Legionnaires’ Disease on a Cruise Ship Linked to the Water Supply System: Clinical and Public Health Implications

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The occurrence of legionnaires’ disease has been described previously in passengers of cruise ships, but determination of the source has been rare. A 67-year-old, male cigarette smoker with heart disease contracted legionnaires’ disease during a cruise in September 1995 and died 9 days after disembarking. Legionella pneumophila serogroup 1 was isolated from the patient’s sputum and the ship’s water supply. Samples from the air-conditioning system were negative. L. pneumophila serogroup 1 isolates from the water supply matched the patient’s isolate, by both monoclonal antibody subtyping and genomic fingerprinting. None of 116 crew members had significant antibody titers to L. pneumophila serogroup 1. One clinically suspected case of legionnaires’ disease and one confirmed case were subsequently diagnosed among passengers cruising on the same ship in November 1995 and October 1996, respectively. This is the first documented evidence of the involvement of a water supply system in the transmission of legionella infection on ships. These cases were identified because of the presence of a unique international system of surveillance and collaboration between public health authorities.

Legionnaires’ disease is a severe pneumonia that has been described as occurring in passengers of cruise ships [1–7]. Morbidity is substantial and fatalities have occurred, often because of the delay in identification of the pathogen. It is difficult to establish a link between the disease and a presumptive site of infection because environmental investigation and the presence of clinical and environmental Legionella isolates are required. This link has been exhaustively documented in only one outbreak involving passengers of cruise ships [7]. However, legionellae have been found in water and air-conditioning systems of ships where associated legionellosis was not evident [8, 9].

Materials and Methods

Epidemiological Investigation

On 1 November 1995, in the context of the European surveillance of legionnaires’ disease associated with travel [10], a fatal case of legionnaires’ disease in a British passenger on an Italian cruise ship was reported to the Italian representative of the European Working Group on Legionella Infection (EWGLI) at the Central Laboratory for Legionellosis (Istituto Superiore di Sanità, Rome, Italy) by the coordinator of the EWGLI at the Communicable Disease Surveillance Centre (CDSC), Public Health Laboratory Service (PHLS), London, UK. The ship cruised in the Mediterranean Sea from 1 to 11 September, and the cruise started and ended in Genoa.

On 16 November, the Italian Ministry of Health reported the case to the local health authorities, and, on 23 November, public health officials of the Regional Epidemiologic Center met the ship in Genoa to perform environmental and epidemiological investigations. The ship was kept in the port to allow collection of samples. The ship’s board-hospital records were checked to detect other cases of respiratory symptoms among passengers and crew members from the 3 months preceding the reported case to the date of sampling. A questionnaire was also submitted to 116 randomly selected crew members, which included questions about fever, respiratory symptoms, cough,
diarrhea, and the period they had been working on the ship. In addition, a serological investigation for antibody titers to *L. pneumophila* serogroup 1 was performed for these subjects. However, since the ship was leaving the port to cruise in warmer seas during winter when the environmental investigation was performed, blood samples were collected when the ship returned 5 months later.

**Environmental Sampling**

Cold and warm water and incrustations were collected from taps and showers at different sites on the ship. Specimens were also obtained from the saltwater whirlpool spa and from material inside the climatization system. Samples were sent to the Central Laboratory for Legionellosis in Rome for evaluation.

