

Occurrence of *Legionella Pneumophila* in Water Distribution Systems in Dental Practices in Latvia

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Abstract

Legionella pneumophila is the major agent of Legionnaire's disease and Pontiac fever. Legionellosis are normally acquired by inhalation or aspiration of legionellae from a contaminated environmental source. The pathogens present in dental unit waterline could be spread by aerosols created by dental equipment, presenting a risk for both a patient and members of a dental team.

The aim of the study was to investigate the occurrence of *Legionella* contamination of water distribution systems in dental practices. A total of 185 samples were collected from 74 dental practices. Samples were taken from water taps in dental practices (n = 79) and from dental unit waterline (n = 106). Overall, 20 out of 74 (27%) of dental practices were found *Legionella pneumophila* positive. Occurrence of *Legionella pneumophila* was significantly higher in samples from water taps than in samples from dental unit waterlines – 25 of 79 (25%) and 5 of 106 (5%), accordingly. From all *Legionella pneumophila* positive samples, 23 (92%) represented *L. pneumophila* serogroup 2-15. Two samples from dental practices in Rīga were contaminated with *L. pneumophila* serogroup 1. The level of contamination of samples from water taps ranged from 2×10^2 CFU/L to 1.1×10^4 CFU/L, and the level of contamination of samples from dental unit waterlines ranged from 3×10^2 CFU/L to 2.4×10^3 CFU/L. Both samples from water taps and dental unit waterlines were positive in three dental practices (4%). In two cases, samples from water taps were negative, though *Legionella pneumophila* was found in samples from dental unit waterlines.

The study showed no correlation between the year of installation of dental unit and occurrence of *Legionella pneumophila*, since it was isolated from samples taken from dental units installed in the year 1998 and up to the year 2013. *Legionella pneumophila* was found in one dental practice dental unit waterline with independent distilled water supplying system.

Keywords: *Legionella pneumophila*, dental units, Latvia.

Introduction

Legionella pneumophila is a facultative intracellular bacterium that multiplies within phagocytic cells [Diederer, 2008]. *Legionella pneumophila* is the major agent of Legionnaire's disease and Pontiac fever. Legionellosis are normally acquired by inhalation or aspiration of legionellae from a contaminated

environmental source. Moreover, *Legionella* strains can survive in moist environments for long periods and can be ubiquitously found in natural moist environments and man-made systems. In natural environments, *Legionella* is present in low density but its concentration can significantly increase in artificial habitats depending on the type of materials, on the presence of biofilms and available nutrients [Veronesi, 2007]. Bacterial biofilm in dental unit waterlines (DUWL) is a widespread problem [Tuttlebee, 2002]. Each dental chair unit (DCU) is equipped with an elaborate loom of interconnected narrow-bore flexible plastic tubing called dental unit waterlines (DUWLs), which supply water to all of the DCU-supplied instruments [O'Donnel, 2011]. The water used in DUWL acts as a coolant for high speed drills and as irrigant during dental procedures, most often it is supplied directly from municipal water supplies [Walker, 2004]. The general problem of microbial contamination of DUWL is well known [Atlas, 1995; Pankhurst, 1998]. Due to the texture and composition of the plastic tubing, microbial biofilms form readily, resulting as high bacterial contaminations in outputs water. The pathogens present in DUWL could be spread by aerosols created by dental hand-pieces, presenting a risk for both a patient and members of a dental team [Laheij, 2012].

Aim

The aim of the study was to investigate the occurrence of *Legionella* contamination of water distribution systems in dental practices, and whether dental treatment might pose a risk for patients and for dental team. In addition, analysis of hot tap water samples for presence of *Legionella* were carried out in order to assess the prevalence of *Legionella* in water supply system in the entire building.

