

Environmental Survey of *Legionella pneumophila* in Hot Springs in Taiwan

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Acquisition of sporadic community-acquired legionnaires' disease has been linked to hot springs and whirlpool baths. Outbreaks of hot spring-associated legionnaires' disease were reported in Japan in the last few years. Although the mode of transmission is unclear, the presence of *Legionella* in hot springs may discourage hot springs resort visits by the general public. An environmental survey was conducted to determine the presence of *Legionella* in hot springs in Taiwan. In total, 55 water samples were collected from 19 hot springs resorts; 21% (4/19) of the hot spring resorts sampled yielded *L. pneumophila* in the public hot springs bath. *Legionella pneumophila* serogroups 1 and 6, *L. pneumophila* serogroup 3, *L. pneumophila* serogroup 5, and *L. pneumophila* serogroup 7 were isolated from four different resort spas, respectively. The total sample positivity rate for *L. pneumophila* was 11% (6/55). The risk of occurrences of legionnaires' disease outbreaks associated with hot springs water in general public is unknown, and epidemiologic investigations should be conducted for locating the potential sources of *Legionella* for those cases of community-acquired legionnaires' disease. Disinfection of hot springs for *Legionella* may be necessary if the risk of contracting legionnaires' disease from hot springs can be validated by an evidence-based approach.

Acquisition of sporadic community-acquired legionnaires' disease has been linked to hot springs (Mashiba et al., 1993;

Miyamoto, 2003; Miyamoto et al., 1997; Nakamura et al., 2003a, 2003b; Suzuki et al., 2002) and whirlpool baths (Benin et al., 2002; Benkel et al., 2000; Fields et al., 2001; Jernigan et al., 1996; Martinelli et al., 1993). In 2002, several outbreaks of hot spring-associated legionnaires' disease were reported in Japan (Matsumoto et al., 2004; Nakamura et al., 2003a, 2003b). Seventy-one cases of legionnaires' disease (29 confirmed, 42 probable) were documented, including 4 deaths. The mode of transmission was unclear. However, drinking of hot spring water as the mode of transmission has been suggested in a case report (Tominaga et al., 2001).

Hot springs bathing is a popular form of relaxation in Asia. The outbreaks in Japan cause concern in Taiwan although there have been no documented outbreaks of legionnaires' disease linked to hot springs in Taiwan. The source of the legionnaires' disease can easily go unrecognized since hospitals do not routinely investigate the sources of the infecting *Legionella* in patients who contract legionnaires' disease.

The answer to whether *Legionella* is present in the hot spring water is the first step in assessing the likelihood of legionnaires' disease being acquired from hot springs. Thus, an environmental survey was conducted to determine the presence of *Legionella* in hot springs in Taiwan. To our knowledge, this is the first study of the presence of *Legionella* in hot springs resorts in Taiwan.

MATERIALS AND METHODS

Study Sites

All 19 resorts sampled in this study were registered with the local government and had permits to operate hot spring baths for public use. These resorts were geographically located as

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FIG. 1. Sampling sites of *Legionella* environmental surveillance in Taiwan.

follows: eight resorts in the northern region of Taiwan, two resorts in the central region, two resorts in the southern region, six resorts in the eastern region, and one resort on a rural island (Figure 1). Each resort was assigned a code that was not linked to the geographical distribution.

Sampling Collection

A sterile screw-cap bottle was inserted into the hot spring bath. The screw-cap was open and collected water sample at about 10 cm above the bottom for a full bottle. The sampling bottle was screwed securely and stored and transported at approximately 4°C.

Sample Process and Analysis

Water samples were concentrated by passing 50 ml of the original sample through a sterile 0.2- μ m polycarbonate filter (Whatman by Fisher Scientific, Hampton, NH). The filter was resuspended in 5 ml sterile deionized water and vortexed.