**Laboratory Methods**

The sputum isolate was sent from the Respiratory and Systemic Infection Laboratory, PHTL, London, to the Central Laboratory for Legionellosis in Rome. Water was concentrated by filtration (Isopore membrane, 0.2 μm pore size; Millipore, Italy). Incrustations and other materials were ground in a mortar and vortexed for about 2 minutes with 1–2 mL of sterile distilled water. Diluted and undiluted specimens were plated on buffered charcoal yeast extract (BCYE) non-selective and glycine-vancomycin-polymyxin B-cycloheximide (GVPC) selective agar (Oxoid, Italy), with and without heat pretreatment, following the usual procedures for the isolation of *Legionella* species. Specimens were also observed by direct immunofluorescence assay (DFA) after staining with *L. pneumophila* serogroups 1 to 6 polyvalent and serogroup 1 monovalent fluorescein-labelled antisera (SCIMEDX, distributed by Dasit, Italy; and BIOS, distributed by Dalton, Italy). Identification of *Legionella* isolates was preliminarily performed using the Legionella latex test kit (Oxoid, Italy). Strains belonging to the *L. pneumophila* species were then confirmed by DFA with a species-specific monoclonal antibody (MAb) (Diagnostics Pasteur, France) and with the above-mentioned polyclonal reagents (SCIMEDX and BIOS). MAbs (kindly supplied by J. H. Helbig, Institut für Medizinische Mikrobiologie und Hygiene, Dresden, Germany) were also used with indirect immunofluorescence (IFA) to confirm the identification at the serogroup (1 to 14) level [11]. Strains that reacted with the *Legionella non-pneumophila* latex reagent were further assessed with polyvalent and monovalent fluorescent antisera (BIOS), examined for blue-white autofluorescence, and analyzed for fatty acid profile by use of gas-liquid chromatography [12]. To show the link between clinical and environmental *L. pneumophila* serogroup 1 isolates, strains were further subtyped using the Dresden MAbs 3/1 and 26/1, which recognize the subtypes Pontiac and Olda, respectively ([13] and J. H. Helbig, personal communication), and analyzed by use of pulsed-field gel electrophoresis (PFGE) of genomic DNA digested with *NotI* and *SfiI* restriction enzymes [14], and by arbitrarily primed PCR (AP-PCR) with six single non-specific primers [15].

**Results**

**Epidemiological Investigation**

The ship’s capacity was 1,400 passengers and 480 crew members, but during the September 1995 cruise only 900 passengers and 300 crew members were on board. None of the 116 crew members randomly enrolled in the serological study 5 months later had had respiratory symptoms or a flu-like illness during the preceding 8 months. Fifty-six of them were on board when the case occurred. Antibody titers to *L. pneumophila* serogroup 1 were very low, ranging from <16 to 64. Titers of 64 were detected in only 3 subjects (2.6%), who had stayed on board during the September 1995 cruise.

The ship was built in 1966 and had been used for cruises in the Mediterranean, Caribbean, and South Atlantic seas during warm seasons until the end of 1996. The 67-year-old passenger was a cigarette smoker and had had a coronary bypass in 1985. He went on the cruise with his wife, who remained in good health during the cruise and thereafter. During the cruise they landed at different places for day excursions, but they never stayed ashore overnight.

No other confirmed case of legionnaires’ disease from this ship was reported in 1995, nor had cases associated presumptively with a stay on this ship been reported to the EWGLI from 1986 (when the surveillance program was established) to 1995. A 65-year-old male passenger on the subsequent cruise, 22 November to 12 December 1995, was examined by the ship’s medical officer on 30 November when he presented with vomiting, diarrhea, and fever. He then developed a pulmonary illness, but there was no evidence of a legionella infection by serological evaluation of two specimens obtained 2 weeks apart. A urinary antigen test was not performed. The patient stated that he had heard of several other individuals among the cruise passengers who had similar illnesses. Another case of legionnaires’ disease was diagnosed by use of a urinary antigen test (*L. pneumophila* serogroup 1) and seroconversion in a 53-year-old man who had been a passenger on the same ship from 12 to 22 October 1996. The patient was hospitalized on 30 October for severe pneumonia, and he developed renal problems as a result of the infection. Predisposing factors for this patient are unknown, and cultures of clinical specimens did not yield *Legionella* isolates for comparison with the 1995 strains.

The two patients for whom legionnaires’ disease was confirmed and the patient for whom the diagnosis was presumptive were staying in different cabins on the ship.
Table 1. Isolation and characterization of clinical and environmental Legionella strains associated with the cruise ship.

