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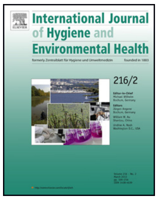

Modelling characteristics to predict *Legionella* contamination risk – Surveillance of drinking water plumbing systems and identification of risk areas

Sebastian Völker*, Christiane Schreiber, Thomas Kistemann

Institute for Hygiene and Public Health, University of Bonn, Sigmund-Freud-Str. 25, 53105 Bonn, Germany

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ABSTRACT

For the surveillance of drinking water plumbing systems (DWPS) and the identification of risk factors, there is a need for an early estimation of the risk of *Legionella* contamination within a building, using efficient and assessable parameters to estimate hazards and to prioritize risks. The precision, accuracy and effectiveness of ways of estimating the risk of higher *Legionella* numbers (temperature, stagnation, pipe materials, etc.) have only rarely been empirically assessed in practice, although there is a broad consensus about the impact of these risk factors.

We collected $n = 807$ drinking water samples from 9 buildings which had had *Legionella* spp. occurrences of >100 CFU/100 mL within the last 12 months, and tested for *Legionella* spp., *L. pneumophila*, HPC 20 °C and 36 °C (culture-based). Each building was sampled for 6 months under standard operating conditions in the DWPS.

We discovered high variability (up to 4 $\log_{10}$ steps) in the presence of *Legionella* spp. (CFU/100 mL) within all buildings over a half year period as well as over the course of a day. Occurrences were significantly correlated with temperature, pipe length measures, and stagnation. Logistic regression modelling revealed three parameters (temperature after flushing until no significant changes in temperatures can be obtained, stagnation (low withdrawal, qualitatively assessed), pipe length proportion) to be the best predictors of *Legionella* contamination (>100 CFU/100 mL) at single outlets (precision = 66.7%; accuracy = 72.1%; $F_{0.5}$ score = 0.59).

1. Introduction

Legionella poses a threat to public health. Legionnaires' disease is normally acquired when water which is contaminated with opportunistic pathogenic *Legionella* is inhaled, resulting in pulmonary colonization and subsequent infection. In the alveolar lung, the bacteria are phagocytized by macrophages within which they can multiply (Alleron et al., 2013). Engineered water systems like piped drinking water, cooling towers, fountains and humidifiers are known to be important sources for Legionellosis cases and outbreaks (Craun et al., 2010).

Legionella outbreaks have resulted in the classification of the disease as a public health priority in Germany, whereby a technical threshold level (TTL) for *Legionella* spp. (>100 colony forming units (CFU)/100 mL) is specified in the German drinking water ordinance (TrinkwV, 2001). Samples exceeding this level are classified as contaminated (DVGW, 2004), on the common sense basis that the presence of *Legionella* in higher proportions in any water sample signals a possible health threat (Hoebe and Kool, 2000). This policy is the consequence of the lack of a reliable dose–response model which can identify an unacceptable risk of infection. The TTL is regarded as a minimum level, above which technical interventions are required. A proposed cut-off level of 30% positive samples to estimate the risk of *Legionella* in drinking water plumbing systems (DWPS) (Lin et al., 2011) has recently been questioned because it is not sufficiently precise or sensitive (Pierre et al., 2014).

Possible sources of human infection are shower heads, faucets, spa baths, and evaporative condensers. Contaminated DWPS are common and were identified in public buildings in a national survey in Germany (Kistemann et al., 2010): approximately every second monitored hospital, every fourth nursing home, and every seventh sports facility in Germany was contaminated with *Legionella* at least once within a 4 year period (2003–2006). Because of the ubiquity of *Legionella*, the task is not to identify whether the bacteria are

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* Corresponding author.
E-mail addresses: Sebastian.Voelker@ukb.uni-bonn.de (S. Völker), Christiane.Schreiber@ukb.uni-bonn.de (C. Schreiber), Thomas.Kistemann@ukb.uni-bonn.de (T. Kistemann).

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present, but to identify which circumstances are risk factors promoting growth of the bacteria (Hoebe and Kool, 2000).

Legionella is dependent on various characteristics of the water supply. Although *Legionella* needs specific nutrients, the bacteria are generally able to survive in environments with very low nutrient concentrations (Flemming et al., 2014). Indirect influences on *Legionella* growth are the hardness and pH of water (Wingender and Flemming, 2011; Huang et al., 2010; Lin et al., 2002). Amongst others, pH influences the corrosive potential within a DWPS. Rough, porous or corroded pipe walls such as cast iron promote the development of biofilms. In half-technical trials *Legionella* prefers PVC and polyethylene pipes over stainless steel pipes under the same water conditions (Flemming et al., 2014), in single-family residences in suburbs in two cities in Germany Mathys et al. (2008) found plumbing systems with copper pipes were more frequently contaminated than those made of synthetic materials or galvanized steel.

