OCCURRENCE OF *LEGIONELLA PNEUMOPHILA* IN POTABLE WATER SUPPLY SYSTEMS IN APARTMENT BUILDINGS IN RIGA AND EVALUATION OF SAMPLING STRATEGIES

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The prevention and control of legionellosis in water supply systems involves environmental comprehensive sampling. Our aim was to investigate occurrence of *Legionella* spp. in water supply systems in different administrative districts of Riga, and to find out if there exists a correlation between *Legionella* spp. positive cases and water source, type of sample, sampling point and temperature of hot water, received at the point of consumption. A total 467 samples were collected from 94 apartment buildings in different administrative districts of Riga. Water samples n=302) were taken from heating units in apartment buildings, from taps and shower heads in private apartments and sediment samples were taken in private apartments (n=165). Water temperature was measured during sampling. *L.pneumophila* was found in 50 out of 94 apartment buildings (53%). From all *Legionella* spp. positive samples, 19% represented *L.pneumophila* serogroup 1 and 81% *L.pneumophila* serogroup 2.-14.(15.). *L.pneumophila* was found in 44% of hot water samples from heating units and in 52% of hot water samples from private apartments. Data analysis confirmed observation that temperature of hot water significantly affects the frequency of *L.pneumophila* positive cases ($p<0,05$). *L.pneumophila* was found in 5% of cold water samples from heating units and in 17% of cold water samples from private apartments. *L.pneumophila* was isolated from 4% of sediment samples. However, investigation of sediment samples did not show presence of *Legionella* spp. in 79% of cases when at least one water sample from the same location was *L.pneumophila* positive.

Key words: *Legionella pneumophila*, drinking water, sampling plan

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INTRODUCTION

Legionellosis became actual in 60's - 80's of the 20th century. Legionellosis is caused by *Legionella pneumophila* and related bacteria (Stout et al. 1985). Clinical manifestations of the disease vary from mild fever (Pontiac's fever) to potentially lethal pneumonia (Legionnaire's disease) (Stout et al. 1992). Primary ecological niche of *Legionella* appears to be a parasite of protozoa, but humans may become secondarily infected after inhaling or aspirating organisms (Chiaraviglio et al. 2008). Main natural habitats of *Legionella* genus are freshwater and humid soil sources, but the major reservoirs are man-made aquatic environments, particularly warm water systems. They have been found in hot water taps and tanks, creeks, ponds, water cooling towers and evaporative condensers and whirlpool spas (WHO 2007). Human infection with *Legionella* spp. is known to result from the inhalation of aerosols (Ø5 mm) filled with numerous infectious bacteria (Fields et al. 2002, Bollin et al. 1985). Over 90% of legionellosis cases are caused by *Legionella pneumophila*. Most cases of legionellosis can be traced to man-made aquatic environments where the water temperature is higher than ambient temperature (Diederen 2008). Very low concentrations of legionellae in natural habitats can increase markedly in engineered hot water systems where water temperatures are below 55 °C (Mathys et al. 2008). After entering the water supply system, *Legionella* can be isolated at all stages of the system - from preparation to delivery to the consumer.

The prevention and control of legionellosis in water supply systems involves environmental sampling, among other measures. The data obtained from sampling constitute an important means of risk assessment and provide a valid basis on which to plan remedial and preventive actions (Ditommaso 2010). Significant increase of Legionellosis cases (from 0.13 cases per 100 000 residents in 2009 up to 2.20 cases per 100 000 residents in 2011) was observed in Latvia (Rozentale et al. 2011). Our aim was to investigate occurrence of *Legionella* spp. in water supply systems in different administrative districts of Riga, and to find out if there exists a correlation between *Legionella* spp. positive cases and water source, type of sample, sampling point and temperature of hot water, received at the point of consumption.

MATERIALS AND METHODS

Sample collection

Drinking water in Riga is supplied from two sources. Majority of consumers on the left bank of river Daugava in Riga receive treated surface water. However, the underground resource "Baltezers – Zaķumuiža" ensures centralized water supply to consumers residing on the right bank of the Daugava.

