Bio-Psycho-Socio-Ecological Risk Factors for Legionella Infection among Japanese 24-Hour Hot Water Bath Users and Non-Users

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Abstract: Bio-Psycho-Socio-Ecological Risk Factors for Legionella Infection among Japanese 24-Hour Hot Water Bath Users and Non-Users: Masahiro Irie, et al. Department of Mental Health, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health—The purpose of this study was to examine the risk of Legionella pneumophila (Lp) infection due to the use of a 24-h hot water bath (24HHWB), which is popular for bathing in Japan because of its water conservation through the use of recycled bath water, and to investigate other possible factors that may be associated with Legionella infection. The risk of anti-Legionella antibody formation associated with the 24HHWB use and various bio-psycho-socio-ecological factors was estimated based on the logistic model among 204 employees (161 men, 43 women) who were either 24HHWB users or non-users. There was a significantly higher rate of anti-Legionella antibody titers (≧8) in the 24HHWB users than in the non-users, particularly against Lp serogroup (SG) 5 (odds ratio, 16.3; 95% CI, 2.1–126.5; p<0.01) and Lp SG 6 (odds ratio, 2.9; 95% CI, 1.3–6.5; p<0.01). However, most of the titers were <64, and no clinical cases of Legionnaires’ disease among the 24HHWB users were found. No significant correlations were observed between the duration of the 24HHWB use and the increased antibody titers. The impossibility of relieving stress, a decrease in age, the use of artificial marble bathtubs, and a decrease in the bathing frequency per week were also related to the increased antibody titers against Lp SG 6 (odds ratio, 5.9; 95% CI, 1.6–22.1; p<0.01), Lp SG 3 (odds ratio, 0.9; 95% CI, 0.8–1.0; p<0.01; odds ratio, 10.5; 95% CI, 1.4–76.9; p<0.05), and Lp SG 1 (odds ratio, 1.9; 95% CI, 1.2–3.2; p<0.05), respectively. The anti-Legionella antibodies were considered to be IgM dominant, because most of the antibodies were sensitive to 2-mercaptoethanol treatment. In conclusion, the use of a 24HHWB was associated with Legionella sensitization, but not with clinically apparent legionellosis. Psychological, environmental, and lifestyle factors also seemed to have causal relations to antibody formation.

(J Occup Health 2000; 42: 205–212)

Key words: Legionella, Infection, Antibody, Recycled bath water, Stress, Psychoneuroimmunology

There is growing evidence that legionellae inhabit natural water environments and man-made water apparatus, such as hot spring spas, whirlpool spas, hot water tanks, shower heads, and cooling towers. The occurrence of legionellosis has been related to these factors, probably when airborne water droplets containing legionellae were inhaled. Therefore, it is necessary to keep water systems as clean as possible to prevent legionellosis.

Recently, the shortage of water resources has become a serious problem in Japan. For the sake of water conservation and convenience, a 24-h hot water bath (24HHWB), which can be used at any time of the day, was invented and has been in use mainly in the homes of the general public. The bath filters and circulates water to be reused. From the early 1980’s, more than 1.2 million units have been sold in Japan. Since the number of households in Japan is approximately 45 million, roughly 3% of households own a 24HHWB.

It is considered that the 24HHWB cannot disinfect all of the circulating water, because its cleaning mechanism largely depends on biological activation of microorganisms. To complement this drawback, many
24HHWB units are equipped with a physical cleaning device that applies heat, ultraviolet, or ozone disinfection to the water after it passes a filtering section, for the purpose of decreasing the number of hazardous bacteria. Nevertheless, Yabuuchi et al. recently reported that *Legionella pneumophila* (Lp) was isolated in 24HHWB water at a concentration of $10^4$–$10^6$ colony forming units/dl. Among the Lp serogroups (SGs) found in the 24HHWB water, LP SG 5 was the most prevalent. Ever since this finding, there has been an intensified concern regarding *Legionella* infection among consumers and sales of the 24HHWB have fallen off considerably. Some companies which deal in the 24HHWB have gone bankrupt. Although it is clear at present that legionellae can exist in a 24HHWB, it has never been examined whether legionellae could actually infect users and cause Legionnaires' disease or Pontiac fever.

