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Occurrence of *Legionella* in hot water systems of single-family residences in suburbs of two German cities with special reference to solar and district heating

Werner Mathys*, Juliane Stanke, Margarita Harmuth, Elisabeth Junge-Mathys

Institute for Hygiene, University of Muenster, Robert-Koch-Str. 41, 48129 Muenster, Germany

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Dedicated to the memory of University Prof. Dr. Friedrich Tiefenbrunner, Innsbruck, Austria

Abstract

A total of 452 samples from hot water systems of randomly selected single family residences in the suburbs of two German cities were analysed for the occurrence of *Legionella*. Technical data were documented using a standardized questionnaire to evaluate possible factors promoting the growth of the bacterium in these small plumbing systems. All houses were supplied with treated groundwater from public water works. Drinking water quality was within the limits specified in the German regulations for drinking water and the water was not chlorinated. The results showed that plumbing systems in private houses that provided hot water from instantaneous water heaters were free of *Legionella* compared with a prevalence of 12% in houses with storage tanks and recirculating hot water where maximum counts of *Legionella* reached 100,000 CFU/100 ml. The presence of *L. pneumophila* accounted for 93.9% of all *Legionella* positive specimens of which 71.8% belonged to serogroup 1. The volume of the storage tank, interrupting circulation for several hours daily and intermittently raising hot water temperatures to $>60\,^{\circ}C$ had no influence on *Legionella* counts. Plumbing systems with copper pipes were more frequently contaminated than those made of synthetic materials or galvanized steel. An inhibitory effect due to copper was not present. Newly constructed systems (<2 years) were not colonized. The type of hot water preparation had a marked influence. More than 50% of all houses using district heating systems were colonized by *Legionella*. Their significantly lower hot water temperature is thought to be the key factor leading to intensified growth of *Legionella*. Although hot water systems using solar energy to supplement conventional hot water supplies operate at temperatures $3\,^{\circ}C$ lower than conventional systems, this technique does not seem to promote proliferation of the bacterium. Our data show convincingly that the temperature of the hot water is probably the most important or perhaps the only determinant factor for multiplication of *Legionella*. Water with a temperature below $46\,^{\circ}C$ was most frequently colonized and contained the highest concentrations of legionellae. It is evident that the same factors affecting colonization by *Legionella* in large buildings also exist in small residential water systems. If temperatures are low there is no difference between large and small systems and Legionella counts are high in both. Since private residences are an important source of community-acquired legionellosis, these findings emphasize the need for preventive control measures in small residential buildings. In some situations it may be necessary to install filtration devices at the point-of-use.

Keywords: *Legionella*; Solar heating; District heating; Private homes; Hot water; Temperature

*Corresponding author. Tel.: +49 251 8355376; fax: +49 251 8355341.
E-mail address: mathys@uni-muenster.de (W. Mathys).

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Introduction

Legionellae exist as part of the natural microbial flora of many aquatic ecosystems such as surface and ground waters (Riffard et al., 2001). Very low concentrations of legionellae in natural habitats can increase markedly in man-made hot water systems where water temperatures are below 55 °C. Hot potable water (30–55 °C) that is dispersed by shower-heads, faucets etc. (Marrie et al., 1992) is the most common source of nosocomial (Mathys et al., 1999) and community-acquired legionellosis (Pedro-Botet and Sabria, 2005) worldwide.

There are numerous reports of colonization of warm water systems in buildings such as hospitals, nursing homes and large apartment buildings whose complex structure is believed to provide optimal conditions for the growth of the bacteria (Lück et al., 1993; Codony et al., 2002). As a consequence, technical guidelines have been published (DVGW worksheet W 551, 2004; ASHRAE standard, 2000) which focus mainly on warm water systems in large buildings. On the other hand, and especially so in Germany, little is known about the growth of *Legionella* in small residential units where the volume of stored warm water is either relatively small or absent as in instantaneous, point-of-use water heaters (Codony et al., 2002; Arnow et al., 1985).

The aim of the study reported here was to investigate the extent to which *Legionella* contamination in hot water systems supplying only a single family is a potential source of community acquired legionellosis and to evaluate factors promoting the growth of the bacterium in these small hot water systems.

Materials and methods

Geographical areas studied

The areas studied comprised the city suburbs of Münster and Bielefeld in North-Rhine-Westphalia, Germany. A total of 452 single family houses, a very common type of dwelling in this region, were randomly selected for the study. All houses were supplied by treated ground water from the public water works. The water delivered to all households was of drinking water quality, non-chlorinated and analysis results were within the limits of the German drinking water regulations. Sampling took place from April 2000 to February 2003.