Another risk factor is stagnation, which is defined as motionless water. In a system with no circulation pump, the water sits in the pipes until an outlet is used and the stagnant water is dispersed. Water stagnation may be one cause of proliferation of *Legionella* in PWH (hot drinking water) systems, because of the time bacteria obtain to grow, and the difficulty of maintaining high temperatures and disinfectant concentrations (Bartram et al., 2007). Hydraulic or physical dead legs and perpetual stagnation zones are ecological niches for bacterial growth. Biofilm formation is supported, and reduced oxygen and nutrient supply of the biofilm can result in a stress state in biofilm bacteria and a detachment of bacteria into the water (Flemming et al., 2014). For this reason numerous regulations call for the removal of any stagnating areas and other structural factors causing stagnation within the DWPS to control the proliferation of *Legionella* (Bédard et al., 2015: 54–56).

Many studies have shown a statistical correlation between temperature in DWPS and *Legionella* incidence and growth. *Legionella* finds optimal conditions at a temperature range between 35 °C and 46 °C (Buse et al., 2012). Temperatures consistently exceeding 60 °C can inhibit growth and detection of *Legionella* (Flannery et al., 2006; Völker et al., 2010). In contrast, PWH temperatures under 50 °C (Borella et al., 2004) or 55 °C (Mathys et al., 2008; Völker and Kistemann, 2015) significantly encourage growth of *Legionella*. Recent studies and guidelines stress the importance of appropriate hydraulic balance to ensure homogenous temperature regimes (Bédard et al., 2015).

Various other indirect risk factors can be found in the research. The distance of an outlet can also be an indirect risk factor, as the furthest outlets within a DWPS accumulate various risk factors such as stagnation, temperature loss, and increased biofilm formation (Borella et al., 2004). Flannery et al. (2006) identified a building's height of over 10 floors as a risk factor, whereas Borella et al. (2004) and Mathys et al. (2008) identified the age of a plumbing system.

Regulations rely on culture-based methods to assess the presence of *Legionella* (in CFU) in DWPS. Control focusses on eliminating favourable conditions for *Legionella* growth and eliminating risk factors. Regulations and guidance documents require a detailed description of the characteristics of the plumbing system along with environmental monitoring as first steps to evaluating the risk for *Legionella* contamination in the PWH system (Bartram et al., 2007). Data from a DWPS description is the foundation for identifying risk areas and interpreting monitoring results.

The precision, accuracy and effectiveness of ways of estimating the risk of *Legionella* contamination (temperature, stagnation, pipe materials, etc.) have only rarely been empirically assessed in practice. For the surveillance of DWPS and the identification of risk areas, there is a need for an early estimation of the risk of *Legionella* contamination within a building, using efficient and assessable variables/parameters to estimate hazards and to prioritize risks.

This paper aims to answer the following research questions:

– In a contaminated drinking water plumbing system, how intense does the presence of *Legionella* at an outlet change over short and long term intervals under standard conditions?
– Which outlet specific parameters are pertinent for an early prediction of the risk of *Legionella* contamination at single outlets?

2. Methods

2.1. Selection of buildings

To answer these questions we needed to select buildings where *Legionella* was already present in the DWPS. We cooperated with the local public health authority of Cologne to identify 9 buildings with *Legionella* occurrences of >100 CFU/100 mL over the previous 12 months, covering a wide range of building characteristics (e.g. type of use, use patterns, age, pipe materials, calorifier volumes) (Table 1). All calorifiers were heaters with storage and recirculation. The research was approved by the ethics committee of Bonn University (no. 268/10).

2.2. Data and sample collection

Before sampling the drinking water, we recorded the structures and characteristics of the DWPS in detail. This was done by systematic inspections of each building by trained and experienced observers who used relevant guidelines, an inspection protocol and knowledge gained in previous projects. We evaluated installation plans and operating condition records, and conducted repeated qualitative interviews with owners, technical staff, personnel, and users. Photographic documentation was applied. Any changes concerning water use during the half-year samplings were recorded in a log book. The inspections revealed several potential risk factors within specific risk categories (distance, temperature, stagnation, and other).

A variable "stagnation (qualitative) low withdrawal" was determined during the inspection of the buildings, where an individual stagnation risk was assigned to every outlet. This was assessed qualitatively by considering user traffic, number of users, frequency and regularity of use, and general remarks by staff, operators and users. Experienced observers can also identify possible stagnation from the state of the tap and its surroundings. The inspection requires hygiene experience and access to the information gathered from building managers, operators and users. We were not able to identify any parameters for measuring stagnation quantitatively (e.g. flow rate, temperature differences at sampling). However, we considered stagnation in practice and used a qualitative approach, which showed the significance of stagnation problems for *Legionella* contamination risk.

In buildings A and B some outlets were equipped with decentralized anti scald thermostatic mixing valves, so that PWM (mixed drinking water with PWH and PWC (cold potable water)) samples were drawn. All buildings were supplied by the same water company so incoming drinking water quality was assumed to be standardized for all buildings.

The pipe lengths in the investigated buildings differed strongly. Thus, the parameter "pipe length proportion" was calculated as a proxy for distance, using the pipe length between the calorifier and the furthest outlet in the DWPS as the 100% distance. For every single outlet the location in relation to the calorifier was determined using this parameter and the pipe lengths of different buildings were compared. We used the 9th decile of the pipe length proportion as parameter, because in this range showed significantly higher *Legionella* counts in an ANOVA analysis ($F = 2.2$; $p = 0.02$; $n = 559$).

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