The aim of this study was to clarify the possibility that 24HHWB use could be a cause of legionellosis, by measuring the anti-*Legionella* serum antibody titers of employees who were either 24HHWB users or non-users. The relationships between having significant antibody titers, 24HHWB use, and the occurrence of legionellosis were investigated. In addition, we also examined how subjects could become seropositive by other internal and external potential factors. The internal factors included individual psychological stress and lifestyles that are known to influence immune functions, which are supposed to result in seropositive. The external factors included the frequency of contacts with legionella-inhabiting environments, such as various water environments, air-conditioning environments, and soil.

**Materials and Methods**

**Subjects**

We recruited 210 subjects (164 men, 46 women) from regular users of 24HHWB units in the 24HHWB manufacturing and marketing corporations (Showa Manufacturing Co., Ltd., and Sanwa Service Co., Ltd.) and non-users with equivalent ages and sexes who worked at the same corporations. The 24HHWB unit utilized in this study cleans the bathtub water with the aid of microorganisms adhering to carriers composed of activated charcoal, filter gravel, and either coral or granite porphyry. Furthermore, the 24HHWB was equipped with an additional cleaning system composed of an ultraviolet lamp (sterilamp). Informed consent for the present study was obtained from the prospective subjects. A total of 204 subjects (161 men, 43 women) were finally enrolled in the present study, because six subjects refused to participate in the study at the time of investigation. They ranged in age from 20 to 64 yr (mean ± S.D., 42.66 ± 11.13). Eight non-user subjects were found to have had a history of using a 24HHWB after the investigation. Consequently, the number of present users turned out to be 102, that of past users, 8, and actual non-users, 94. There were no subjects who had immunosuppressing conditions (e.g., cancer, HIV infection/AIDS, organ or bone marrow transplantation, kidney dialysis, use of immunosuppressive medications).

**Data collection**

To these subjects, a self-administered questionnaire was provided in July, 1997. The questions addressed demographic factors, present and past disease histories of the subjects and their family members, bathing-related factors, contacts with legionella-inhabiting environments, lifestyles regarding cigarette smoking and alcohol drinking, the 60-item version of the General Health Questionnaire (GHQ) and its subscales (somatic symptoms, anxiety and insomnia, social dysfunction, severe depression), and the possibility of relieving considerable stress. The questions about disease histories included physician-diagnosed pneumonia (supposed to include Legionnaires’ disease) and influenza-like symptoms (supposed to include Pontiac fever), and other diseases, such as bronchitis, gastroenteritis, and fever of unknown origin. The 24HHWB users were given additional questions regarding the duration of use. Each question had from three to eight options. In addition, fasting blood samples were taken early in the morning on weekdays at the same time as the questionnaire investigation. Additionally, the numbers of legionellae inhabiting the 24HHWB water were investigated 13.7 ± 6.2 (means ± S.D.) times during the half year preceding the present study in 31 24HHWB units.

**Determination of serum antibody titers**

For the measurement of anti-*Legionella* serum antibody titers, a microplate agglutination test (MPAT), which was recently established for the serological diagnosis of legionellosis, was applied according to the methods previously described. A total of 204 serum samples were heated for 30 min at 56°C, and diluted 1:4 with sterile PBS supplemented with 2% of normal rabbit sera. The antigens were prepared from Lp SG 1 (ATCC 33152), SG 3 (ATCC 33155), SG 4 (ATCC 33156), SG 5 (ATCC 33216), and SG 6 (ATCC 33215). These serogroups are known to exist in 24HHWB units. The bacteria were cultured on buffered charcoal-yeast extract agar supplemented with α-ketoglutarate (BCYEα) at 37°C for 48 h, harvested, and suspended in phosphate-buffered saline (PBS, 136.8 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄) to give an optical density equivalent to 1.0 at 650 nm. The suspensions were heated in boiling water for 1 h and were used as the antigens in the MPAT.