Material and methods

A total of 185 samples were collected from 74 dental practices from February 2014 until June 2014. Samples were taken in Rīga (n = 71) and four regions of Latvia, randomly representing Latgale (n = 40), Kurzeme (n = 34), Vidzeme (n = 25) and Zemgale (n = 15). The samples were taken from water taps in dental practices (n = 79) and from dental unit waterline (n = 106). Water samples were collected in sterile bottles before routine working hours. At least two samples were collected in each dental practice, one sample from DUWL (cup filler) and one hot tap water sample from the sink in the same room. In dental practices, which have more than one or two dental chair units, up to 10 DUWL samples were taken per practice. During the sampling, the dental personnel was asked for additional information about the year of installation of DCU and methods for treatment of DUWL incoming water.

Isolation and identification of *Legionella pneumophila* was carried out by using standard ISO 11731. One litre of water sample was filtrated and concentrated using membrane filtration with 0.45 µm pore-size polyamide filter (Millipore, USA). The filter membranes were cut into pieces and resuspended in 5 ml sterile distilled water, then shaken for two minutes (Vortex Genie) and kept in room temperature for 10 minutes. A total of three 0.1 ml untreated, heat-treated and acid-treated aliquots of the sample were spread on Buffered Charcoal Yeast extract medium (GVPC, Oxoid, UK). The plates were incubated at 36 °C in a humidified environment for 10 days, and examined every day beginning on day 3. At least three characteristic colonies from each GVPC plate were selected for subculture onto plates Buffered Charcoal Extract agar medium with L-cysteine (BCYE, OXOID, UK) and Buffered Charcoal Extract agar medium without L-cysteine (BCYE-Cys, OXOID, UK) and incubated for at least 48 hours at 36 °C. Colonies grown on BCYE were subsequently identified by latex agglutination test (*Microscreen Legionella CE*, Microgen Biologics, UK). *Legionella* Rapid Latex Test Kit allows for separate identification of *L. pneumophila* serogroup 1 and serogroups 2-15 and identification of 10 non-*Legionella pneumophila* species. Colonies from all plates were counted, confirmed and the estimated number of *Legionella* was expressed as CFU/litre *Legionella* species and serogroup.

Microbiological analysis was carried out in Laboratory of Medical Microbiology (Institute of Food Safety, Animal Health and Environment "BIOR").

Results

Overall, 20 out of 74 (27%) dental practices were found *Legionella* positive (Fig. 1, Fig. 2). However, *Legionella* was not found in samples from dental practices in Zemgale. In other districts, the occurrence of *Legionella* ranged from 13% in Latgale up to 48% in Rīga (Table 1).

Samples were taken in different administrative districts of Rīga (Figure 2), where *Legionella* was found in 9 of 15 (60%) administrative districts.

Overall, *Legionella* was isolated in 25 out of 185 samples (14%). The occurrence of *Legionella* was significantly higher ($p = 0.04$) in samples from water taps than in samples from dental unit waterlines – 25 of 79 (25%) and 5 of 106 (5%), accordingly (Table 1).

From all *Legionella* positive samples, 23 (92%) represented *L. pneumophila* serogroup 2-15. Two samples from dental practices in Rīga were contaminated with *L. pneumophila* serogroup 1. The level of contamination of samples from water taps ranged from 2×10^2 CFU/L to 1.1×10^4 CFU/L, and the level of contamination of samples from dental unit waterlines ranged from 3×10^2 CFU/L to 2.4×10^3 CFU/L (Table 2).

Both samples from water taps and dental unit waterlines were positive in three dental practices (4%). In two cases, samples from water taps were negative, though *Legionella* was found in samples from dental unit waterlines.

Table 1. Occurrence of *Legionella* in samples from water taps and dental equipment in regions of Latvia

District	Number of dental practices, n	Number of practices with at least one positive sample, n (%)	Samples from water taps. Number of samples / positive samples, n (%)	Samples from dental unit waterline. Number of samples / positive samples, n (%)
Rīga	23	11 (48)	27 / 11 (41%)	44 / 3 (7%)
Latgale	16	2 (13)	16 / 2 (13%)	24 / 0 (0%)
Kurzeme	15	3 (20)	15 / 3 (20%)	19 / 2 (11%)
Vidzeme	13	4 (31)	14 / 4 (31%)	11* / 0 (0%)
Zemgale	7	0 (0)	7 / 0 (0%)	8 / 0 (0%)
TOTAL	74	20 (27)	79 / 20 (25%)	106 / 5 (5%)

* In two dental practices in Vidzeme, only tap water samples were taken.