Sampling

Samples of hot water were drawn in each house into a 1 l sterile glass bottle, in most cases from a hot-water faucet in the bathroom. To avoid the effect of contamination from distal sources (e.g., tap), we removed all devices from the tap, heated the tap with a gas burner and discarded the first 5 l of hot water before sampling. The temperature of the sampled water was measured using an electronic thermometer (TFF 200, ebro, Germany) and samples sent within 3 h to the laboratory for processing.

Analysis for copper

Copper was measured in an acidified sample (1% nitric acid) using flame atomic absorption spectroscopy (Solaar 939, Thermo, Dreieich, Germany).

Microbiological analysis

Samples of 100 ml of water were concentrated by membrane filtration according to ISO 11731-2 (2004) using a 0.45 μm pore size cellulose nitrate filter (Millipore, Molsheim, France) and treated with acid buffer. The filter and two 1 ml aliquots of the original sample were placed on selective agar plates (MWY-agar supplemented with 3 g/l glycine, 50,000 IE polymyxin-B, 1 mg/l vancomycin, 80 mg/l anisomycin, Heipha, Germany) and incubated for 14 days at 36 °C in a humidified environment. Suspected colonies were subcultured on buffered charcoal yeast extract (BCYE-α, Heipha) agar (with cysteine) and charcoal yeast extract agar (cysteine-free). Colonies growing only on BCYE with cysteine were tested for *L. pneumophila* serogroup 1, *L. pneumophila* serogroup 2–14 and *Legionella non-pneumophila* using the agglutination test (Legionella Latex Test, Oxoid, Wesel). Counts are given as the number of colony forming units (CFU) per 100 ml of water.

Questionnaire

A detailed standardized questionnaire was developed to evaluate risk factors possibly associated with colonization: age of building, type of hot water preparation, volume of storage tank, material used in the construction of the plumbing system, mode of operation.

Statistical analysis

The statistical analyses including analysis of variance (ANOVA), determination of averages, medians, percentiles, standard deviations (SD) and linear regression were performed using the Statgraphics Plus statistics pack (Statpoint Inc., USA).

Results and discussion

All plumbing systems in houses using point-of-use, instantaneous water heaters were free of *Legionella*. The mean temperature of the hot water in these houses was considerably higher than in households using hot-water storage tanks (Table 1). This finding is in agreement with the investigations of Martinelli et al. (2000) who observed significantly lower rates of colonization in systems with instantaneous devices compared with those employing storage tank systems. In the further analysis of the data we therefore excluded households using point-of-use heaters.

Legionella was found in 12% of hot water systems of households using a storage tank and recirculation. This rate of colonization is considerably less than in hot water systems of large buildings (Lück et al., 1993; Arnow et al., 1985; Borella et al., 2005). However, in small hot water systems, the maximum bacterial counts ranged up to 100,000 CFU/100 ml, a value comparable with that of heavily colonized hot water systems in large buildings. The counts in the hot water systems of single households were considerably higher than those reported by Arnow et al. (1985) for apartment buildings and those of Borella et al. (2004) for private residences in Italy. In other studies the prevalence of *Legionella* colonization in single households ranged from 6.4% in Pittsburgh (Stout et al., 1992) to 32.7% in Quebec (Alary and Joly, 1991) and 8% in European houses (Tiefenbrunner et al., 1993).

Most (93.9%) *Legionella* positive specimens were identified as *L. pneumophila*, 71.8% of which belonged to serogroup 1 (Table 1). A similarly high proportion of colonization with *L. pneumophila* SG 1 has been reported by others (Borella et al., 2004; Pedro-Botet et al., 2002).

This and other similar studies demonstrate that *L. pneumophila* is a common contaminant in residential hot water systems and the main factors promoting growth of the bacterium need to be identified. Examination of the material used in the construction of the plumbing systems of single households (Table 2) showed that water systems with copper pipes were colonized significantly more often than those with galvanized steel or plastic pipes, despite the fact that the temperature of the hot water in these systems was similar. Also, there was a positive correlation between the concentration of copper in hot water and growth of *Legionella.* Hot water systems containing >0.5 mg Cu/l (Table 2) were more frequently colonized and had a higher degree of colonization than those with copper concentrations of 0.5 mg/l or less. This is in accordance with the findings of Tiefenbrunner et al. (1993) for single family residences. These authors were able to isolate legionellae only from hot water systems using copper pipes and found no correlation between chemical constituents in the hot water and the incidence of *Legionella*. On the other hand, Borella et al. (2004) found an inverse relationship between copper levels and *Legionella* counts indicating that in their study copper concentrations had an inhibitory effect on the growth of these bacteria.

