

Pontiac Fever Due to *Legionella micdadei* from a Whirlpool Spa: Possible Role of Bacterial Endotoxin

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During January 1998, a cluster of illnesses occurred among hotel guests in Wisconsin. Ill persons had been exposed to the hotel's whirlpool spa and swimming pool. Symptoms included headache, fever, chills, myalgia, shortness of breath, and fatigue. A diagnosis of Pontiac fever was made, based on serologic evidence of acute infection with *Legionella micdadei*. High concentrations of heterotrophic bacteria were recovered from the spa, despite apparently high disinfectant levels. *L. micdadei* was isolated from the swimming pool filter and water from the spa after heat enrichment but not from pools and spas at nearby hotels. Water from hotel pools and spas was tested to determine endotoxin levels; water from the spa of the implicated hotel contained the highest concentration of endotoxin (14,400 endotoxin units/mL). Additional studies are needed to determine the role of endotoxin from legionellae or other bacteria in the pathogenesis of Pontiac fever.

Pontiac fever is an acute, self-limited form of legionellosis characterized by fever, chills, myalgia, and headache [1]. Transmission of Pontiac fever is thought to occur after inhalation of aerosolized water colonized with legionellae, and disease has been associated with 4 *Legionella* species [1–4]. Epidemiologically, Pontiac fever differs from legionnaires' disease, the more common and serious form of legionellosis, in the characteristic short incubation period (usually 6–48 h), high attack rate, and absence of mortality or long-term complications associated with the former. The pathogenesis of Pontiac fever is poorly understood, and why exposure to legionellae may result in these 2 clinically and epidemiologically distinct syndromes is not known. One theory is that Pontiac fever is caused by hypersensitivity to a cellular component of either legionellae or the protozoan hosts of the bacteria [5]. Investigation of a 1992 outbreak associated with a whirlpool spa suggested that live *Legionella pneumophila* in the spa filter were killed on exposure to biocides in the whirlpool tub, and exposure to high concentrations of dead *L. pneumophila* aerosolized from the spa tub resulted in the disease [6]. Here we describe an outbreak of Pontiac fever associated with a whirlpool spa, in which water samples were tested for total heterotrophic bacteria, legionellae, and endotoxin levels.

Methods

Background. In January 1998, the Wisconsin Division of Public Health was notified by a hotel guest of a cluster of illnesses among guests who stayed at a particular hotel (hotel A) during the weekend of 23–25 January 1998. Symptoms of the illness included headache, fever, chills, myalgia, shortness of breath, and fatigue. Participants and attendees of an ice hockey tournament were the only guests at hotel A during 23–25 January 1998. Hotel guests represented members of 38 families.

Case-control study. A standardized questionnaire was administered to each hotel guest. Data collected included signs and symptoms of illness, history of illness before arriving at the hotel, whirlpool and swimming pool use, shower history, and attendance at a party associated with the tournament. Non-ill guests served as unmatched control subjects for the case patients.

Case definitions. A case of Pontiac fever was defined as an illness in a person who stayed at hotel A for ≥ 1 night during 23–25 January who experienced 4 of 5 symptoms (fever, chills, fatigue, headache, and myalgia) within 3 days of arrival at the hotel. Cases were further categorized as “definite” by a ≥ 4 -fold rise in antibody to *Legionella micdadei* between acute-phase and convalescent-phase serum samples, to a titer of $\geq 1:128$; “probable” by a < 4 -fold rise, to a titer of $\geq 1:128$; and “possible” if the antibody titer was $< 1:128$ or if serum samples were not submitted for testing.

Environmental and laboratory testing. On 28 January, the whirlpool spa and swimming pool at hotel A were inspected; water samples were obtained from both pools and were cultured at the Wisconsin State Laboratory of Hygiene (WSLH), to test for total heterotrophic bacteria and legionellae, by standard methods [7]. On 3 March, a sand and water mixture was collected from the filters of both the swimming pool and the whirlpool at hotel A for use in culture. On 18 April, water samples were collected from hotel A and 3 comparison hotels (B–D) within a 10-mile radius of hotel A. Stored aliquots of water collected from hotel A in January and