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Specimen</th>
<th>Legionella (cfu/L)</th>
<th>Legionella isolates</th>
<th>MAb subtype</th>
<th>Genomic type¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient (from cabin B)</td>
<td>Sputum</td>
<td></td>
<td>Lp1</td>
<td></td>
<td>Pontiac A</td>
</tr>
<tr>
<td>Cabin A</td>
<td>Tap water</td>
<td>$7.1 \times 10^7$</td>
<td>Lp2, Legionella species¹</td>
<td>Lp5, L. longbeachae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shower head incrustations</td>
<td></td>
<td>Lp1, Lp3, Legionella species¹</td>
<td>Pontiac A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tap incrustations</td>
<td></td>
<td>Lp2, Legionella species¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cabin B</td>
<td>Shower water</td>
<td>$3.5 \times 10^3$</td>
<td>Lp1</td>
<td></td>
<td>Pontiac A</td>
</tr>
<tr>
<td></td>
<td>Shower head incrustations</td>
<td></td>
<td>Lp1, Lp2, L. longbeachae, Legionella species³</td>
<td>Pontiac A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tap incrustations</td>
<td></td>
<td>Lp3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Material from outlet of air-conditioner</td>
<td></td>
<td>Lp3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Board hospital</td>
<td>Tap 1 water (cold)</td>
<td>$3.0 \times 10^5$</td>
<td>Lp1, Lp2</td>
<td></td>
<td>Pontiac A</td>
</tr>
<tr>
<td></td>
<td>Tap 1 incrustations</td>
<td></td>
<td>L. longbeachae, Legionella species¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tap 2 water (warm)</td>
<td>$1.6 \times 10^3$</td>
<td>Lp1, Lp2, L. longbeachae, Legionella species³</td>
<td>Pontiac A</td>
<td></td>
</tr>
<tr>
<td>Air-conditioning station</td>
<td>Incrustations from external air inlet and filter 1</td>
<td></td>
<td>Not isolated</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Incrustations from internal air inlet and filter 2</td>
<td></td>
<td>Not isolated</td>
<td>Pontiac A</td>
<td></td>
</tr>
<tr>
<td>Whirlpool spa</td>
<td>Marine water</td>
<td></td>
<td>Not isolated</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Lp = L. pneumophila; MAb = monoclonal antibody.
¹ Legionella species = Legionella non-pneumophila (species not identified).
² Obtained by combining PFGE and AP-PCR results.
³ Culture-negative specimens were also negative at the DFA test.

Environmental Investigation

Of 14 samples of water and materials collected from different sites on the ship, cultures of 10 samples yielded legionellae (table 1). Specimens for which cultures yielded no isolates were also negative by use of DFA staining. Samples from the ship’s freshwater system showed a legionellae concentration of $3 \times 10^2$ to $7 \times 10^3$ cfu/L. The free residual chlorine concentration was not measured at the time of water sampling. Legionellae were not isolated from the whirlpool spa. The isolates were identified as L. pneumophila of different serogroups, as Legionella longbeachae, or remained unidentified (although they demonstrated a typical Legionella fatty acid composition). L. pneumophila serogroup 1 was isolated from all the samples obtained from sites of the ship’s water system.

L. pneumophila Serogroup 1 Subtyping

L. pneumophila serogroup 1 strains matching the patient’s isolate either antigenically (belonging to the monoclonal subtype Pontiac) or by their genomic fingerprints (PFGE and AP-PCR) were isolated from various sites of the ship’s water supply system (table 1 and figure 1). It is of interest that the water sampled from different outlets in the cabin where the patient had stayed yielded two types of L. pneumophila serogroup 1; one matched the patient’s isolate (lanes 3, figure 1), and the other belonged to a different monoclonal subtype (Olda) and showed a different genomic pattern (lanes 2, figure 1).

Discussion

Legionnaires’ disease has been linked to passenger-ship cruises in several reports [1–6], but the source of the organism was clearly demonstrated in only one outbreak [7]. In the present case, we established that the source of the organism was the ship’s freshwater supply. In fact, cultures from the whirlpool spa and the air-conditioning system were negative, whereas cultures of 80% of water samples obtained from different sites yielded L. pneumophila serogroup 1. The isolate from the patient was identical to the water supply isolates after analysis by monoclonal subtyping and two genomic fingerprinting methods (PFGE and AP-PCR).

The precise mode of transmission was uncertain, since the organism could not be isolated from mechanical aerosol-generating devices. Aspiration is a possible mode of transmission [16], given that aerosol transmission generally leads to outbreaks, whereas aspiration occurs in selected individuals with the following risk factors: elderly age, cigarette smoking, and underlying disease. Despite case-finding among 1,200 individuals (900 passengers and 300 crew members) potentially exposed to the infection during the 10-day cruise in September 1995, only one contracted the disease, and this individual presented with major predisposing factors.