The volume of the storage tanks (Table 2) seems not to be an important factor for growth of *Legionella*. Hot water systems with small tanks (<150 l) tend to show less colonization than larger ones. Hot water systems in houses with relatively new plumbing systems (<2 years old) were not colonized despite the fact that warm water temperatures did not differ from those in older systems.

Table 1. Descriptive data on the occurrence of *Legionella* in hot water systems of single family residences

	Houses with point-of-use instantaneous water heaters ($n = 52$)			Houses with hot water storage tanks and recirculation of hot water ($n = 400$)		
	Legionella (CFU/100 ml)	Percentage of *Legionella*-positive specimens	Temperature of hot water (°C)	*Legionella* (CFU/100 ml)	Percentage of *Legionella*-positive specimens	Temperature of hot water (°C)
Mean	0	0	54.9	387	12.0	50.6
Median	0		54.0	0		50.2
Maximum	0		62.0	100,000		70.0
Standard deviation	0		3.7	5040		7.1
Lower quartile	0		52.0	0		45.4
Upper quartile	0		58.0	0		55.4
Species					*L. pneumophila*: 93.9% *L. non-pneumophila*: 6.1%	
Serogroups of *L. pneumophila*					Serogroup 1: 71.8% Serogroup 2–14: 28.2%	

Table 2. Analysis of the influence of material, copper concentration in water, volume of storage tank and age of property/system on growth of *Legionella* in hot water systems with recirculation in single family residences using analysis of variance (ANOVA); SD = standard deviation, $p<0.05$ means statistically significant at the 95% confidence level

	N	Mean count (CFU *Legionella*/100 ml) ± SD	Percentage of *Legionella* positive specimens ± SD	Mean of contamination level (0 = <1; 1 = 1–99, 2 = 100–999, 3 = 1000–9999, 4 = ⩾10,000 CFU *Legionella*/100 ml) ± SD	Mean of hot water temperature (°C) ± SD
Factor: material of the hot water plumbing system					
Copper	245	609 ± 6432	17.1 ± 37.8	0.37 ± 0.87	51.0 ± 7.1
Synthetics	74	19 ± 151	2.7 ± 27.0	0.07 ± 0.42	49.5 ± 6.8
Galvanized steel	30	1 ± 6	3.3 ± 18.2	0.03 ± 0.18	50.5 ± 9.4
p-value		0.64	<0.05	<0.05	0.39
Factor: concentration of copper					
⩽0.5 mg/l	153	10 ± 102	2.0 ± 13.9	0.04 ± 0.30	51.6 ± 8.3
>0.5 mg/l	44	2395 ± 15063	15.9 ± 37.0	0.41 ± 1.02	50.0 ± 8.3
p-value		0.052	<0.05	<0.05	0.25
Factor: volume of hot water storage tank					
<150 l	57	58 ± 248	12.3 ± 33.1	0.23 ± 0.68	50.9 ± 7.0
150–299 l	209	620 ± 6926	16.3 ± 36.9	0.34 ± 0.85	50.6 ± 7.2
⩾300 l	36	578 ± 1856	16.7 ± 37.8	0.44 ± 1.03	50.0 ± 7.2
p-value		0.81	0.75	0.46	0.86
Factor: age of house/plumbing system					
<2 years	45	0 ± 0.0	0.0 ± 0.0	0.00 ± 0.00	49.1 ± 6.6
⩾2 years	352	440 ± 5372	13.6 ± 34.3	0.30 ± 0.80	50.5 ± 7.6
p-value		0.58	<0.05	<0.05	0.25

It can be concluded that legionellae are transported into the residential systems by the public water supply and that the presence of *Legionella* can only be detected after an interval. Of the 45 new and not yet colonized hot water systems, 16 had copper pipes and 24 had plastic pipes showing no evidence that copper is the cause of the – up to 2 years – delay in the growth of *Legionella* as claimed by van der Kooij et al. (2005) on the basis of data from model hot water systems.

Neither interrupting the hot water circulation for some hours at night, nor raising the temperature of the whole hot water system on a regular basis (mostly weekly) to 60 °C had a significant effect on *Legionella* counts. On the contrary, raising hot water temperatures to 60 °C only periodically and for very short time intervals seems to favour growth of *Legionella* and cannot be recommended from the results obtained in this study.