Quantitative agglutination was performed by using a 96-well U-bottom microplate for each antigen. Within each well of the plate, 0.025 ml of PBS was placed, and
then 0.025 ml of the serum sample was added to the first well of each row. Each row of serum was diluted by the microdilution technique with 0.025 ml microdiluters. Antigen, in 0.025 ml volumes, was added to every well, and the plates were placed on a vertical vibrator and mixed for 15 s. The plates were covered with plastic microtiter plate sealers, and were incubated overnight (16 h) at room temperature (25°C). A positive reaction consisted of a mat of agglutinated cells with no definite button. A negative reaction consisted of a definite button of organisms. The end point is the highest total dilution of serum (after the addition of antigen) resulting in a mat of cells with no button or with only a very slight trace of a button. The agglutination reaction of each serum sample was observed in serial dilutions of 8. Anti-Legionella IgG antibody titers were examined after the treatment of the serum with an equal volume of 0.2 M 2-mercaptoethanol (2ME) for 2 h at 37°C.

Statistical methods

Data analysis was performed with the use of SAS, version 6.11 (SAS Institute Inc, Cary, NC). Several analyses, such as Pearson’s product-moment correlations, Spearman’s rank correlations, and chi-square tests with Yates’ continuity correction, were used to estimate the relationships between antibody formation, in dilutions of 8, of each serogroup and a total of 35 variables (age, gender, body mass index, cigarette smoking, alcohol drinking, frequency of bathing in a week, duration of bathing time, types of heating methods of bath water, materials of bathtub, use of air-conditioners, use of humidifiers, use of well water, use of hot-water supply system, contacts with soil during gardening, GHQ score, possibility of relieving considerable stress) including multiple choices and the GHQ subscales in the questionnaire. Selected factors that were related to antibody formation with a significance of less than 10% probability level were analyzed further. If the items had multiple choices, then the factors that had a relationship to the formation of antibodies with a trend of less than 10% probability level were selected. For each of the factors, the odds ratio influencing antibody formation was calculated, and its 95% confidence interval (CI) was confirmed by simple logistic regression analysis with the use of a Bonferroni’s correction for multiple comparisons. The options in each variant were grouped to be either three or four, in order to have similar numbers of answers. The 24HHWB users included both present and past users in the logistic regression analysis. With regard to Lp SG 4, the odds ratio in relation to the 24HHWB use could not be calculated because the antibody positive subjects consisted only of non-users.

In order to evaluate a combinational, i.e., not individual, relation of each factor to antibody formation, multiple logistic regression analysis using a backward elimination technique was applied to each serogroup, with positive or negative antibody formation in dilutions of 8 as the dependent variable and the items in the questionnaire as the independent variables. The items which were related to the antibody formation at a significance level of 15% were used in the multiple analysis. Answers concerning 24HHWB use were inevitably included in the calculation, even if the 24HHWB use was not selected by the multiple logistic regression analysis. In the multiple analysis, the variables were selected with a significance level of 20% for staying in the model or being added to the model.

A chi-square test with Yates’ continuity correction and Wilcoxon signed-ranks test were used to compare the distribution of anti-Legionella pneumophila antibody titers between the 24HHWB users and non-users. The Spearman’s rank correlation coefficient was utilized to examine the relationship between the average number of legionellae existing in the 24HHWB water during the half year preceding the blood collection and the antibody titers in dilutions of 8 for each serogroup.

Results

Distribution of anti-Legionella pneumophila antibody titers

There were no significant differences in age, gender distribution, type of work, and work position between the 24HHWB users and non-users in a chi-square test with Yates’ continuity correction and Student’s t-test (Table 1). Among the 24HHWB users, 49.1% showed aggregation with at least one serogroup in dilutions of 8. On the other hand, aggregation was observed in only 18.1% of non-users, showing a significant difference (χ²=20.1; p<0.001). Table 2 shows the comparisons of the titers of each antibody between the two groups. The subjects were found to have a significantly larger amount of anti-Lp SG 5 antibody in the 24HHWB users who showed aggregation in dilutions of 8 (χ²=12.9; p<0.001), as compared with the non-users. Also, against Lp SG 6, there was a significantly higher rate of antibody formation in the 24HHWB users than in the non-users (χ²=17.9; p<0.001). The titers of the anti-Lp SG 6 antibody were significantly higher than those of the anti-Lp SG 5 antibody, irrespective of the 24HHWB use (Wilcoxon signed-ranks test, p<0.001). Most of the serum antibody titers were less than 64, with only one case of 256 and two cases of 128.

