen P, Grunnet K, Uldum SA, Andersen BH, Madsen H. Pontiac fever at a sewage treatment plant in the food industry. Scand J Work Environ Health 1999;25(3):291—295.

Background and objectives During a hot and humid summer period workers became ill with fever and flu-like symptoms after repairing a decanter for sludge concentration at a sewage treatment plant. The work took place over a period of 10 days in a small closed room, while another decanter was in operation and was consequently emitting aerosol to the environment, to which the workers were exposed. The aim of this study was to determine the cause of this outbreak of febrile illness so that additional cases could be prevented.

Methods All 5 patients were seen and examined in the Department of Occupational Medicine. Furthermore, 2 of the workers had recurrent illness and were examined during hospitalization. As Pontiac fever (nonpneumonic legionellosis) was suspected, antibodies to legionellae were measured in blood samples. After positive antibody titers to Legionella pneumophila were found, samples of the sludge were collected for legionellae culture.

Results and conclusions The clinical picture agreed with that described for Pontiac fever, and positive antibody titers to L. pneumophila serogroup 1 were found in blood from all 5 patients. L. pneumophila serogroup 1 was cultured in high amounts from sludge from the decanter. It was concluded that the fever was caused by L. pneumophila emitted to the environment by the uncovered decanter. Procedures for preventing new cases were established.

Key terms legionellosis, sludge, occupational disease.

Legionellosis demonstrates 2 clinical patterns, one being Legionnaires' disease, a severe multisystem illness with pneumonia as the most prominent feature, and Pontiac fever, a self-limited illness with a high attack rate characterized by fever, headache, myalgia, and fatigue.

Occupational legionellosis has been associated with hot water systems, cooling water systems used for air-conditioning purposes, cooling systems used for industrial purposes, industrial coolants used for grinding machine lubrication, the cleaning of steam turbine condensers with compressed air, and municipal water supplies (1—5). Legionellosis has, to our knowledge, not been reported after exposure to sewage and sludge, but it has recently been described after a basement flooding (6).

This study comprises 5 work-related cases of Pontiac fever after exposure to an aerosol emitted during the sludge concentration process in a sewage plant in the food industry. The plant treated only organic industrial waste water from the company and no domestic sewage.

This report concerns the first work-related outbreak of legionellosis described in Denmark.

Subjects and methods

Background. Five workers were seen in the Department of Occupational Medicine, Køge Hospital, on suspicion of a work-related infectious disease. They had all been working on the same task in the sewage treatment plant and had experienced a flu-like disease. Later, 2 other workers, who had shown no symptoms but had worked with the 5 ill workers, were examined.

The exposure took place during a hot and humid period with a mean temperature >20°C in August 1997 during the repair of one of the decanters on the first floor in a decanter house, where the sewage sludge from the treatment plant was concentrated by centrifugation in 2 uncovered decanters, which caused the production of an aerosol to the surrounding environment. The sludge...
The outbreak. On day 1, 2 workers (workers 1 and 2) disassembled the defective decanter, while the other decanter was operating. They worked at this task for about 2.5 hours. Both workers wore a half-mask, type B2, a filtering device with a charcoal filter, ie, a filter not meant for aerosol protection.

On day 2 the same 2 workers cleaned the decanter outside the decanter house by means of high-pressure washing. For this task worker 2 used a B2 filter mask, while worker 1 wore no mask. Afterwards the decanter was transported to the workshop and disassembled by the same 2 workers with assistance from worker 6.

Worker 1 developed flu-like symptoms, including malaise, nausea, pressure in the chest, and a feeling of fever 37 hours after the exposure to the decanter aerosol. He recovered spontaneously after 41 hours.

Worker 2 developed flu-like symptoms, including headache, pressure in the chest, dyspnea, and a feeling of fever 46 hours after the same exposure. He recovered spontaneously after 36 hours.

The 2 workers recovered during a weekend and began working again on the following Monday (day 6).

Worker 6 developed no clinical symptoms.

On day 8 the decanter was assembled and remounted, while the other decanter was stopped. Workers 1 and 2 performed the work, both now wearing air-line equipment (ie, breathing protection with fresh air also protective against aerosols).

On day 9 worker 1, wearing a B2 filter mask, inspected the decanter briefly, while worker 2, wearing air-line equipment, worked a few hours in the decanter house.

On day 10 workers 1 and 2 once more repaired the first decanter over a period of 1 hour with assistance from workers 3 and 4. Worker 5 was occupied for 5 hours with electrical installations in the decanter house. During the repair the other decanter was in operation. All the workers wore half-masks, type B2.