Contamination by *Legionella* is strongly influenced by the type of hot water production. The bacterium could be isolated in more than 50% of houses that heat water using heat exchangers supplied by district heating systems compared to only 5.5% of houses with conventional hot water systems (Table 3). Hot water temperatures in plumbing systems using district heating were significantly lower (47.9 °C versus 50.7 °C) than in other systems, and this is likely to be the main factor promoting growth of *Legionella*. Similar high levels of colonization by *Legionella* in hot water systems with district heating have also been reported by Martinelli et al. (2000).

There are no published reports on the possible influence of solar heating on the growth of *Legionella* in hot water systems. We expected such systems to be more frequently colonized by *Legionella* because of the anticipated lower temperature of the hot water. However, although the supportive use of solar energy (dual storage tanks) produces hot water which is 3 °C lower (47.4 °C versus 50.7 °C) than in the other systems, we found no evidence for increased colonization (Table 3). On the contrary, these alternative systems are colonized to a lesser degree than conventional systems suggesting that high elevations in water temperature in the storage tanks of solar systems inhibits the growth of *Legionella*.

Our data show convincingly that the temperature of the hot water is an independent and probably the most important determinant for the multiplication of *Legionella* (Table 4). Water with a constant temperature below 46 °C was most frequently colonized and contained the highest concentrations of legionellae. On the other hand, no legionellae were isolated from hot water systems with a constant temperature of 60 °C or higher. The strong correlation between the temperature of the

Table 3. Analysis of the influence of intermittent high temperatures, interruptions in circulation and mode of heating on growth of *Legionella* in hot water systems with recirculation in single family residences using analysis of variance (ANOVA); SD = standard deviation, $p<0.05$ means statistically significant at the 95% confidence level

	N	Mean count (CFU *Legionella*/100 ml) ± SD	Percentage of *Legionella* positive specimens ± SD	Mean of contamination level (0 = <1; 1 = 1–99, 2 = 100–999, 3 = 1000–9999, 4 = ⩾10,000 CFU *Legionella*/100 ml) ± SD	Mean of hot water temperature (°C) ± SD
Factor: interrupting hot water circulation for more than 6 h daily					
No	126	170 ± 611	14.3 ± 35.1	0.32 ± 0.85	48.9 ± 7.1
Yes	246	542 ± 6413	12.1 ± 32.8	0.26 ± 0.75	51.2 ± 7.6
p-value		0.52	0.57	0.65	<0.05
Factor: intermittently raising hot water temperatures >60 °C					
No	284	166 ± 790	13.4 ± 34.1	0.29 ± 0.78	50.4 ± 7.8
Yes	55	1946 ± 13475	16.4 ± 37.3	0.36 ± 0.93	50.1 ± 6.9
p-value		0.03	0.56	0.53	0.84
Factor: type of hot water preparation					
No district heating	343	340 ± 5414	5.5 ± 22.9	0.12 ± 0.53	50.7 ± 7.5 (median = 50.6)
Using district heating	57	667 ± 1384	50.8 ± 50.4	1.10 ± 1.22	47.9 ± 6.4 (median = 46.9)
p-value		0.65	<0.05	<0.05	<0.05
Without solar energy	352	409.4 ± 5355	13.1 ± 33.7	0.28 ± 0.77	50.7 ± 7.6 (median = 50.6)
Using solar energy	48	223 ± 1230	4.2 ± 20.1	0.13 ± 0.60	47.4 ± 5.3 (median = 47.3)
p-value		0.81	0.07	0.188	<0.05

Table 4. Analysis of the influence of water temperature in hot water systems with recirculation in single family residences on growth of *Legionella* using analysis of variance (ANOVA); SD = standard deviation, $p<0.05$ denotes a statistically significant difference at the 95% confidence level

Level of hot water temperature	N	Mean count (CFU *Legionella*/100 ml) ± SD	Percentage of *Legionella* positive specimens ± SD	Mean of contamination level (0 = <1; 1 = 1–99, 2 = 100–999, 3 = 1000–9999, 4 = ⩾10,000 CFU *Legionella*/100 ml) ± SD	Maximum count (CFU *Legionella*/100 ml)
1 = ⩽45 °C	78	1756 ± 11352	28.2 ± 45.3	0.69 ± 1.18	100,000
2 = 46–54 °C	225	73 ± 372	9.8 ± 30.0	0.19 ± 0.63	3600
3 = 55–59 °C	56	23 ± 161	7.1 ± 26.0	0.13 ± 0.51	1200
4 = ⩾60	41	0	0 ± 0.0	0.0	0
p-value		<0.05 for level 1—other levels	<0.05 for level 1–other levels	<0.05 for level 1–other levels	

