Short Communication

Bathwater-Associated Cases of Legionellosis in Japan, with a Special Focus on Legionella Concentrations in Water

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SUMMARY: To evaluate the relationship between the incidence of legionellosis and Legionella concentrations in bathwater, we sent a questionnaire to 76 prefectural and municipal public health laboratories in Japan and found that 35 had encountered cases of legionellosis and had implemented investigations to determine the sources of the infections. Based on the results of the questionnaire, we were able to analyze various characteristics of the patients, of the facilities that were thought to be associated with the cases, and of the species and serogroups of the isolates and concentrations of Legionella. Ninety-six cases were included in this study. The median age was 67 years (range, 13 - 89 years). The most prevalent underlying medical condition among patients was diabetes, and the second most prevalent was high blood pressure. Concentrations of Legionella in bathwater ranged from 10 to 160,000 CFU/100 ml. Ten episodes were selected in which causative strains were found in the suspected source environment, and were then confirmed by pulsed-field gel electrophoresis analysis, enabling us to provide an estimated infectious concentration range of Legionella of 90 to 140,000 CFU/100 ml. It was thus suggested that the current Japanese regulatory safety level for Legionella in bathwater, which is set below the detection limit of culture techniques (10 CFU/100 ml), should be appropriate to prevent bathwater-associated legionellosis. In tandem with the above-mentioned research, a review of literature concerning bathwater-associated legionellosis and typical cases was undertaken.

Legionellae are the aetiologic agents of legionellosis caused by the inhalation of aerosols or the microaspiration of water contaminated with the bacteria, and they are ubiquitous in not only natural but also man-made water systems, such as cooling towers, spas, artificial fountains, and drinking water supply systems (1). Legionellosis has been classified as one of the Category IV notifiable infectious diseases in the Law Concerning the Prevention of Infectious Diseases and Medical Care for Patients of Infections (the Infectious Diseases Control Law) in Japan. Since the Infectious Diseases Control Law was enacted in April of 1999, the number of reported legionellosis cases in Japan each year has steadily increased from 56 (in 1999) to 668 (in 2007). Many of the cases of legionellosis in which an infection source was established have been associated with bathing facilities in Japan, suggesting that the infection occurs via the inhalation of aerosols proceeding from bathwater (2-4).

The risk of infection apparently depends on many complex factors, among which are: the susceptibility of patients to illness in general; water-system configurations; disinfection methods; the concentration and virulence of the bacteria in the water; the number and size of the sloughed off pieces of biofilm containing the bacteria that are suspended in the water; and the frequency of diagnostic testing for legionellosis among patients (5). Among these factors, the concentration of the bacteria in the water is certainly one of the most critical influences upon community-acquired legionellosis, as, in large outbreaks of bathwater-associated legionellosis in Japan, high concentrations of the bacteria in bathwater have been reported (3,6-8). However, there is no established dose–response relationship for Legionella infections, and the concentration of legionellae necessary to cause an outbreak is unknown (9).

In this study, data on patients and environmental investigations from 76 prefectural and municipal public health laboratories in Japan and generated between October of 2001 and June of 2007 were collected via a questionnaire. All of these cases were either thought or proven to be bathwater-associated legionellosis. With reference to the guidelines for surveillance, several of the data acquired from questionnaires were confirmed by the National Epidemiological Surveillance of Infectious Diseases. The information collected included the age and gender of the patients; date of onset; latent period; symptoms; underlying medical conditions; testing methods for diagnosis; species and serogroups of Legionella from patient and suspected source environment; suspected source facilities; concentrations of Legionella in suspected source environment; chlorination of bathwater; suspected routes of infection; and results of pulsed-field gel electrophoresis (PFGE) analysis for the Legionella isolates.

Thirty-five public health laboratories investigated 92 sporadic and 4 outbreak cases of legionellosis to determine the sources and routes of infection. The median age of the cases was 67 (age range of 13 - 89 years; mean of 64.8), with 84 males (88.4%) and 11 females (11.6%). The gender of one patient could not be confirmed. Among the 96 patients analyzed, 88 (91.7%) patients were older than 49 years. The most prevalent age group, which included 39 patients, was the group containing patients aged 60 to 69 years (40.6%).