Next, acid treatment was used by mixing 2 ml of the concentrated sample with 2 ml of 0.2 M HCl-KCl buffer for 5 min to suppress the non-*Legionella* organisms. Then 0.1 ml of the mixture was plated onto buffered charcoal yeast extract and differential glycine-vancomycin-polymyxin B media (Ta et al., 1995). Culture plates were incubated at 37°C for 5 d and then evaluated for *Legionella* colony growth. A direct fluorescent antibody testing kit was used for *Legionella* speciation and serogroups (sg) (m-Tech, Alpharetta, GA).

RESULTS

In total, 55 water samples were collected. Twenty-six percent (5/19) were sulfur-based hot spring located in the northern region, and 74% (14/19) were carbonate-based hot springs throughout the country. Of the 19 hot spring resorts sampled, 21% (4/19) yielded *L. pneumophila* in the hot spring pools. *Legionella pneumophila* sg-1 and 6 were isolated from one resort (resort A, central region), *L. pneumophila* sg-3 was isolated from one resort (resort G, eastern region), *L. pneumophila* sg-5 was isolated from one resort (resort J, southern region), and *L. pneumophila* sg-7 was isolated from one resort (resort O, northern region) (Table 1). Eleven percent (6/55) of water samples collected were positive for *L. pneumophila*. All positive samples were from carbonate-based hot spring water.

DISCUSSION

Hot spring resorts are a rapidly growing business in Taiwan, and now one of the most popular vacation activities in Taiwan. Although legionnaires' disease is at high risk for individuals who have underlying diseases and are cigarette smokers, the discovery of the presence of *Legionella* in hot springs water may discourage visits by the general public to hot spring resorts. Shortly after the reported outbreaks in Japan, a nationwide environmental survey for Japan showed that 28.7% (204/710) of the hot springs sampled yielded *Legionella* from the hot spring water; *L. pneumophila* was the predominant species, and serogroups 1 and 5 were frequently isolated (Furuhata et al., 2004).

In our study, 21% (4/19) of hot spring resorts were positive for *L. pneumophila*. Only 11% (6/55) of samples were positive for *L. pneumophila*. Our sample positivity rate appears to be lower than that in Japan. The most virulent strain, *L. pneumophila* serogroup 1, was found in less than 4% (2/55) of the samples. Based on limited data in this study, a large outbreak of legionnaires' disease is less likely to occur compared to Japan. It is noteworthy that all positive samples in this study were found in carbonate hot springs. A similar finding was also reported by Japanese investigators. The *Legionella* positivity rate was 4.9% at pH 3 or lower. When pH was 7.6 or higher, the positivity rate was 24.8% (Furuhata et al., 2004). To our knowledge in literature review, there is no information available to explain this observation and further research is needed.

TABLE 1
Percent Sample Positivity and *L. pneumophila* Serogroup in Hot Springs Resort

Resort	Percent of samples positive for <i>Legionella</i>	<i>Legionella pneumophila</i> serogroup
Resort A	40% (2/5)	<i>L. pneumophila</i> serogroups 1 and 6
Resort G	25% (1/4)	<i>L. pneumophila</i> serogroup 3
Resort J	66% (2/3)	<i>L. pneumophila</i> serogroup 5
Resort O	33% (1/3)	<i>L. pneumophila</i> serogroup 7

Specialized laboratory techniques for detecting *Legionella* from water samples are critical. Hot springs water contains a very diverse population of microorganisms and chemical compounds. The warm water temperature (40–43°C) of hot springs further enhances the growth of microorganisms. Combinations of acid pretreatment of water samples and the use of antibiotic-containing selective culture media have been successful in isolating *Legionella* (Ta et al., 1995).

The risk of acquiring *Legionella* from hot springs water is unknown due to the following factors:

1. The host susceptibility is a major risk factor for acquiring legionnaires' disease. Most vacationers are healthy immunocompetent individuals, while a small portion of hot spring visitors are elderly who can be at higher risk.
2. Direct ingestion of hot springs water is minimal since the hot springs water is considered nondrinkable; thus, the chances of aspiration are minimal.
3. The intensity of aerosols in the hot spring pool may contribute to an increased risk of acquiring the disease.