hot water and *Legionella* counts is shown in Fig. 1, where there is maximum growth at temperatures between 30 and 46 °C. The figure also shows that at temperatures higher than 55 °C there is a break point, and this finding agrees with observations by other investigators who report that the range of 55–60 °C is a critical temperature region above which the proliferation of legionellae in the hot water systems of larger buildings is inhibited (Darelid et al., 2002; Arnow et al., 1985). Studies on the hot water systems of hospitals have identified a strong association between colonization by *Legionella* and the temperature of the water (Plouffe et al., 1983). Several authors have demonstrated that lower hot water temperatures are also closely associated with the contamination of domestic hot water systems. In surveys from Pittsburgh (Stout et al., 1992; Lee et al., 1988) and Quebec (Alary and Joly, 1991), the hot water temperature at both the distal site and the hot water tank were significantly lower in homes which were *Legionella*-positive than in homes free of *Legionella*. Codony et al. (2002) report much greater contamination at temperatures below 50 °C.

From these reports and from our results it is clear that the factors responsible for *Legionella* colonization in hot

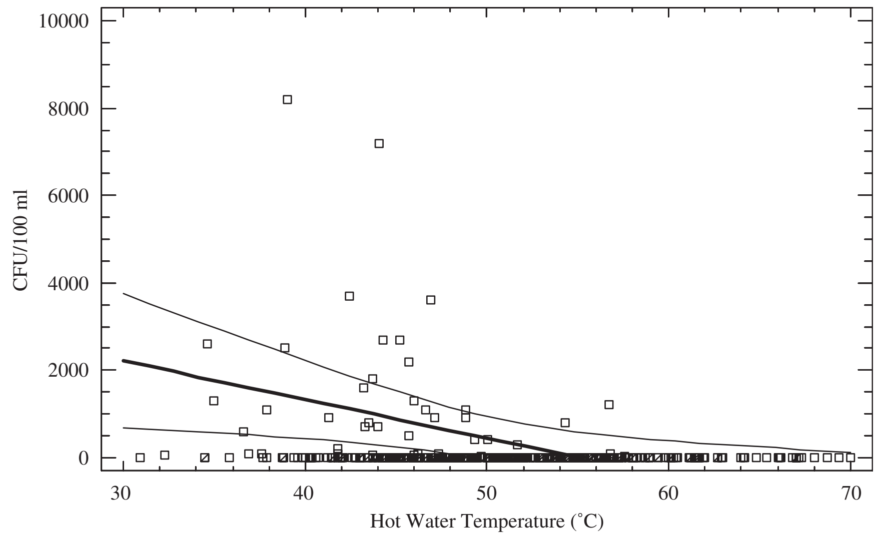

Fig. 1. Linear regression showing the relationship between the concentration of *Legionella* and water temperature in hot water systems with recirculation (black: regression line, dotted lines: confidence limits at the 95% confidence level).

water systems of large buildings also exists in smaller residential systems. Low temperatures favour growth of legionellae in both large and small hot water systems, and very high counts present a legionellosis risk for elderly and immuno-compromised members of the community.

The incidence of community-acquired legionellosis is considerably underestimated. The introduction of the *Legionella* urinary antigen test dramatically increased the number of cases detected (Pedro-Botet and Sabria, 2005). Although an infrequent cause of pneumonia in the past, legionellosis now ranks among the three most frequent causes of community-acquired pneumonia of bacterial origin (Pedro-Botet and Sabria 2005).

Private residences such as flats, apartments and single family homes are an important source of community-acquired legionellosis (Bornstein et al., 1986; Lim et al., 2003; Roig et al., 2003; Verhoef et al., 2004; Pedro-Botet et al., 2002; Straus et al., 1996). Prospective clinical studies have demonstrated a direct link between the presence of the bacterium in the residential water supply and the occurrence of disease (Stout et al., 1987). These authors concluded that the overall risk of acquiring Legionnaires′disease from exposure to the bacterium in residential water systems is generally low, but that some individuals, because of their low health status, are at greater risk, e.g., those who have recently undergone organ transplantation (Sax et al., 2002; Chen et al., 2002). Persons at risk would clearly benefit from preventive measures which reduce the proliferation of *Legionella* in hot water systems or which remove the bacteria at the point-of-use, e.g., by filtration devices. Such point-of-use devices have proved to be highly effective in minimizing the risk of infection in hospitals (Mathys et al., 1999) and could be installed in private homes during the period when the health status of a resident is severely compromised.

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