Figure 1. Sampling points and *Legionella* positive results in Latvia

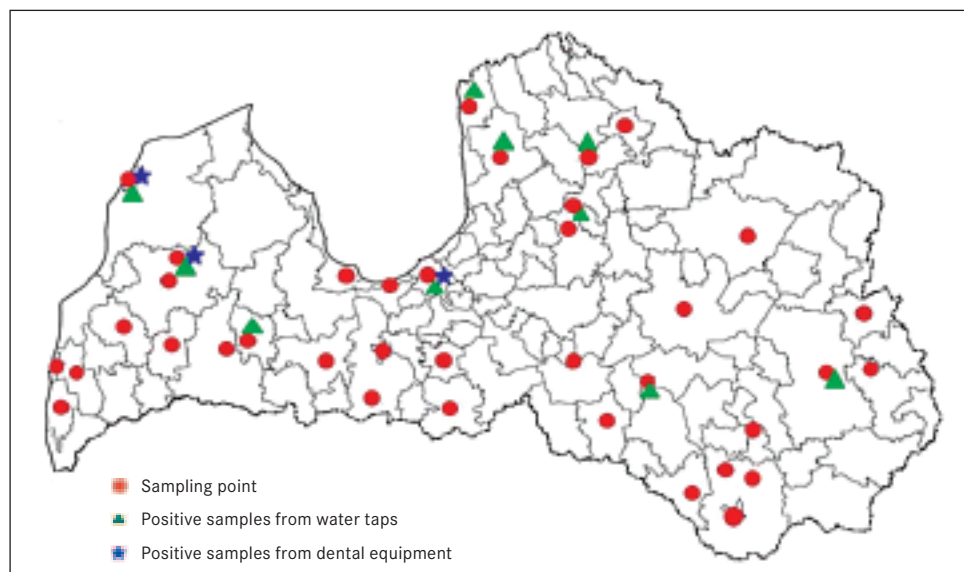


Figure 2. Sampling points and *Legionella* positive results in districts of Riga

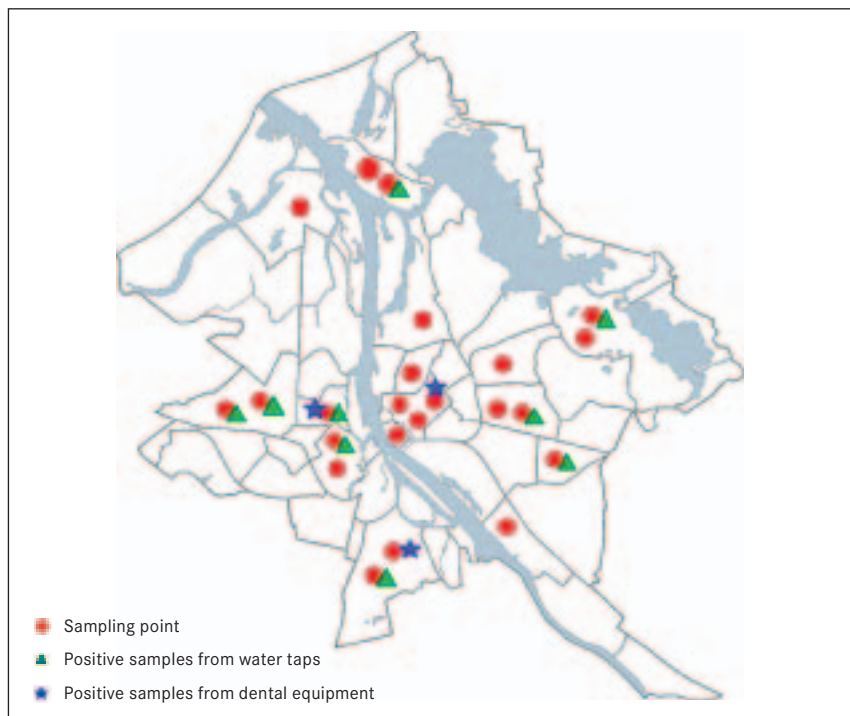


Table 2. *Legionella* positive samples and level of colonisation

ID number	District	Samples from hot water taps		Samples from dental unit waterline	
		CFU/L	Serogroup	CFU/L	Serogroup
19	Kurzeme	4×10^3	2-15	1×10^3	2-15
20	Vidzeme	6×10^3	2-15	ND	ND
21	Latgale	9×10^2	2-15	ND	ND
22	Vidzeme	3.5×10^3	2-15	ND	ND
36	Vidzeme	1×10^3	2-15	ND	ND
37	Vidzeme	6×10^2	2-15	ND	ND
38	Latgale	3×10^3	2-15	ND	ND
39	Kurzeme	8×10^2	2-15	3×10^2	2-15
46	Riga	7×10^3	2-15	ND	ND
47	Riga	1.1×10^4	2-15	ND	ND
49	Riga	8×10^3	1	ND	ND
50	Riga	ND	ND	2.4×10^3	2-15
51	Riga	2.5×10^3	1	ND	ND
54	Riga	4×10^2	2-15	ND	ND
58	Riga	8×10^2	2-15	ND	ND
61	Riga	2×10^2	2-15	ND	ND
		4×10^2	2-15	ND	ND
		9×10^3	2-15	1.1×10^3	2-15
66	Riga	7×10^2	2-15	ND	ND
68	Riga	6×10^3	2-15	ND	ND
70	Riga	ND	ND	1.2×10^3	2-15
74	Kurzeme	2×10^3	2-15	ND	ND

ND - not detected.

Table 3. Installation period of dental chair units included in sampling plan

District	Period of installation, years	Average age of DCU, years
Rīga	2000–2014	4.7
Latgale	2000–2009	8.3
Kurzeme	1998–2014	6.4
Vidzeme	1995–2014	7.5
Zemgale	1997–2013	6.9

The study showed no correlation between the year of installation of dental unit and the occurrence of *Legionella*, since it was isolated from samples taken from dental units installed in years between 1998 and 2013. Some DCUs use independent water reservoir bottles to provide distilled water to the DUWLs. Thus, *Legionella* was found in one dental practice DUWL with such water supplying system. The only method used for municipal water additional treatment, were filters. No influence of additional filters on occurrence of *Legionella* was observed.