Worker 1 once again developed flu-like symptoms 48 hours after the reexposure on day 9 and 24 hours after the reexposure on day 10. This time the symptoms were more severe than on the first occasion. His body temperature was 39.2°C, and he was admitted to the hospital. He had no lung symptoms, and his chest X-ray was normal. He was also treated with penicillin and gentamicin intravenously because of fever of uncertain origin, and he recovered within the next 24 hours.

Worker 3 developed flu-like symptoms, including chills, nausea and headache, 24 hours after the exposure on day 10. He had no respiratory symptoms. He recovered spontaneously after a few days.

Worker 4 developed malaise, myalgia, and chills 23 hours after the exposure on day 10. His body temperature was 39°C. He recovered within 48 hours.

Worker 5 developed flu-like symptoms, including chills, headache, nausea and fever, 36 hours after the exposure on day 10. He had slight dyspnea. He recovered spontaneously within 48 hours.

Worker 7 had visited the decanter room only briefly every day (day 1—10). Furthermore, he had performed hard physical labor in the decanter room for 3.5 hours on day 10. He had worn a mask with a B2 filter every time. He developed no clinical symptoms.

After occupational health professionals in the Department of Occupational Medicine had seen the workers, they contacted the factory and subsequently met with the safety organization. The meeting resulted in a temporary plan to prevent new cases. All personnel were required to wear air-line equipment when entering the decanter house.

Measurements for identifying the cause of the fever were planned. The endotoxin concentration of the air was determined, and the workers' blood was tested for antibodies to legionellae. Later, after positive antibody titers to \textit{L pneumophila} were found, urine samples were collected for \textit{Legionella} urinary antigen tests, and sludge was submitted to legionellae culture.

Laboratory tests. For all 5 ill workers laboratory tests were performed, including tests for white blood cell count (WBC), sedimentation rate (SR) and C reactive protein (CRP). In addition, on suspicion of legionellosis, up to 6 blood samples were drawn from each of the ill workers over a period of 8—9 months. Only the results for 3 specimens from each worker are shown in table 1. One sample was drawn from each of the 2 workers without symptoms. Antibodies to legionellae were measured by the indirect immunofluorescence antibody test (IFAT), according to the guidelines of the Centers for Disease Control and Prevention (CDC) (7) with the following heat-killed bacterial antigens, as previously described (8): \textit{L pneumophila} (serogroups 1—6), \textit{L miedaei}, and \textit{L bozemani} serogroup (sg) 1. Furthermore antibodies to one of the \textit{L pneumophila} sg 1 isolates cultured from the sludge were measured. Urine samples were collected from each ill worker approximately 12 weeks after the onset of symptoms and analyzed for \textit{L pneumophila} soluble antigen with an in-house enzyme-linked immuno sorbent assay (ELISA) (9).
Endotoxin measurement at the workplace. Because endotoxin exposure was suspected as the cause of fever, measurements of the endotoxin concentration in the air were performed inside (ground floor and first floor) and outside the decanter house. The measurements took place on day 20, when the weather was still warm but the temperature had fallen (temperature 17.5°C outside and 19.0°C inside with a relative humidity of 70%). The specimens were sampled on a 0.8 μm membrane filter and analyzed for endotoxins by the Limulus amoebocyte lysate (LAL) test (10).

Isolation of legionellae from sludge. A sludge sample was collected from the decanter 83 days after the first exposure and cultured for legionellae. Three grams of the sludge sample was transferred to a measuring cylinder and sterile hard water (MgCl₂ 119 mg/l, CaCl₂ 277 mg/l, NaHCO₃ 280 mg/l) was added to a total volume of 30 ml. The sludge was suspended by vigorous shaking for 2 minutes. The suspension was diluted in sterile hard water to 10⁻². To 100 ml of this dilution, 900 ml of an acid buffer pH 2.2 (26 mM HCl, 173 mM KCl) was added, and the solution was subsequently incubated for 5 minutes at room temperature. Of this 0.1 ml suspension was inoculated on each of 2 GVPC (buffered charcoal-yeast extract agar) plates (11) and incubated for 7 days at 36°C. The number of Legionella colony-forming units (cfu) were counted on each plate.

Serogrouping of legionellae isolates. Subcultures from 4 legionellae-like colonies isolated from the sludge were serogrouped by the Oxoid Legionella Latex Test (Unipath Limited, Basingstoke, Hampshire, England). Three of the 4 isolates were further serogrouped and subgrouped by ELISA with the Dresden monoclonal antibody (MAb) panel to L pneumophila lipopolysaccharide (12).