This case has relevant clinical implications for physicians. First, it has been well established that Legionella species are often overlooked as a cause of community-acquired pneumonia [17]. Thus, physicians, especially board medical officers, should always consider this diagnosis. In addition, community
Figure 1. Genotyping of *Legionella pneumophila* serogroup 1 strains. (A) the macrorestriction analysis of chromosomal DNA digested with *SfiI* and *NotI*, and separated by pulsed-field gel electrophoresis (PFGE). (B) the arbitrarily primed PCR (AP-PCR) patterns generated with six oligonucleotides designated AP5 (5'-TCCGCTGCCG-3'), AP12 (5'-CGGCCCCTGC-3'), CD1 (5'-GGATCTGAC-3'), 1247 (5'-AAGAGCCCGT-3'), 1253 (5'-GTTTCCGCC-3'), and 1283 (5'-GCGATCCCCA-3'). Lane 1, isolate from cabin A (tap incrustations); lane 2, isolate from cabin B (tap water); lane 3, isolate from cabin B (shower water); lane 4, isolate from the board infirmary (warm water from tap 2); lane 5, isolate from the patient who stayed in cabin B during the September 1995 cruise. Lanes *M*, *M'* and *M''* are DNA size markers, as indicated on the right side of each electrophoreogram.

physicians should always inquire about recent travel as part of their patient’s history for an accurate anamnesis.

Second, since detection of *L. pneumophila* requires special laboratory methods, such methods should be made more widely available to community physicians [18]. Our case would not have been diagnosed had the culture not been sent to an appropriately equipped laboratory. Recent travel history should therefore stimulate the application of specialized tests for legionnaires’ disease.

Third, this investigation demonstrates the power of combining molecular methodologies with epidemiologic investigation. In our study the combination of antigenic and genomic typing methods provided consistent evidence that the ship’s freshwater system, which was contaminated by *L. pneumophila* serogroup 1, was the
source of the infection. The total concentration of \textit{Legionella} species in water specimens obtained from the ship’s system ranged from $3 \times 10^2$ to $7 \times 10^4$ cfu/L, a level that is believed to cause one case per year in susceptible people [19]. It should, however, be considered that for all but one specimen the total count included more than one serogroup or species of legionellae. Only water obtained from the shower in cabin B yielded one single species and serogroup (i.e., \textit{L. pneumophila} serogroup 1, $3.5 \times 10^3$ cfu/L), which was also isolated from the patient.

Finally, the results of this investigation have broad public health implications. The benefits of a surveillance scheme for travel-associated legionnaires’ disease [10] and an international laboratory collaboration within the EWGLI allowed the correct diagnosis to be made and the source of the organism to be discovered. Such international cooperation is uncommon but has obvious public health benefits for a society in which worldwide travel is commonplace.

Although maintenance of water supplies and adherence to sanitation standards are uniformly advocated for prevention of legionnaires’ disease, such approaches are not always useful in minimizing \textit{Legionella} contamination of water supplies [20]. Since outbreaks of legionnaires’ disease are highly disruptive and prohibitively expensive—given the intense negative media exposure, the panic among passengers, and subsequent litigation—prevention of such outbreaks would be ideal.

Given the fact that the freshwater supply was implicated as the source of infection in this cruise ship as has been the case for hospitals and hotels, the application of preventive measures is feasible. Water brought on board from different sources during the ship’s cruise is a risk factor that is difficult to control. Cruise-ship companies must be aware of the risk of legionnaires’ disease associated with the presence of \textit{L. pneumophila} in ships’ freshwater supplies. Cultures of water specimens that are positive for \textit{Legionella} species would raise the index of suspicion among cruise ships’ medical personnel who encounter patients with respiratory symptoms. Earlier diagnosis combined with appropriate antibiotic therapy could be expedited. Because \textit{Legionella} species can often be isolated from the freshwater supply of ships [3, 8, 9], it is likely that cases of legionnaires’ disease that are now overlooked might be uncovered and treated successfully. A scientific evaluation of disinfection measures successfully applied to large-volume water systems [21–23] should be considered to determine their efficacy when applied to the smaller water systems of ships. International guidelines or recommendations [24, 25] for the safety and proper maintenance of ships’ water systems are needed.

Acknowledgments

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