Discussion

This study showed that 27% of dental practices had at least one *Legionella* positive sample. Overall, *Legionella* was found in 5 of 106 DUWL samples, which is significantly lower than in other countries with a higher average annual temperature, where the occurrence of *Legionella* in DUWL systems varied from 16.1% in Greece [Mavridou, 2006], 33% in South Africa [Singh, 2005], 33.3% in Italy [Montagna, 2006] and 86.7% in Jordan [Ma'ayeh, 2008].

Water temperature could be the main reason for significant differences. It is difficult to maintain cool water temperature below 20 °C in countries with high average air temperature. Optimum temperature range for proliferation of legionellae is 32–35 °C [Levesque, 2004; Wadovsky, 1985]; however, in Latvia, cold water temperature rarely exceeds 20 °C. In some countries with similar climate, the results may vary. No *Legionella* positive dental unit reservoir samples were found in Poland [Szymanska, 2004]; in London and Northern Ireland the prevalence of *Legionella* was very low (0.37%) [Pankhurst, 2003]; however, a significantly higher occurrence was observed in Sweden (15%) [Dahlen, 2009], Switzerland (20%) [Barben, 2009] and Germany (27.8%) [Arvand, 2013]. Differences in the occurrence of *Legionella* can be explained by different sampling strategies. In the retrospective study, DUWL samples were mainly taken from cup-fillers, while in other researches samples were taken from high-speed hand-piece tube, syringe or micromotors. It has been confirmed that cup-filler samples can be twice less contaminated with *Legionella* than samples from instrument channels [Arvand, 2013].