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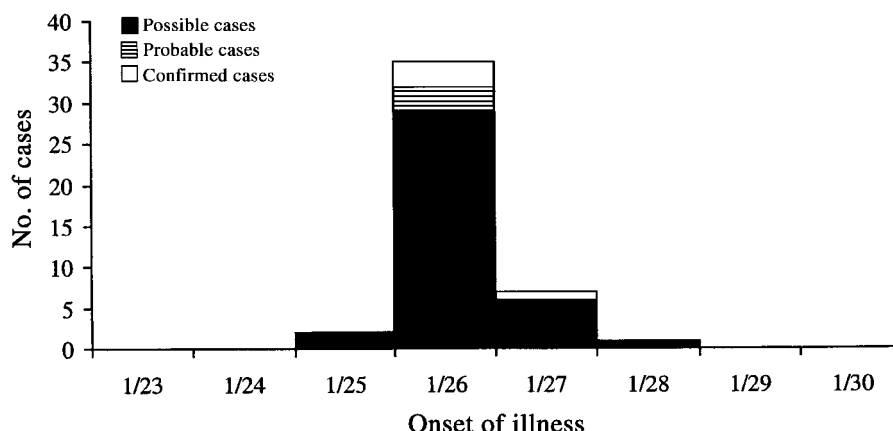


Figure 1. No. of confirmed, probable, and possible cases of Pontiac fever, by date of onset of illness (month/day), among guests who stayed at Wisconsin hotel A during 23–25 January 1998.

March and fresh specimens collected from hotels A and D in April also were sent to the Centers for Disease Control and Prevention (CDC) and tested for legionellae by standard methods and by “heat enrichment” weekly culture of water samples at 35°C [7, 8].

The endotoxin activity of water samples was determined by the *Limulus* Amebocyte Lysate (LAL) turbidimetric assay and Pyros software (version 1.5; Associates of Cape Cod). Serial dilutions (undiluted, 1:10, 1:100, and 1:1000) were prepared in LAL reagent (endotoxin-free) water. Standards (0.0313, 0.0625, 0.125, 0.25, 0.5, 1.0, and 2 ng/mL) prepared from *Escherichia coli* O113:H10 and control standard endotoxin lot 71 (Associates of Cape Cod) were included in each run to generate a standard curve [9]. Results are reported in endotoxin units (EU)/mL (activity) as opposed to weight (ng/mL or mg/mL); the conversion factor is 13.8 EU/ng.

Blood samples were obtained from 12 case patients during the acute phase of illness. A second blood sample was obtained from 11 case patients 3–4 weeks later. The paired serum samples were tested at the WSLH by indirect fluorescent antibody assay (IFA) for antibody specific to *L. pneumophila* serogroups 1–6, influenza A and B viruses, parainfluenza virus types 1–3, adenovirus, and respiratory syncytial virus. Throat swab specimens were obtained for virus culture from 8 of the 12 persons and tested at the WSLH for influenza A and B viruses, parainfluenza virus types 1–3, adenoviruses, and respiratory syncytial virus. Urine samples were collected from 5 case patients and tested by EIA (Binax) for *L. pneumophila* antigen at the Bureau of Laboratories, City of Milwaukee Health Department (Milwaukee, WI).

Serum samples were tested at the CDC by IFA for antibody to *L. micdadei*. The antigen was prepared using the strain of *L. micdadei* isolated from the hotel A whirlpool in a suspension of 0.6% formalin/saline. Serum samples were tested for combined IgG, IgA, and IgM to *L. micdadei* by standard IFA procedures, as described elsewhere [10].

Analytic and statistical methods. We used Epi Info software (version 6.4; CDC) to determine attack rates by exposure to the whirlpool, the swimming pool, and the adjacent area and the odds ratio of illness with 95% confidence interval.

Results

Case findings and case-control study. Questionnaires were returned by 77 hotel A guests; 45 (58%) reported illness that met the case definition. Of 12 case patients for whom ≥ 1 serum specimen was available, 4 had definite and 3 had probable Pontiac fever; the remaining 38 case patients had possible Pontiac fever. All 11 paired serum samples had negative results of testing for respiratory viruses and *L. pneumophila* serogroups 1–6. All throat swab specimens were culture negative for influenza A and B viruses, parainfluenza virus types 1–3, adenovirus, and respiratory syncytial virus. All urine samples had negative results of testing for *L. pneumophila* antigen.