A total 467 samples were collected from 94 apartment buildings in different administrative districts of Riga from January 2011 through December 2012 (Fig. 1). Hot water samples were taken from heating units in apartment buildings (n=100), from taps and shower heads in private apartments (n=63). Cold water samples were taken from heating units (n=85) and from taps in private apartments (n=54). Sediment samples were taken from water taps and shower heads in private apartments (n=165). A total 1 litre of water per sampling site was collected in sterile bottles. Water temperature was measured in all sampling sites.

Microbiological analysis

Isolation and identification of *Legionella pneumophila* was carried out by using the ISO 11731 standard (Anonymus, 1998). A total one litre of water sample was filtrated and concentrated using membrane filtration with 0,45μm pore-size polyamide filter (Millipore, USA). The filter membranes were cut into pieces and resuspended in 5 ml sterile distilled water and shaken for two minutes (Vortex Genie) and kept in room temperature for ten minutes. Heat treatment and acid treatment were used to reduce the growth of other bacteria. A total three 0,1ml

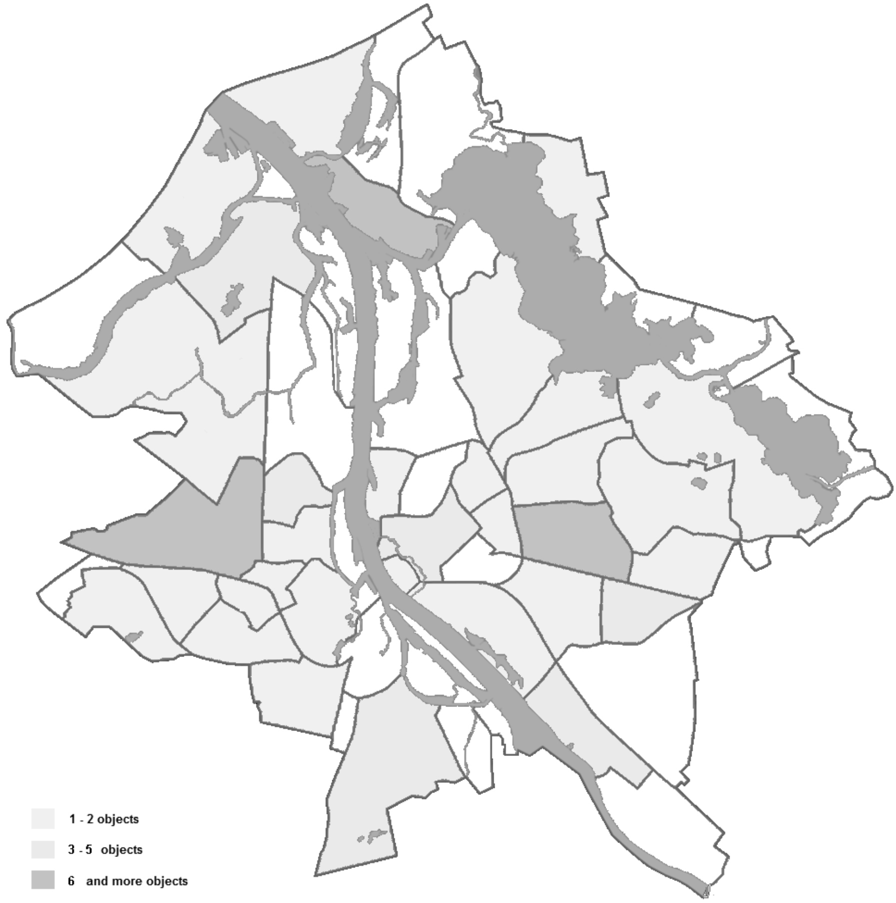

Fig. 1.Distribution of sampling sites in Riga city districts (Map: WikiMedia).

