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THE COLONIZATION OF HOT WATER SYSTEMS BY *LEGIONELLA*

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Abstract: This study surveyed *Legionella* in 805 samples taken from 18 hot water systems under operating conditions. The results were analyzed and discussed in relation to water temperature, legislative requirements and optimization of the systems. The temperature of most samples (71%) ranged from 45–60°C. The highest levels of colonization by *L. pneumophila* were found at water temperatures from 30–35°C. At temperatures above 50°C there was a large decrease in the number of positive samples, as well as the number of *Legionella* in individual samples. However, *L. pneumophila* was found in some samples having a temperature of 55–60°C. These results indicate that the legislative requisite temperature of 50°C for hot water systems is insufficient. A system operating temperature of 55°C might be a better optimum, given the economical and safety limitations of temperatures as high as 60°C. If it is impossible or ineffective to use classical method superheating (70–80°C) then it is necessary take into account the chemical decontamination of water.

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INTRODUCTION

The aim of this study was to survey the frequency of occurrence and numbers of *Legionella* in hot water systems in a range of types of buildings, in relation to the legislative requirements of water temperature and optimization of the systems, the econmics of water-heating costs and the safety factors inherent in higher water temperatures.

Elevated water temperature is one of the methods used to eradicate *Legionella* from hot water systems. It is well known that water temperature has a large influence on the colonization of hot water systems by *L. pneumophila* [2, 4, 31].

The actual operational temperature in hot water systems is a balance between that required by national and international legislature, and the demands of safety, bearing in mind the economy of energy costs to heat the water. Different hot water systems can be found varying widely in their operational conditions in relation to temperature and physical organization of the system, both of which influence colonization by *Legionella*. Hot water systems in buildings are governed by the Decree 194/2007 cool. – in the Czech Republic that specifies a water in temperature of 45–60°C; §4 [7]. Safety considerations mean that temperatures as high as 60°C are never used, and in some specific systems, such as those described by Decree 137/1998; §59 cool., it is necessary to maintain a permanent water temperature lower than 50°C. For example, in schoolrooms the temperature of tap water must be lower than 45°C [5]. However, from a health standpoint, one should not decrease hot water temperatures below 50°C (optimally above 55°C), according to Decree 252/2004 cool.; Annex 2. [6], on the ground of minimization of the development of *Legionella*. National and European legislature [2002/91/EC] [9] both require hot water systems to be operated within certain economic boundaries, and the simplest means to accomplish this is to reduce the operational temperature of the water system. At the same time, it should be remembered that European Directive [98/83/EC] [12] demands a specified water quality destined for human consumption. Thus,

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there is a balance between the demands of safety and economics in relation to reducing the operational temperature of hot water systems.

This study surveyed *Legionella* in 805 samples taken from 18 hot water systems under operating conditions. The results were analyzed and discussed in relation to water temperature, legislative requirements and optimization of the systems.

The temperature of most samples (71%) ranged from 45–60°C. Highest levels of colonization by *L. pneumophila* were found at water temperatures from 30–35°C.

MATERIALS AND METHODS

Samples (805) were collected from outlet taps of 18 separate systems, following a period of one minute discharge, when the water temperature was also measured [10, 13]. Samples were transferred immediately to the laboratory of the Institute of Public Health, Centre of Hygienic Laboratories, Jihlava, Czech Republic, for analysis. Investigation of *Legionella* was carried out according to ISO 11731 Water quality – detection and enumeration of *Legionella* [10, 36]. Samples were cultured by routinely plating on BCYE agar-medium with charcoal, yeast extract and a selective suplement (medium includes N-(2 acetamido)-2-aminoethansulfonic acid – (ACES), L-cysteine and a selective suplement – glycine, polymyxin B sulphate, vancomycin hydrochloride and cycloheximide), and incubated at 36 ± 1°C [10, 36]. To confirm *Legionella*, individual colonies were inoculated on BCYE medium without cysteine, which is indispensable for *Legionella*, and further confirmed by using the latex agglutination test which discriminates between *L. pneumophila* serogroups 1 and 2–14, as well as other *Legionella* species.

The method EN 27888 was used for determination of electrical conductivity [34]. Water hardness was estimated in our laboratory according to CSN ISO 7980 using the atomic absorption spectrophotometric method [35].

The samples were taken from hot water systems varying from those in living, office and operational multi-storey buildings during the period 2003–2008.

Hot water systems in 4 different objects were analyzed in more detail. These objects were used for the same purpose. Every object included living, office and operational multi-storey buildings. Hot water systems in these 4 objects had similar configurations. In all buildings various materials were used for water pipes – plastic, zinc-coated and copper. In all 4 analyzed systems hot water was distributed from a central boiler-room to single buildings via conduit pipes by pumping.

Temperature of cold water supplied to systems was in the range 6–10°C. There is a very low probability of *Legionella* occurrence in a water supply with water temperature lower than 15°C [3].