Simple regression analysis

Table 3 shows the various factors that have a relationship to the rate of each anti-Lp antibody titers (8) in a simple logistic regression analysis with the use of a Bonferroni’s correction for multiple comparisons. It was demonstrated that a decrease in the average number of baths in a week significantly led to antibody formation against Lp SG 1. Antibody formation against Lp SG 5 was observed to be significantly related to 24HHWB use. Significant relations were found between antibody formation against Lp SG 6
and several factors, such as the 24HHWB use, an increase in contact with garden trees and flowers, and a decrease in the prospect of relieving stress. The 24HHWB use was not associated with the other factors in the questionnaire. Also, the 24HHWB use was unrelated to age, sex, body mass index (BMI), cigarette smoking, and alcohol drinking, even when simple logistic regression analyses were performed without the use of a Bonferoni’s correction for multiple comparisons.

Multiple regression analysis

A multiple logistic regression analysis (Table 4) showed that a decrease in the average number of baths in a week was significantly related to the rate of antibody titers (Table 2).
8) against Lp SG 1. An increase in age indicated a slight increase in antibody formation against Lp SG 1. The antibody was formed less frequently against Lp SG 3 in the subjects who had an increased age, and more frequently in the subjects who used bathtubs made of artificial marble. Antibody formation against Lp SG 3 also tended to occur when the subjects were more impaired in their social activity (GHQ subscale). The use of the 24HHWB was significantly associated with Lp SG 5 antibody formation. Antibody formation against Lp SG 6 was significantly related to 24HHWB use and to a decrease in the prospect of relieving stress. There was also a tendency for an increase in contact with garden trees and flowers or an aggravation of a depressive state (GHQ subscale) to be related with antibody formation against Lp SG 6.

**Other factors and outcome**

No significant relations between 24HHWB use as well as antibody formation and several diseases, such as pneumonia, influenza-like symptoms, bronchitis, and acute gastroenteritis were found. No clinical cases of physician-diagnosed pneumonia were observed among the 24HHWB users. There were two cases who had

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### Table 3. Factors associated with antibody formation of each *Legionella* serogroup by simple logistic regression analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number</th>
<th>Regression coefficient</th>
<th>SE</th>
<th>OR (95%CI)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. pneumophila</em> serogroup 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease in the average number of baths in a week</td>
<td>204</td>
<td>1.74</td>
<td>0.61</td>
<td>5.70 (1.73, 18.82)</td>
<td>0.004</td>
</tr>
<tr>
<td><em>L. pneumophila</em> serogroup 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24HHWB use</td>
<td>204</td>
<td>2.86</td>
<td>1.04</td>
<td>17.41 (2.25, 134.53)</td>
<td>0.011</td>
</tr>
<tr>
<td><em>L. pneumophila</em> serogroup 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24HHWB use</td>
<td>204</td>
<td>1.45</td>
<td>0.33</td>
<td>4.27 (2.23, 8.19)</td>
<td>0.004</td>
</tr>
<tr>
<td>Increase in contact with garden trees and flowers</td>
<td>203</td>
<td>0.88</td>
<td>0.26</td>
<td>2.42 (1.47, 3.99)</td>
<td>0.014</td>
</tr>
<tr>
<td>Decrease in possibility of relieving stress</td>
<td>157</td>
<td>1.74</td>
<td>0.58</td>
<td>5.68 (1.82, 17.69)</td>
<td>0.039</td>
</tr>
</tbody>
</table>

GHQ=General Health Questionnaire. 24HHWB=24-h hot water bath. *Bonferoni corrected significance.