Attack rates were 66% among 68 persons who went into the whirlpool/swimming pool area and 71% among 49 persons who used the whirlpool spa during their stay. None of the 9 persons who did not go into the whirlpool/swimming pool area became ill. The majority of illnesses began on 26 January, suggesting a point-source exposure (figure 1). The incubation period for the illness was estimated to be 9–67 h (mean, 38 h). Reported symptoms and signs included headache (100%), myalgia (100%), fatigue (98%), chills (89%), fever (86%), cough (62%), nausea (46%), sore throat (36%), and shortness of breath (33%).

Whirlpool use, swimming pool use, and exposure to the whirlpool/swimming pool area were each more common among case patients than among control subjects (table 1). Stratified analysis did not distinguish a differential risk among the 3 risk factors.

Environmental and serologic laboratory testing. Bromine was continuously added to both the swimming pool and whirlpool spa for disinfection, with occasional shock chlorination; the most recent additions of chlorine had been made on 23 and 25 January. Bromine concentrations in water collected on 28 January were 13 ppm in the swimming pool and 17 ppm in the spa. These concentrations and bromine concentrations measured by hotel staff over the weekend of 23–25 January exceeded the minimum

Table 1. Association of whirlpool spa use, swimming pool use, and exposure to the whirlpool spa/swimming pool area with illness, Wisconsin, January 1998.

Exposure	Odds ratio	95% CI	P
Used whirlpool spa	4.5	1.5–13.9	.0048
Used swimming pool	3.5	1.1–11.1	.024
Entered spa/pool area	Undefined	3.4 ^a	.00017

^a Odds ratio and upper bound of 95% confidence interval (CI) are undefined because 1 cell of the 2 × 2 contingency table contained 0.

bromine concentration of 5 ppm in whirlpools and 3 ppm in swimming pools recommended in Wisconsin.

All water samples had negative results of standard culture for legionellae [7]. Samples from the whirlpool water obtained from hotel A on January 28 contained 90 cfu/mL of *L. micdadei* after heat enrichment. The sand and water mixture collected on 3 March from the filter of the swimming pool contained 150 cfu/mL of *L. micdadei* after heat enrichment (table 2). The hotel A whirlpool spa water collected on 28 January had an endotoxin concentration >4-fold higher than that of any of the other samples tested (14,400 EU/mL; table 2). The 2 samples from which *L. micdadei* was recovered had the highest endotoxin levels. Endotoxin concentrations were measured sequentially during heat enrichment, and concentrations changed little during incubation (data not shown).

Discussion

Evidence that illness in this outbreak was Pontiac fever acquired from a whirlpool spa includes the epidemiologic link to exposure to the spa, the presence of *L. micdadei* in the spa water, laboratory evidence of recent exposure to *L. micdadei* among ill hotel guests, the absence of laboratory evidence of recent infection with other pathogens, and the lack of other common exposures among ill guests during 23–25 January 1998. Of 11 ill hotel guests from whom paired serum samples were obtained,

4 (36%) had a ≥4-fold rise in *L. micdadei* antibodies between acute- and convalescent-phase serum samples, reflecting recent exposure to the organism. Of the remaining 8 patients for whom ≥1 serum specimen was available for testing, 3 (38%) had elevated levels of antibody (≥1:128). By comparison, antibody levels ≥1:64 are found in only 1.5%–8% of healthy adults [11, 12]. *L. micdadei* has been implicated in ≥3 outbreaks of Pontiac fever associated with whirlpool spas [2, 13, 14].