Thus, linking the presence of *Legionella* in hot springs to cases of community-acquired Legionnaires' disease is needed before institution of regulations that may be expensive and unnecessary. The health departments should consider epidemiologic investigations for locating the potential sources of *Legionella* for those cases of community-acquired legionnaires' disease that are culture confirmed. This would allow an assessment of risk for hot springs water, as well as assessing risks for other domestic water sources. Until such information is available, the authorities and the hot spring operators may follow the published guideline for good maintenance practices and periodical pool disinfection. Warnings can be posted in hot spring facilities for elderly and immunocompromised individuals before the use of hot springs as precaution.

If the presence of *Legionella* does impose a public health threat, continuous disinfection of hot springs water may be an option for prevention of the disease. Chlorination may not be an ideal disinfection method for *Legionella* (Lin et al., 1998). *Legionella pneumophila* is known to be resistant to chlorine, and visitors to the hot springs may find the odor of chlorine objectionable. *Legionella* likely resides in biofilms (or "microbial mats") throughout the hot spring bath and its

plumbing, so point-of-entry disinfection methods (ultraviolet light and filtration) may be unsuccessful (Lin et al., 1998). Copper–silver ionization, chlorine dioxide, and chloramines are new methods for *Legionella* disinfection. Ionization may not be optimal for carbonate hot springs because the elevated pH may compromise the efficacy of ionization (Lin et al., 2002). Chlorine dioxide and chloramines may be attractive because both compounds have better penetration into biofilms than chlorine and minimal odor. Furthermore, the efficacy of chlorine dioxide and chloramines is not affected by elevated temperature (unlike chlorine) and elevated pH (unlike ionization). Nevertheless, the efficacy of these new methods must be scientifically validated before its adoption especially since these systems can be expensive and must be maintained.

One limitation of our study is that the sample size may be small. Hot spring resort operators are reluctant to participate in the surveillance because of concerns about negative publicity from the media, with loss of customers. This environmental survey is confidential, with a limited number of hot spring resorts, to protect their identity. Whether *Legionella* was present in the hot spring source water was not determined; instead, we sampled the baths. It is possible the hot spring source water is free of *Legionella*, because water temperature at hot spring source is between 70 and 95°C, which may kill *Legionella*. The *Legionella* isolated from the hot spring water may come from blended water that is added to hot spring (e.g., tap water or groundwater). Further investigation can be conducted to look for the presence of *Legionella* in tap water and groundwater in areas where the baths of the hot spring resort are positive for *Legionella*.

In summary, we present the results of the first environmental surveillance of the hot spring water in Taiwan. However, our findings are not meant to cause public panic nor to encourage disinfection of hot springs water. Before rational recommendations can be implemented, a scientific, evidence-based approach should be taken in determining the risk of contracting Legionnaires' disease from hot springs. Good maintenance practices in pool quality and precaution for susceptible hosts may be useful for prevention of legionnaires' disease, and continuous disinfection of hot springs for *Legionella* may be necessary if an epidemiological link can be established and the risk can be identified.