### Table 4. Factors associated with antibody formation of each *Legionella* serogroup by multiple logistic regression analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Partial Regression Coefficient</th>
<th>SE</th>
<th>OR (95%CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. pneumophila</em> serogroup 1 (n=200)</td>
<td>- 0.45</td>
<td>1.28</td>
<td>0.64 (0.05, 7.79)</td>
<td>0.723</td>
</tr>
<tr>
<td>24HHWB use</td>
<td>0.65</td>
<td>0.26</td>
<td>1.92 (1.16, 3.17)</td>
<td>0.011</td>
</tr>
<tr>
<td>Increase in age</td>
<td>- 0.10</td>
<td>0.05</td>
<td>0.90 (0.81, 1.01)</td>
<td>0.062</td>
</tr>
<tr>
<td><em>L. pneumophila</em> serogroup 3 (n=199)</td>
<td>- 0.27</td>
<td>0.66</td>
<td>0.77 (0.21, 2.82)</td>
<td>0.688</td>
</tr>
<tr>
<td>24HHWB use</td>
<td>- 0.11</td>
<td>0.04</td>
<td>0.89 (0.83, 0.97)</td>
<td>0.004</td>
</tr>
<tr>
<td>Increase in age</td>
<td>2.35</td>
<td>1.01</td>
<td>10.53 (1.44, 76.86)</td>
<td>0.020</td>
</tr>
<tr>
<td>Use of artificial marble bathtub</td>
<td>0.33</td>
<td>0.20</td>
<td>1.40 (0.95, 2.05)</td>
<td>0.090</td>
</tr>
<tr>
<td><em>L. pneumophila</em> serogroup 5 (n=204)</td>
<td>2.79</td>
<td>1.05</td>
<td>16.30 (2.10, 126.47)</td>
<td>0.008</td>
</tr>
<tr>
<td>24HHWB use</td>
<td>0.99</td>
<td>0.74</td>
<td>2.70 (0.63, 11.60)</td>
<td>0.182</td>
</tr>
<tr>
<td><em>L. pneumophila</em> serogroup 6 (n=153)</td>
<td>1.06</td>
<td>0.41</td>
<td>2.89 (1.30, 6.46)</td>
<td>0.010</td>
</tr>
<tr>
<td>24HHWB use</td>
<td>1.05</td>
<td>0.67</td>
<td>2.86 (0.76, 10.69)</td>
<td>0.119</td>
</tr>
<tr>
<td>Use of artificial marble bathtub</td>
<td>0.61</td>
<td>0.33</td>
<td>1.84 (0.96, 3.51)</td>
<td>0.065</td>
</tr>
<tr>
<td>Decrease in possibility of relieving stress</td>
<td>1.78</td>
<td>0.67</td>
<td>5.90 (1.58, 22.07)</td>
<td>0.008</td>
</tr>
<tr>
<td>Increase of depression in the GHQ test</td>
<td>0.36</td>
<td>0.20</td>
<td>1.43 (0.98, 2.10)</td>
<td>0.066</td>
</tr>
</tbody>
</table>

24HHWB=24-h hot water bath. GHQ=General Health Questionnaire.
anamnesis of fever of unknown origin after they started to use the 24HHWB. They were either a 24HHWB user or a family member of a 24HHWB user, and the fever thereafter subsided. The duration of 24HHWB use did not show a distinct association with antibody formation in any serogroup.

After the treatment of sera with 2ME, the anti-Legionella antibody titers that were 8 decreased in 24HHWB users against Lp SG 5 (14.5% vs. 2.7%) and Lp SG 6 (44.5% vs. 10.9%), and in non-users against Lp SG 6 (16.0% vs. 3.2%), respectively. Anti-Legionella antibody titers (8) against Lp SG 1, Lp SG 3, and Lp SG 4 were not observed after the treatment of sera with 2ME.

Legionella pneumophila was isolated in the 24HHWB water at concentration of 10^8-10^9 colony forming units/dl in 31 24HHWB units. Among the users, 20 subjects showed antibody titers in dilutions of 8. Spearman’s rank correlation coefficients between the average number of legionellae existing in the 24HHWB water in the past and the antibody titers in dilutions of 8 of each serogroup in the 20 24HHWB users were -0.179 (Lp SG 1), 0.014 (Lp SG 3), 0.356 (Lp SG 5), and -0.212 (Lp SG 6), although no statistical significance was obtained for any serogroup.