Results

Laboratory tests. All the patients had an elevated white blood cell count, sedimentation rate, and CRP; all the test parameters had normalized after 8—10 days, however. The Legionella IFAT results for the serum samples from the 7 workers are shown in table 1. The serum samples from 5 patients (workers 1—5) showed a significant rise in antibody titers from ≤16 to titers ≥256 against the L pneumophila sg 1 routine antigen (strain Knoxville). Of the 2 workers with recurrent illness, worker 1 had an equivocal titer of 64 and worker 2 had a positive titer of 512 to the routine L pneumophila sg 1 antigen in their first blood samples. For both patients no significant changes in antibody levels were seen in later samples. All 5 patients (workers 1—5) had serum samples with antibody titers ≥256 to the environmental isolate. Although no general significant differences in the results with the 2 antigens were seen, serum samples from worker 5 showed significantly higher antibody titers to the isolate than to the routine antigen (table 1). Unfortunately, all the samples were not available for testing with the environmental isolate. Serum samples from worker 2 also showed reactions with L pneumophila sg 4 (titer 256) and serum from worker 4 also reacted with L pneumophila sg 3 (titer 128). No other cross-reactions were seen. The antibody titers for the unexposed and non-ill worker 6 were ≤16 for both antigens. However, the antibody levels to both antigens were elevated in serum from the exposed and non-ill worker 7 (table 1). Urine samples from all the patients (workers 1—5) were negative in the Legionella urinary antigen test.

The endotoxin concentrations in the decanter house were 4.4 ng/m³ on the ground floor, 7.3 ng/m³ on the first floor, where the decanters are placed, and ≤0.4 ng/m³ outside the decanter house.

Isolation and serogrouping of legionellae from the sludge sample. On the 2 inoculated plates, 167 and 140

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**Table 1.** Results of the indirect immunofluorescence antibody test for antibodies to legionellae in blood samples from 7 workers who had worked on the same work task at the sewage treatment plant. (SG1 = Legionella pneumophila serogroup 1 strain Knoxville, ENV = environmental isolate of Legionella pneumophila serogroup 1 subgroup ODA/Oxford, Ab = antibody, ND = not done)

| Number of worker | Onset of symptoms (day) | First blood sample | Second blood sample | Third blood sample |
|------------------|-------------------------|--------------------|---------------------|--------------------|
|                  | Sampling day            | SG1 Ab titers      | ENV Ab titers       | Sampling day       | SG1 Ab titers | ENV Ab titers | Sampling day | SG1 Ab titers | ENV Ab titers |
| 1                | 2—3 and 11              | 16                 | 64                  | ND                 | 65              | 128          | 256         | 99              | 128          | 512          |
| 2                | 3 and 11                | 16                 | 512                 | 1024               | 65              | 512          | 1024        | 85              | 512          | 512          |
| 3                | 11                      | 15                 | ≤16                 | ≤16                | 64              | 256          | 256         | 88              | 256          | 256          |
| 4                | 11                      | 15                 | ≤16                 | ≤16                | 64              | 128          | ND          | 86              | 256          | 256          |
| 5                | 11—12                   | 13                 | ≤16                 | ND                 | 71              | 64           | 256         | 94              | ≤32          | 256          |
| 6                | Not ill                 | 166                | ≤16                 | ≤16                | ND              | ND           | ND          | ND              | ND           | ND           |
| 7                | Not ill                 | 129                | 128                 | 84                 | ND              | ND           | ND          | ND              | ND           | ND           |

* Days for onset of symptoms and collection of the blood specimens are indicated in relation to the first exposure of workers 1 and 2 in the decanter house.

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colonies of legionellae were found (mean 154 cfu/plate). Thus the sludge suspension contained 1.5 x 10^8 cfu/ml and consequently the concentration of legionellae in the sludge equaled 1.5 x 10^7 cfu/g. All 4 investigated colonies were L pneumophila serogroup 1, as determined by the OXoid Latex Test. Three of the colonies were sero-
grouped and subgrouped by ELISA with the Dresden monoclonal antibodies. All 3 revealed the L pneumophila sg 1 monoclonal subgroup OLDA/Oxford.

A second sludge sample was collected from the decan-
ter 65 days after the first sample (in January 1998). It was not possible to isolate any legionellae from this sample. A check of the company log revealed that this sample contained sludge from the anaerobic digestion tanks. Subsequently, 2 samples of sewage from the presedimentation basin were collected, as aerobic sludge was not available. The first sample was collected in mid-
February and the second in mid-April of 1998. L pneumo-
phia was not detected in any of these samples. However low counts of L londiniensis were detected in the first sample (data not shown).