REFERENCES

- Benin, A. L., Benson, R. F., Arnold, K. E., Fiore, A. E., Cook, P. G., Williams, L. K., Fields, B., and Besser, R. E. 2002. An outbreak of travel-associated Legionnaires disease and Pontiac fever: The need for enhanced surveillance of travel-associated legionellosis in the United States. *J. Infect. Dis.* 185:237–243.
- Benkel, D. H., McClure, E. M., Woolard, D., Rullan, J. V., Miller, G. B., Jenkins, S. R., Hershey, J. H., Benson, R. F., Pruckler, J. M., Brown, E. W., Kolezak, M. S., Hackler, R. L., Rouse, B. S., and Breiman, R. F. 2000. Outbreak of Legionnaires' disease associated with a display whirlpool spa. *Int. J. Epidemiol.* 29:1092–1098.
- Fields, B. S., Haupt, T., Davis, J. P., Arduino, M. J., Miller, P. H., and Butler, J. C. 2001. Pontiac fever due to *Legionella micdadei* from a whirlpool spa: Possible role of bacterial endotoxin. *J. Infect. Dis.* 184:1289–1292.
- Furuhata, K., Hara, M., Yoshida, S., and Fukuyama, M. 2004. Distribution of *Legionella* spp. in hot spring baths in Japan. *Kansenshogaku Zasshi* 78:710–716.
- Jernigan, D. B., Hofmann, J., Cetron, M. S., Genese, C. A., Nuorti, J. P., Fields, B. S., Benson, R. F., Carter, R. J., Edelstein, P. H., Guerrero, I. C., Paul, S. M., Lipman, H. B., and Breiman, R. 1996. Outbreak of Legionnaires' disease among cruise ship passengers exposed to a contaminated whirlpool spa. *Lancet* 24(347):494–499.
- Lin, Y. S., Stout, J. E., Yu, V. L., and Vidic, R. D. 1998. Disinfection of water distribution systems for *Legionella*. *Semin. Respir. Infect.* 13:147–159.
- Lin, Y. E., Vidic, R. D., Stout, J. E., and Yu, V. L. 2002. Negative effect of high pH on biocidal efficacy of copper and silver ions in controlling *Legionella pneumophila*. *Appl. Environ. Microbiol.* 68:2711–2715.
- Martinelli, F., Carasi, S., Scarcella, C., and Speziani, F. 2001. Detection of *Legionella pneumophila* at thermal spas. *N. Microbiol.* 24:259–264.
- Mashiba, K., Hamamoto, T., and Torikai, K. 1993. A case of Legionnaires' disease due to aspiration of hot spring water and isolation of *Legionella pneumophila* from hot spring water. *Kansenshogaku Zasshi* 67:163–166.
- Matsumoto, N., Matsumoto, Y., Ashitani, J., Katoh, S., and Nakazato, M. 2004. An outbreak of Legionnaires' disease associated with a circulating bath water system at a public bathhouse. *Nihon Kokyuki Gakkai Zasshi* 42:75–79.
- Miyamoto, H. 2003. Prevention measures against *Legionella* infection in a circulating hot water bath. *J. UOEH* 25:61–77.
- Miyamoto, H., Jitsurong, S., Shiota, R., Maruta, K., Yoshida, S., and Yabuuchi, E. 1997. Molecular determination of infection source of a sporadic *Legionella pneumonia* case associated with a hot spring bath. *Microbiol. Immunol.* 41:197–202.
- Nakamura, H., Yagyū, H., Tsuchida, F., Sudou, A., Watanabe, O., Kioi, K., Kishi, K., Oh-ishi, S., Kiguchi, T., Yamaguchi, K., and Matsuoka, T. 2003a. A major outbreak of Legionnaire's disease due to a public bathhouse: Clinical examination. *Nihon Kokyuki Gakkai Zasshi* 41:325–330.
- Nakamura, H., Yagyū, H., Kishi, K., Tsuchida, F., Oh-Ishi, S., Yamaguchi, K., and Matsuoka, T. 2003b. A large outbreak of Legionnaires's disease due to an inadequate circulating and filtration system for bath water—Epidemiologic manifestations. *Intern. Med.* 42:806–811.
- Suzuki, A., Ichinose, M., Matsue, T., Amano, Y., Terayama, T., Izumiyama, S., and Endo, T. 2002. Occurrence of *Legionella* bacteria in a variety of environmental waters—From April, 1996 to November, 2000. *Kansenshogaku Zasshi* 76:703–710.
- Ta, A. C., Stout, J. E., Yu, V. L., and Wagener, M. M. 1995. Comparison of culture methods for monitoring *Legionella* species in hospital potable water systems and recommendations for standardization of such methods. *J. Clin. Microbiol.* 33:2118–2123.
- Tominaga, M., Aoki, Y., Haraguchi, S., Fukuoka, M., Hayashi, S., Tamesada, M., Yabuuchi, E., and Nagasawa, K. 2001. Legionnaires' disease associated with habitual drinking of hot spring water. *Intern. Med.* 40:1064–1067.