Although live legionellae were recovered from environmental sources in previous outbreaks of Pontiac fever, this does not preclude the possibility that numerous dead bacteria were also present in these settings. If nonviable legionellae or cellular components of killed legionellae are capable of causing Pontiac fever, outbreaks may be most likely when many bacteria are present and disinfectant levels are high enough to kill numerous organisms. The spa in this outbreak was in heavy use during 23–25 January 1998 and had been shock-chlorinated on 23 and 25 January. However, even the additional disinfectant did not completely eliminate heterotrophic bacteria from water sample cultures, suggesting that extremely high concentrations of both viable and killed bacteria were present in the water (table 2). Heat enrichment of water samples collected on 28 January from the whirlpool and on 3 March from the sand and water mixture of the swimming pool filter aided in the isolation of *L. micdadei*. Many water samples contain protozoa that can serve as hosts for intracellular multiplication of legionellae. Incubation of aliquots of water allowed remaining viable legionellae to multiply in these protozoa to a concentration that could be detected by culture. Endotoxin levels did not appear to correlate directly with the number of *L. micdadei* recovered; however, the number of colonies in the aliquots of water that were heat-enriched does not accurately reflect the actual number of legionellae in water at the time of exposure or the concentration of killed organisms.

To our knowledge, this is the first investigation of Pontiac fever to examine a potential role of environmental endotoxin in the

Table 2. Results of testing of water samples from hotels A, B, C, and D and the city well for total bacteria, legionellae, and endotoxin, Wisconsin, 1998.

Water source	Date collected, month/day	Total heterotrophic bacteria, cfu/mL	Legionellae, cfu/mL	Endotoxin, EU/mL
Hotel A				
Swimming pool	1/28	8900	0 ^a	494 ^a
Whirlpool spa	1/28	68,000,000	90 ^a	14,400 ^a
Swimming pool filter	3/3	153,000,000	150 ^a	3610 ^a
Sand from spa filter	3/3	175,000,000	0 ^a	3440 ^a
Whirlpool spa	4/18	98	0 ^a	106 ^a
Spa water heater	4/18	50	0	147
Swimming pool	4/18	<1	0	28
Pool heater	4/18	<1	0	ND
City well	4/18	59	0	0.16
Hotel B: swimming pool	4/18	2	0	101
Hotel C: swimming pool	4/18	8200	0	473
Hotel D				
Whirlpool spa	4/18	600	0 ^a	17.8 ^a
Swimming pool	4/18	7	0 ^a	2195 ^a

NOTE. EU, endotoxin units; ND, not determined.

^a Results after 6 weeks of heat enrichment.

disease. Water from the whirlpool spa at hotel A contained more endotoxin than samples from other sites in the hotel or from swimming pool and spa samples collected at nearby hotels. This finding suggests that endotoxin may play some role in the pathogenesis of Pontiac fever. There are 2 possible mechanisms. First, high levels of endotoxin from either live or dead legionellae may be responsible for this syndrome. *L. pneumophila* lipopolysaccharide (LPS) is highly reactive in the LAL and Schwartzman reaction in rabbits and mice, although it has low potential for interaction with the CD14 receptor of human monocytic cells [15]. The second possibility is that LPS of aquatic bacteria other than legionellae that have similar environmental growth requirements and are growing in concert with legionellae may be the cause of Pontiac fever outbreaks.

Pontiac fever shares many clinical characteristics with acute hypersensitivity pneumonitis and inhalation fevers (e.g., organic dust toxic syndrome) [16, 17]. One outbreak of chronic hypersensitivity pneumonitis has been linked to recurrent exposures to bacterial endotoxin in aerosolized swimming pool water [18]. Legionellae were not recovered from water samples in that investigation, but amoebae appearing to be *Hartmannella* species, a common host for legionellae, were identified in pool water. Use of heat enrichment to enhance recovery of legionellae was not reported. Inhalation fever after exposure to aerosols containing endotoxin has also been reported [17, 19, 20].

In an investigation of workers who developed illness after exposure to aerosols produced by an industrial washer, high levels of endotoxin were detected in recirculated wash water, but no legionellae were recovered [17]. However, a notable difference between Pontiac fever and hypersensitivity pneumonitis/inhalation fevers is the lack of reported recurrent illness after reexposure for persons with Pontiac fever. In addition, the prevalence of antibodies to *L. pneumophila* has not been found to be higher among persons with hypersensitivity pneumonitis; conversely, antibodies to thermophilic organisms known to cause hypersensitivity pneumonitis have not been detected among patients with Pontiac fever [21]. Clearly, additional studies are needed to determine possible overlap between hypersensitivity pneumonitis/inhalation fevers and Pontiac fever and to better define the role of endotoxin in each of these diseases.

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