Eighty of the 96 patients had pneumonia. The remaining 11 patients suffered from symptoms including fever, dyspnea, cough, fatigue, and diarrhea. One patient was diagnosed with Pontiac fever. Information about four patients’ symptoms

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could not be obtained. The latent period ranged between 1 and 16 days, with an average of 6.2 days. Underlying medical conditions included diabetes (14.6%), high blood pressure (5.2%), hyperlipidemia (3.2%), and others (24.0%). Fifty patients (52.1%) were reported not to have underlying medical conditions.

The testing methods for diagnosis are shown in Table 1. Eighty-two patients (85.4%) in total were diagnosed as having legionellosis through urinary antigen detection. Twenty-five Legionella pneumophila strains were isolated from 24 patients (25.0%). Serogroups included serogroup 1 (SG1) (68.0%), SG2 (12.0%), SG3 (8.0%), SG6 (8.0%), and SG4 (4.0%).

Facilities that were suspected to be associated with legionellosis included public baths (41.7%), inns (13.5%), hotels (12.5%), sports facilities (6.3%), nursing homes (4.2%), and other facilities (14.6%). Four patients (4.2%) were reported to have been infected residentially. Two hundred thirty-nine Legionella strains were isolated from the suspected environmental sources of infection in 69 cases. The most prevalent serogroup was L. pneumophila SG1 (26.8%), followed by SG6 (19.7%), 5 (9.2%), and 3 (8.8%). The concentrations of Legionella ranged from 10 to 140,000 CFU/100 ml.

Among 18 episodes (20 cases) in which Legionella strains were isolated from both the patient and the environment, and the concentration of Legionella organisms was determined, the PFGE patterns of the strains were indistinguishable from each other in 10 episodes (12 cases). For these episodes, the PFGE results provided the evidence of the source of infection, and estimated concentrations of Legionella in the source of infection ranged from 90 to 140,000 CFU/100 ml (Table 2).

Table 2 shows that all patients except one were males older than 50 years. Seven out of 12 patients had one or more underlying medical conditions. A circulating bathwater system was used in 8 of 10 episodes, and chlorination of bathwater was insisted in 8 episodes but residual chlorine concentrations were less than 0.2 ppm at sampling time. Two patients suffering from an outbreak of episode no. 10 might have been infected with Legionella by the inhalation of aerosol from a whirlpool spa, even though the concentration of Legionella in the water was quite low. A patient of episode no. 7 was infected with Legionella by aspiration.

A number of case reports indicate that hot tubs in public bath facilities and accommodations are the most common source of legionellosis in Japan (2-4,10-12). According to a review of related studies, the bacterial concentrations found in such sources can range from 520,000 to less than 10 CFU/100 ml (Table 3). The aspiration of bathwater and the use of corticosteroids both appear to increase the risk of legionellosis, even with relatively low concentrations of Legionella in bathwater, such as those under 300 CFU/100 ml. Another noteworthy point in that regard is that even a very low concentration (3 CFU/100 ml) was sufficient to cause Legionnaires’ disease in a near-drowning case. In #4, 3 out of 1,833 cruise ship passengers in total were reported to have contracted Legionnaires’ disease after using the cruise ship hot tub, which, though they lacked the potentially problematic air induction bubble systems and hydrotherapy jet circulation, nonetheless were shown upon testing to have concentrations of 15,000 CFU/100 ml of Legionella (December 23, 2002) (10). In four large outbreaks (#1, #2, #3, and #6, Table 3) associated with hot tubs which did have air induction bubble systems, and/or hydrotherapy jet circulation, and/or hot water-falls, the source of the water being circulating bathwater,
Legionella water concentrations approached and exceeded 10,000 CFU/100 ml. This would seem to suggest that, in the case of the discovery of such a high concentration in a public facility, local public authorities should announce the name and location of the bathing facilities promptly and make all feasible efforts to locate epidemiologically linked cases among area patients presenting with respiratory symptoms. Means ± SE of the attack rates of legionellosis in these outbreaks were 0.13 ± 0.15% (a confirmed case number divided by total visitor days) (#1, #2, #3, and #6, Table 3) and 3.4 ± 1.7% (a compensated case number divided by total visitor days) (#1, #2, and #6, Table 3).