Some DCUs use independent water reservoir bottles to provide water to the DUWLs. These bottles were manually filled with distilled or sterile water.

One of *Legionella* positive samples was taken in dental practice, which does not use municipal water, but DCU is supplied by distilled water from a single reservoir. However, it does not protect against contamination. Even DUWL supplied by sterile or distilled water, at the moment of filling will become colonised to the same extent as those supplied by tap water. Once the bacteria gained access to the system, there will be enough nutrients from the plastic tubing and the turnover of the bacteria themselves to support biofilm growth. This does create difficulties for some practitioners, despite the use of sterile water source [Walker, 2004].

Our results showed no correlation between the year of installation of dental unit and the occurrence of *Legionella*; it was isolated from samples taken from dental units installed between years 1998 and 2013. Most DCUs often are not used for more than 12 hours per day, 5 days per week, and thus water stagnation is a significant contributory factor to DUWLs output water contamination [O'Donnell, 2011]. Historically, the majority of DUWL have been supplied by municipal tap water, which is still the case today in Latvia. With such systems, even within 5 days of installation, the microbial counts can reach 2.0×10^5 CFU/ml in the water at the distal outlets [Walker, 2004; Barbeau, 1996]. Complex design of dental chair equipment, resulting in the stagnation of water within the equipment lines where bacteria, including *Legionella*

pneumophila could proliferate within biofilm is a major factor affecting microbial contamination of water lines [Smith, 2002]. DCU manufacturers can significantly contribute to controlling the problem of DUWL biofilm [Coleman, 2007].

The occurrence of *L. pneumophila* was considerably higher in hot tap water (25%) compared to other European countries, where the occurrence of *Legionella* in water distribution systems varied from 22.6% in Italy [Borella, 2004], 26% in Germany [Zietz, 2001] to 30% in Finland [Zacheus, 1994].

A total of 15 dental practices, where *Legionella* was found in hot tap water samples, were not contaminated in DUWL. This may suggest that incoming municipal water could be a source of infection for DUWL biofilms, which is in accordance with previous studies [Valcina, 2013] and using other sampling strategies and methods of analysis, *Legionella* prevalence in DUWL could be higher. However, it has to be emphasised that the classical cultivation method used in this study did not allow determining the presence of non-cultivable legionellae [Delgado-Viscogliosi, 2005].

Statistically significant differences ($p = 0.02$) were observed in the distribution of *L. pneumophila* in different districts of Latvia. Zemgale was the only region where *Legionella* was not detected in any sample.

From all *L. pneumophila* positive samples, 8% represented *L. pneumophila* serogroup 1 and 92% *L. pneumophila* serogroup 2-15. Both cases of serogroup 1 were observed in Rīga, in territories, which received treated surface water. The data are consistent with results of other studies. In Poland, *L. pneumophila* 2-15 serogroup was isolated from 73% and serogroup 1 from 19.8% of *Legionella* spp. positive samples [Stojek, 2011], in Italy 75.6% and 22.6%, respectively [Borella, 2004].

Currently, only one case has been reported about an 82-year-old woman who died of Legionnaires disease in Italy in 2011 [Ricci, 2011]. Nevertheless, dental personnel and the increasing number of immunocompromised dental patients that present routinely at dental surgeries are being exposed to potentially opportunistic pathogenic bacteria through ingestion and inhalation of dental unit water [Walker, 2004]. The potential occupational hazard to a dental team is considered greater than that of the patient population due to sustained and daily contact with contaminated DUWL aerosols [Pankhurst, 2007].

Conclusions

1. Our study showed that several dental unit water lines contained *Legionella pneumophila* (5%), which poses a risk for both patients and dental team. However, the actual risk of legionellosis based on our results has to be studied further.
2. High contamination of hot tap water with *Legionella pneumophila* (25%) can indicate that incoming water may cause a threat to dental unit water line systems.
3. Regular monitoring of microbial contamination of dental unit waterlines is essential to control and reduce the microbial burden within dental unit water lines as well as to highlight the risk of occupational exposure in general dental practices.

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