Discussion

Our study demonstrates that 24HHWB use is associated with a rate of antibody titers (8) against Lp SG 5 and Lp SG 6. The highest odds ratio in Lp SG 5 was consistent with the previous reports of its prevalence in the 24HHWB8-7. This result seems to reflect the highest correlation coefficient between the average number of legionellae existing in the 24HHWB water in the past and the antibody titers (8) against Lp SG 5, although we only investigated a small number of samples and did not have a statistically significant difference. However, most of the serum antibody titers did not have a value high enough to be an etiologic agent of legionellosis. Of the persons with high titers, we analyzed the factors related to the antibody titers of 8. The antibody titers in this study were not generally higher than those of 98 healthy Japanese subjects whose antibody titers were also measured using the MPAT method15. In addition, none of the subjects experienced Legionnaires’ disease after they started to use the 24HHWB. The 24HHWB users did not show higher rates of influenza-like symptoms than the non-users. Furthermore, longer durations of use did not necessarily mean higher onset risks of legionellosis, because no correlation was observed between the 24HHWB use duration and antibody formation. Therefore, we suggest that Legionella sensitization in the 24HHWB users does not invoke Legionnaires’ disease and Pontiac fever and remains subclinical in the present study. Further consideration will be necessary to clarify whether our findings can be applied to Legionella sensitization due to 24HHWB units with different disinfecting mechanisms.

This hypothesis is supported by the fact that there have been no apparent published reports regarding 24HHWB-caused pneumonia and Pontiac fever in Japan. When the first outbreak of Legionnaires’ disease occurred at the 1976 meeting of the American Legion in Philadelphia, no onset among chronically exposed hotel personnel was observed, except for one case who was an air-conditioner repairman19. Also, the epidemic of whirlpool spa-related Legionnaires’ disease among cruise ship passengers in 1994 did not include any patients from the ships staff20. Our results appear to correspond with these previous findings. Serological study with respect to hot spring spas revealed that no clinical cases of Legionnaires’ disease were found among the therapists who were frequently exposed to the contaminated hot spring water21. Other serological study also indicated that no cases of Legionnaires’ disease were observed among workers exposed to a contaminated cooling tower water22. Accordingly, when healthy subjects are chronically exposed to legionellae, which grow slowly, subclinical sensitization may be likely to occur and thus an onset of legionellosis may be prevented, even in acute or frequent exposures.

The mechanisms underlying the subclinical sensitization of the 24HHWB users are unknown. Cell-mediated immune functions, such as activated macrophages, are considered to have a significant role in preventing legionellosis rather than in antibody formation23. Although we did not examine such cell-mediated immune functions, antibody formation in conjunction with complement was reported to promote phagocytosis of Lp by alveolar macrophages24. The onset of legionellosis, therefore, might be prevented in the 24HHWB users who had higher rate of antibody formation than the non-users. Furthermore, the 24HHWB water-cleaning mechanisms are more complex and are considered to keep the water cleaner than the sand filters in which legionellae were isolated in a previous investigation of whirlpool spa-related Legionella infection25. These issues may help to explain further the subclinical Legionella sensitization in the 24HHWB users. Another possibility which may influence the outcome is the mode of airway transmission of legionellae. The 24HHWB is suspected to produce fewer airborne water droplets containing legionellae compared with air-conditioners, and this might result in the subclinical sensitization of 24HHWB users. However, the cases of fever of unknown origin may need to be studied further for a possible relationship, in spite of the small number of subjects.
In the present study, we measured the IgG antibody titers by treating the sera with 2ME in order to clarify the class of antibody. The IgG antibody titers were lower than those of IgM, in spite of chronic exposure to legionellae. This result is consistent with the previous findings that bacterial lipopolysaccharides (LPSs) induce the secretion of IgM predominantly, because bacterial LPSs have been classified as T-independent antigens. Another report that the levels of IgM antibody against Lp SG 1 were higher than those of IgG in healthy populations in New Zealand also supports our viewpoint.