Water temperature of samples was expressed in 5 degree of intervals from 20–65°C. According to legislative requirements [6, 13], positive samples were regarded as those which exceeded a limit of 100 cfu/100 ml.

RESULTS AND DISCUSSION

The problems of *Legionella* occurrence, its dependence on temperature and the restriction of its numbers in hot water systems are described in various papers. These have been based on work carried out partly under laboratory conditions on culture collections and on clinical cultures [21, 26, 31, 33], and partly on real systems [4, 8, 16, 18, 19, 27, 31]. All authors have identified that the occurrence of *Legionella* depends on temperature.

However, there remains the problem of determining the appropriate temperature to inactivate *Legionella* in functional hot water systems.

It is necessary to take into account those factors that influence the survival of *Legionella* in hot water systems. If bacterial cells find themselves at high density, they may communicate in a way that enables them to better survive in a stressful environment [16, 24, 32]. The influence of temperature has been seen not only on the survival, but also on the virulence of *Legionella*. Although different researchers have utilised various methods to assess virulence, all agree that there is an effect of temperatures, such as that which often occur in hot water systems [11, 22]. Number and virulence properties of *L. pneumophila* may be essential for the risk of *Legionella* infection [25]. Another influential factor is the presence of amoebae [24, 28]. Both water pH and temperature determine how effective *L. pneumophila* is at avoiding ingestion by bacteriolytic amoebae and thus survival of the bacteria in hot water. Minerals play an important role in bacterial metabolism, and it has been shown that a small amount of metals have a significant effect on the survival and growth of *L. pneumophila* in hot water systems. In contrast, higher quantities of minerals are toxic for cells [28]. Biofilm formation may also offer *L. pneumophila* a likely environment for survival and dissemination [1, 14, 23].

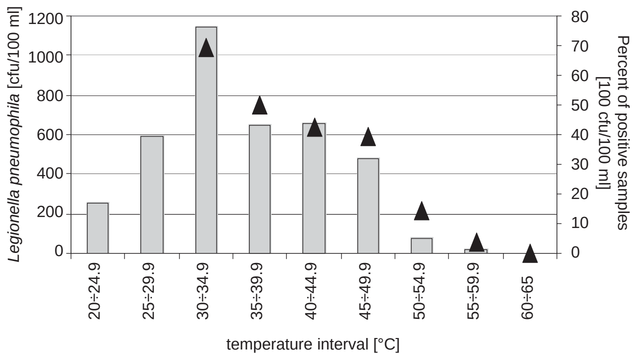

Figure 1. Mean concentration of *L. pneumophila* (cfu/100 ml) (▒) and percent of positive samples (▲) in the samples taken from the hot water systems, for each range of temperature.

Table 1. Number of samples and percentage of positivity of *L. pneumophila* for each range of temperature.

	20–24.9	25–29.9	30–34.9	35–39.9	40–44.9	45–49.9	50–54.9	55–59.9	60–65
number of samples	4	10	26	56	104	149	238	181	37
% positive samples (>100 cfu/100 ml)	*	*	69.2	50.0	42.3	39.6	14.3	3.8	0

* – for lower number of samples no interpret

As far as running hot water systems for human consumption are concerned, it is also necessary to take into account specific legislature relating to these systems, as well as the technical possibilities of the systems. Most such systems were constructed before such legislature had been enacted. The basic European legislature for water quality is given in European Directive 98/83/EC [12] on the quality of water intended for human consumption, which also forms the basis of European Guidelines and other national legislature. For example, European Guidelines for Control and Prevention of Travel associated Legionnaires' Disease 2005 [13], aimed at harmonizing procedures among member states. However, if procedures differs then national regulations take effect. In the Czech Republic, for hospital and other medical accommodation facilities, for shower rooms of public swimming-pools and bathing establishments, it is national Decree 252/2004 cool. that is valid [6]. According to this decree, the hot water temperature must not fall below 50°C (optimally above 55°C), within one minute at outlets, on the grounds of minimisaing the development of *Legionella* in the water distribution system. This legislative requirement stipulates an upper limit for *L. pneumophila* in hot water of 100 cfu/100 ml. Interpretation of obtained results were carried out on norm requirements [6, 13]. In the samples there was dominant occurrence *L. pneumophila* sg. 2–14 (87.1%) and a less numerous occurrence *L. pneumophila* sg. 1 (12.9%), which is in accordance with results presented by different laboratories [17, 29, 30]. The results are shown in Table 1 and in Figures 1–3.

The smallest number of samples collected were those with a temperature interval of 20–30°C (2%). Most samples had a temperature from 45–60°C (71%), precisely (30%) with a temperature of 50–55°C, (22%) from 55–60°C, and (19%) from 45–50°C.

Figure 1 also shows that the highest colonization by *L. pneumophila* was found in water with a temperature from 30–35°C. At temperatures above 50°C colonization with *Legionella* is under the requisite limit of 100 cfu/100 mls, according to legislature [6, 13]. Colonization further decreases with mounting temperature to 55–60°C. These results indicate that that water outlets (taps) should supply water with a temperature above 50°C to take into account the health aspects and requisite limits for the quantity of *Legionella* [6, 13].