The present study has expanded upon the previous data on concentrations of Legionella in bathwater as a source of infection, especially in concentrations ranging from 90 to 3,000 CFU/100 ml. There were 10 episodes within our study in which the PFGE patterns of Legionella strains from the source of infection and from the patients were indistinguishable, and the concentrations of Legionella associated with these 10 episodes ranged from 90 to 140,000 CFU/100 ml (Table 2). Eight of the 10 episodes were linked to a self-contained body of water that was filtered and ‘chemically disinfected’ (a hot tub) (24). Hot tubs are designed for sitting or lying in up to the neck, not for swimming, and are not drained, cleaned, and refilled after each user. In Japan, however, the temperature of the water in hot tubs usually ranges from 40°C to 43°C, which is higher than in Europe (30°C to 40°C). A noteworthy finding was that even a low concentration of Legionella (90 CFU/100 mL) could be linked to the development of legionellosis in a situation of increased aerosol formation.

Advanced age, the state of being male, and other similarly regrettable chronic medical disorders such as end-stage renal disease, cancer, and diabetes are known risk factors for legionellosis (25). Among the 96 patients in the study, 21 were reported to have some kind of underlying medical conditions. The most frequent underlying medical condition was diabetes, and this was followed by high blood pressure. The role of high blood pressure as a risk factor is not clear. Only two patients were reported to be heavy smokers. The habit of smoking, by the way, should be reported in epidemiological investigations along with other underlying medical data, because cigarette smoking is one of the risk factors for legionellosis (26). In a national surveillance report (January 2003 to September 2008; 2,460 cases), the mean age of legionellosis patients in Japan was 65.2 years, and the percentage of males was 83% (27). These trends—the risk of age and gender—were borne out by the findings in the present study as well. The mean age of the patients in the present study was 64.8 years and the percentage of male patients was 88.4%.

Molecular typing and comparison of clinical isolates and suspected environmental source isolates of Legionella promotes the identification of sources of the disease and the subsequent prevention of further cases. Among such methods, PFGE analysis is considered to be one of the most discriminative epidemiological methods for subtyping L. pneumophila strains (28). Selected cases listed in Table 2 confirm that the culturing of clinical and environmental samples and the sub-

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**Table 3. Clinical and epidemiological characteristic of legionellosis cases associated with bathwater: a literature review**

<table>
<thead>
<tr>
<th>Episode no.</th>
<th>Concentration of Legionella (CFU/100 ml)</th>
<th>Legionella spp.</th>
<th>Facility</th>
<th>Confirmed case (Fatal case)</th>
<th>Age (y)</th>
<th>Gender (% male)</th>
<th>Underlying medical condition</th>
<th>Reference no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>520,000</td>
<td>L. pneumophila SG1, L. dumoffii</td>
<td>public bath</td>
<td>46 (7)</td>
<td>mean 63.3 (26-95)</td>
<td>33 males (71.7%)</td>
<td>hypertension, cardiovascular disease, diabetes, renal disease, chronic respiratory disease, smallmalignancy; smoking (76 subjects)</td>
<td>6, 13, 14</td>
</tr>
<tr>
<td>2</td>
<td>130,000</td>
<td>L. pneumophila SG1</td>
<td>public bath</td>
<td>9 (1)</td>
<td>mean 64.9 (52-82)</td>
<td>male</td>
<td>not reported</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>88,000</td>
<td>L. pneumophila SG1</td>
<td>public bath</td>
<td>23 (2)</td>
<td>mean 67.0 (50-86)</td>
<td>21 males (91.3%)</td>
<td>hypertension, hyperlipemia, diabetes, emphysema, gastric ulcer; smoking (22 subjects)</td>
<td>8, 15</td>
</tr>
<tr>
<td>4</td>
<td>15,000</td>
<td>L. pneumophila SG1 &amp; 5</td>
<td>cruise ship</td>
<td>3 (0)</td>
<td>70, 71, 73</td>
<td>2 males (66.7%)</td>
<td>emphysema, hypertension, atrial fibrillation, abnormal glucose tolerance; smoking</td>
<td>10, 16, 17, 18</td>
</tr>
<tr>
<td>5</td>
<td>14,640</td>
<td>L. pneumophila SG6</td>
<td>home</td>
<td>1 (1)</td>
<td>8 days</td>
<td>female</td>
<td>neonate after waterbirth</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>8,420</td>
<td>L. pneumophila SG1</td>
<td>public bath</td>
<td>27 (3)</td>
<td>mean 62.8 (27-85)</td>
<td>18 males (66.7%)</td>
<td>hypertension, diabetes, chronic hepatitis (HCV carrier); smoking (20 subjects)</td>
<td>3, 19</td>
</tr>
<tr>
<td>7</td>
<td>4,500</td>
<td>L. pneumophila SG1</td>
<td>public bath</td>
<td>2 (0)</td>
<td>70, 76</td>
<td>male</td>
<td>not reported</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>4,480</td>
<td>not reported</td>
<td>home</td>
<td>1 (0)</td>
<td>56</td>
<td>female</td>
<td>diabetes, diabetic nephropathy</td>
<td>20</td>
</tr>
<tr>
<td>9</td>
<td>300</td>
<td>L. pneumophila SG6</td>
<td>hotel</td>
<td>1 (0)</td>
<td>59</td>
<td>male</td>
<td>submerging his entire head and face</td>
<td>21</td>
</tr>
<tr>
<td>10</td>
<td>&lt;10 -70</td>
<td>L. pneumophila SG10</td>
<td>hospital</td>
<td>1 (1)</td>
<td>72</td>
<td>female</td>
<td>idiopathic pulmonary fibrosis; treated with methylprednisolone</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>L. pneumophila SG10</td>
<td>Japanese inn</td>
<td>1 (0)</td>
<td>71</td>
<td>female</td>
<td>chronic pancreatitis, old tuberculosis; near-drowning</td>
<td>22, 23</td>
</tr>
</tbody>
</table>