The present study also revealed several important clinical points regarding the mode of transmission of legionellae. Simple and multiple analyses demonstrated that antibody formation against Lp SG 1 had possible associations with the average number of baths in a week. Therefore, unsanitary lifestyles regarding bathing seemed to increase the sensitization by legionellae. As for the material used for the construction of the bathtub, the use of artificial marble showed a positive relation with antibody formation against Lp SG 3 in multiple analysis. The material used for the construction of the bathtub may also influence antibody formation.

Additionally, it was noteworthy that the antibody formation against Lp SG 6 was significantly or marginally related with a decrease in the prospect of relieving stress, an increase in a depressive state (GHQ subscale), and an increase in contact with garden trees and flowers, and had significantly higher titers than those against Lp SG 5, which was reported to be most prevalent in the 24HHWB water. These findings suggest possible sensitization through contacts with legionellae inhabiting soil, and also higher infectivity through stress-induced suppression of immunological activities as well as the use of 24HHWB. Previous studies have demonstrated that psychological stress suppressed immune responses, such as IgA production, which are important to protect the host from infections, and thus increased the risk of respiratory diseases. To the best of our knowledge, however, no evidence has been obtained regarding Legionella infection in association with psychological stress. Our findings suggest that Legionella infection is also influenced by stress, as in cases of mildly pathogenic viruses, such as rhinovirus, respiratory syncytial virus, and corona virus. Among the various manifestations of psychological distress, depression is often reported to suppress immune functions, which are important to prevent infection. Therefore, a longer depressive experience evaluated by the GHQ, which reveals the depressive state during several weeks, would be likely to result in antibody formation. In addition to these findings, there was a reliable association between the prospect of stress relief and antibody formation. This seems to suggest the importance of the probability of overcoming stress, rather than the degree of stress, in the case of Legionella infection. The impossibility of relieving stress is considered to result in long lasting psychological distress, such as depression.

Several limitations must be also considered in discussing our findings. First, the risk of anti-Lp serum antibody formation associated with various bio-psycho-socio-ecological factors might be over- or underestimated, because most of the titers in the 24HHWB users and non-users were low. Second, the power of this study to demonstrate the risk factors for Legionella infection was relatively limited because of small numbers of samples analyzed. Third, determination of antibody titers was performed regarding specific serogroups which were isolated in the 24HHWB water. Whether antibody formation of other legionellae could occur among 24HHWB users is unknown. Fourth, several organisms such as Mycoplasma pneumoniae, Bacteroides fragilis, Pseudomonas spp., and Campylobacter spp., have been reported to cross-react with an anti-Legionella antibody. Thus, the possibility that the MPAT method might include cross-reactivity with other organisms was not completely neglected. Fifth, the causations between the use of a 24HHWB and the elevated antibody titers against Lp SG 5 and Lp SG 6 were not entirely clarified because of the cross-sectional design of the present study. Consequently, further investigations are required to confirm our findings.

Despite the limitations, the current study seems to demonstrate subclinical Legionella sensitization, possibly due to the use of a 24HHWB. Although the 24HHWB users should be aware of the possibility of Legionella infection and keep their bath water as clean as possible, 24HHWB use did not necessarily lead to clinically apparent legionellosis. On the other hand, the 24HHWB manufacturing corporations must develop better cleaning systems to decrease the inhabiting number of legionellae. The 24HHWB manufacturing and marketing corporations in the present study improved their cleaning systems after learning of the results of this investigation. It is also necessary to consider other psychological, environmental, and lifestyle factors that seem to have causal relations to Legionella infection. We believe this study offers valuable suggestions concerning Legionella infection caused by environmental and domestic water systems, as well as other comprehensive factors.

Acknowledgments: This work was funded by a grant from the Foundation for Total Health Promotion, Tokyo, Japan. The authors thank Megumi Matsuo, Shizuko Hirayama, Teruhiko Fujino, and Keiji Mizuguchi (Showa Manufacturing Co. Ltd.), and Tadashi Takeuchi (Sanwa Service Co. Ltd.) for their help in the investigation, and Prof. Uki Yamashita (Department of Immunology, School of Medicine, University of Occupational and Environmental Health) for helpful comments on immunological findings.
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