In order to investigate the possibility of influencing the configuration of the hot water system, 4 systems were selected for further study. In fact, a large proportion (385/805) of the total samples were obtained from these 4 hot water systems. The results are summarized in Figure 2.

Figure 2 shows that colonization by *L. pneumophila* is generally less at temperatures above 50°C, as demonstrated by systems 1, 2 and 4. However, the properties of the hot water system also has a role to play, because the results for system 3 were different, in which the average colonization by *Legionella* did not fall below 100 cfu/100 ml until the temperature was 55–60°C (Fig. 3.).

The technical details of the 4 selected systems were essentially the same in relation to their design and construction, therefore some other factor must be responsible for the differences in *Legionella* colonization. One possibility is that the water properties differ, such as the conductivity or water hardness [20]. The mean values water conductivities of the systems 1–4 were 470, 210, 350 and 210 µS/cm, respectively. The mean values water hardness for systems

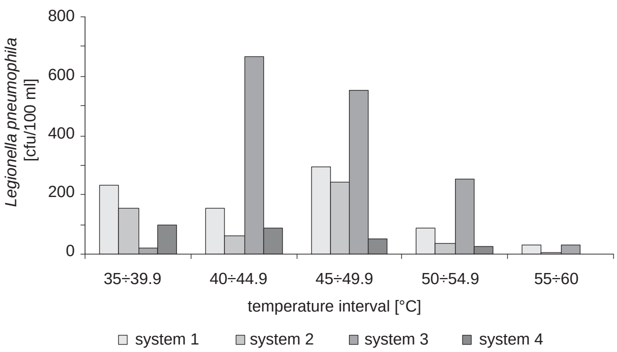

Figure 2. Mean concentration of *L. pneumophila* (cfu/100 ml) in the samples taken from the 4 selected hot water systems, for each range of temperature.

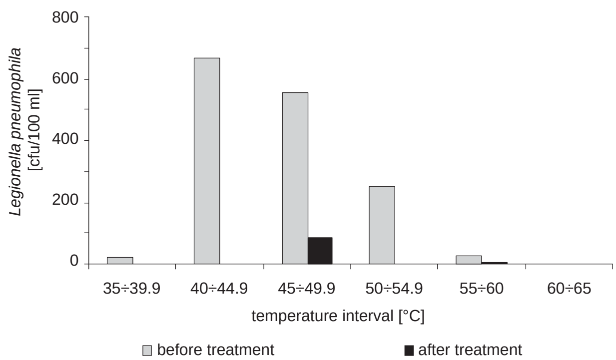

Figure 3. Mean concentration of *L. pneumophila* (cfu/100 ml) in the samples taken from system 3, before and after chemical decontamination in relation to temperature rise.

1–4 were 2.7, 1.2, 1.7 and 1.1 mmol/l, respectively. It was not possible to view directly the inside surfaces of pipe-work or to analyze deposits there directly. In the systems followed the relation between conductivity and water hardness of *Legionella* numbers was not approved.

As shown in Figure 3, *Legionella* was eliminated by water temperature increase and by the add-on of chemicals [21]. In system 3, shock hyperchlorination of chlorine 40–50 mg/l was used for a period of 2 hours. The method used for *Legionella* elimination depends on local specific conditions, objects area, technical conditions of pipeline, as well as on many other factors. Using this combined treatment, none of the 72 samples assayed exceeded the legislative limit of *Legionella* numbers.

These results exemplify the legitimacy of the requirements of the European Guidelines for hot water pipes, namely: "ideally to give a return temperature to the storage water heater of 55°C, but certainly not less than 50°C".

As concluded from the data in Figure 3, the minimum operating temperature in hot water system should be above 55°C. In such cases, as documented by the results, it is appropriate to perform more frequent verification tests for the presence of *Legionella* in hot water systems and to carry out some of the non-heat decontamination.

The maintaining of high temperature in pipe-work leads to heat losses. Fossil fuels were used as fueling for water heating, thus energy saving for water heating also has a side effect – emission reduction and economy of non-renewable resources. Except for human health monitoring of *Legionella* is important from the point of view of the optimal temperature in the water system.

CONCLUSIONS

Results document that in similar hot water systems *Legionella* may occur with different heat resistence. Estimation of *Legionella* occurrence in relation to temperature will help to determine optimal temperature range, and could support economical and ecological savings. The use of non-heat treatment will probably be necessary if the temperature around 60°C did not sufficiently supress *Legionella*'s numbers and it is not possible or appropriate to use the classical method of superheating (70–80°C). Among people, it is a extensive habit to use tap hot water for preparation of tea or coffee, it is therefore necessary to take into account the chemical decontamination of water. Using the heat method in contradiction to the chemical method one does not damage pipe-work and it is not dangerous for human beings.

Regular monitoring of *Legionella* is important for the optimization of hot water systems.

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