All episodes except for #11 were associated with hot tubs (with circulating bathwater system). Detailed information on the hot spring (#11 case) was not available.

Molecular typing of clinical and epidemiologically linked environmental Legionella isolates was performed by PFGE (#1, #3, #4, #6, #9-#11). In episode #1, a high concentration of L. londiniensis (1,500,000 CFU/100 ml) was detected in a bathwater sample, but this is omitted from this table since neither increase in serum titer against nor isolation of L. londiniensis were observed among patients.
sequent PFGE analysis are mandatory methods for determining the sources of infection and the concentrations of *Legionella* in an environment. Urinary antigen detection is a rapid and easy test and can detect most cases of legionellosis caused by *L. pneumophila* SG1 (Table 1). However, clinical specimens for *Legionella* isolation should always be cultured.

A WHO comprehensive overview of *Legionella* states that legionellae are not distributed normally within the aquatic environment, and that even when high concentrations of the bacteria are detected, the WHO says that this may not be related to health risk (9), citing work by Kool et al. (5) and Bentham (29). First, using multivariate Poisson regression analysis, the number of nosocomial Legionnaires’ disease cases in each hospital is reported to correlate better with the proportion of water-system sites that tested positive for *Legionella* than with the concentration of *Legionella* bacteria in water samples (5). However, it should be kept in mind that this counterintuitive result would not necessarily be applied to cases of community-acquired infection, since the rate of healthy people in communities is expected to be higher than in hospitals and healthy people are generally more resistant to opportunistic pathogens like legionellae. Second, during the monitoring of cooling tower water, culture results from *Legionella* samples taken from the same systems 2 weeks apart were not statistically related, suggesting that determinations of health risks from cooling tower water cannot be reliably based upon single or infrequent *Legionella* tests (29).

*Legionella* concentrations in bathwater can be influenced by operating conditions, numbers of users, and concentrations of disinfectants. One problem when trying to verify an infection source is whether the bacterial concentration when sampled is approximately the same as the concentration during the time of infection. The facilities suspected as the source of infection should be kept as they are (without urgent disinfection), and the relevant bathwater should be investigated as soon as possible by local health authorities. These limitations and caveats notwithstanding, facilities with very high colony counts probably are substantially more likely to be the source of an outbreak than those with lower counts (30) (Table 3). In conclusion, our retrospective findings suggest that the current Japanese regulatory safety level for *Legionella* in bathwater, which is set below the detection limit of culture techniques (10 CFU/100 ml), is appropriate to prevent bathwater-associated legionellosis.

**ACKNOWLEDGMENTS**

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**REFERENCES**