**Discussion**

The workers had been exposed to aerosols created by the centrifugation of sludge (workers 1-5 and 7) and aerosols created by high-pressure washing during the cleaning of the removed decanter (workers 1 and 2). The aerosol created during the high-pressure washing was probably not a source of infection in this outbreak, as 3 of the 6 workers became ill after exposure to the aerosol from the decanter only.

The workers were wearing B2-filter masks when ex-
posed. This type of mask does not protect against smaller particulate matter such as aerosols, but only against chemical substances that are absorbed on the surface. The workers wearing air-line equipment were not exposed to aerosols.

Worker 7 had used a filter mask with a B2 filter and had thus been exposed in short periods during the 10 days, and presumably heavily so on the last day, when he performed hard physical labor beside the decanter. Nevertheless, he developed no symptoms, but his Legionella antibody titer was elevated 120 days after the first exposure (table 1).

Our serological results correspond with a diagnosis of legionellosis (table 1). The incubation periods (23—46 hours), the clinical symptoms, and the high attack rate were all in accordance with those previously reported for Pontiac fever (1, 2, 13, 14). The duration of the illness (24—48 hours), however, was shorter than the 2 to 5 days normally reported (1, 2, 13, 14), but in an outbreak among 10 steam condenser cleaners all the patients recovered within 2 days (3).

L pneumophila sg 1 subgroup OLDA/Oxford was iso-
lated in high counts from a sludge sample from the de-
canter. The serological results, especially with serum from worker 5, indicate that this isolate was the etiologi-
ic agent.

Recurrent illness was observed for workers 1 and 2, who were reexposed. Recurrent illness is rarely reported for Pontiac fever; however it was also seen in the original outbreak of Pontiac fever in Michigan in 1968, but only in 14% of the employees who were reexposed (1). In a presumptive outbreak of Pontiac fever among steam condenser cleaners, recurrent illness was also reported for some of the workers (15). The 2 workers did not show seroconversion, probably because they had already sero-
converted at the time for the first blood sample (day 16 after the first exposure).

The presence of viable L pneumophila in the sludge and of L londiniensis in the presedimentation basin indicates that legionellae can grow in sewage, as also previously described (16), and it can be a potential occupational hazard for sewage workers. Legionella is an aerobic bacterium that proliferates in the temperature range from 22 to 42°C. The hot and humid summer period with a mean temperature of >20°C that preceded the outbreak of Pontiac fever may have caused favorable conditions for the proliferation of L pneumophila in the sewage plant.

We first suspected the outbreak to be due to expo-
sure to endotoxins. The measured concentrations were, however, 3—4 times lower than the level at which irri-
tation in the respiratory tract occurs and approximately 20 times below the level which can cause disease. Most of the workers experienced fever, chills, and chest tight-
ness (ie, the core symptoms in endotoxin fever). As the endotoxin level could have been higher at the time of the outbreak, we retested the workers 2—3 weeks before the measurements, and endotoxin fever cannot be ruled out completely. However, it is not likely that the concentration should have been at least 20 times higher. Although L pneumophila possesses lipopolysaccharide (LPS), which is reactive in the Limu-
lus amoebocyte lysate test, the endotoxic potential of the lipopolysaccharide in L pneumophila is low (17), and probably not the cause of the symptoms seen in Pontiac fever. The capacity of other constituents of legionellae to induce endotoxin-fever-like symptoms must still be investigated. In a murine model it has been shown that co-inhalation of amoebae and L pneumophila enhanced the intrapulmonary levels of gamma interferon and tu-
mor necrosis factor alpha (18). These 2 cytokins are well

known for their fever-inducing capacity and could, at least in part, be the explanation of nonpneumonic legi-
onellosis (Pontiac fever) in immunocompetent persons after environmental exposure to legionellae in associa-
tion with amoebae (personal communication, Birgid Neu-
meister, Universität Tübingen, Germany).
As the serological results and the finding of L. pneumophila in the sludge corroborate with legionellosis, we conclude that the diagnosis of Pontiac fever is most likely.

To prevent new cases the decanter has been enclosed, room ventilation has been established, and the workers are required to wear air-line equipment inside the decanter house.

Acknowledgments
We thank Dr T Harrison and Dr N K Fry of the Respiratory and Systemic Infection Laboratory, PHLS Central Public Health Laboratory, London, for their identification of the species L. londiniensis.

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