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 the day 3. At least three characteristic colonies from each GVPC plate were selected for subculture onto plates BCYE (Buffered Charcoal Extract agar medium with L-cysteine, OXOID, UK) and BCYE-Cys (Buffered Charcoal Extract agar medium without L-cysteine, OXOID, UK) and incubated for at least 48h at 36˚C. Colonies grown on BCYE were subsequently identified by latex agglutination test (*Legionella* Rapid Latex Test Kit, BIOLIFE Italiana S.r.l., ITALY). *Legionella* Rapid Latex Test Kit allows the separate identification of *L.pneumophila* Serogroup 1 and Serogroups 2-15 (until December 2011, there were included 2-14 serogroups) and identification of 10 non pneumophila *Legionella* species: *L.micdadei, L.bozemanii* 1&2, *L.dumoffii, L.longbeachae* 1&2, *L.jordanis, L.gormanii, L.anisa* and *L.feelei*. Colonies from all the plates were counted, confirmed and estimated number of *Legionella* were expressed as CFU/litre *Legionella* species and serogroup. Internal quality control, accuracy and recovery performance were established by using reference strains including *L.pneumophila* serogroup 1 (ATCC 33152, Microbiologics, USA) throughout the study, beginning from the microbiological control of culture media and until the simulation of real laboratory samples with appropriate level of target organisms and

Table 1. Occurrence of *L.pneumophila* in water samples and sediment samples

Type of sample/ sampling point	Number of samples	Number of positive samples	Positive samples, %
Hot water/heating unit	100	44	44
Hot water/apartments	63	33	52
Cold water/heating units	85	4	5
Cold water/apartments	54	11	20
Sediment samples/apartments	165	8	5

the inclusion of background flora. For external quality control periodically (at least once a year) is used proficiency testing schemes provided by Health Protection Agency (UK).

Data analysis

Data were analyzed with MS Excel based Data Analysis tool. Analysis of variance (one-way and two-way ANOVA) and post hoc tests were performed to determine possible significant differences for the occurrence of *Legionella pneumophila* depending on various factors. Correlation analysis was performed to assess significance of correlation between temperature of water and occurrence and level of *Legionella pneumophila* colonization.

RESULTS

Only one *Legionella* species – *Legionella pneumophila* was isolated during this study. *L.pneumophila* was found in 50 out of 94 apartment buildings (53%).

Data showed higher occurrence of *L.pneumophila* in samples from private apartments. *L.pneumophila* was found in 52% of hot water samples and in 17% of cold water samples from apartments, meanwhile 44% of hot water samples and only 5% of cold water samples from heating units were contaminated with *L.pneumophila* (Table 1).

Our data (Table 2) showed that mean difference of temperature of hot water between heating units and private apartments equals 6.4°C (Δ_{min}=8.0 °C; Δ_{max}=22.0 °C) and mean difference of temperature of cold water between heating units and private apartments equals 1.6°C (Δ_{min}=1 °C; Δ_{max}=12.0 °C).

Two-way ANOVA was performed to determine influence of water source and temperature of water on occurrence of *L.pneumophila*. Data analysis confirmed observation that temperature of hot water significantly affects the frequency of *L.pneumophila* positive cases (F=3.78 and p=0.04; $F>F_{crit}$ and $p<0.05$). Tukey's HSD post-hoc test indicated statistically significant differences in occurrence of Legionella at temperatures between 42 °C to 51°C.

Results of two-way ANOVA did not show statistically significant influence of water source on total amount of *L.pneumophila* positive samples ($p>0.05$). However, evaluating occurrence of each serogroup in different districts of Riga, statistically significant difference in occurrence of *L.pneumophila* serogroup 1 in samples from various water sources was observed. From all *Legionella* spp. positive samples, 19% represented *L.pneumophila* serogroup 1 and 81% *L.pneumophila* serogroups 2.-14.(15.) Results of one-way ANOVA showed that occurrence of *L.pneumophila* serogroup 1 is significantly higher in surface water (F=3.67 and p=0.03; F>Fcrit and $p<0.05$).

Correlation analysis was performed to evaluate correlation between water temperature and occurrence and colonization of *L.pneumophila*. Moderate correlation between temperature and

Table 2. Colonization of *L.pneumophila* and characteristics of water samples

Type of sample/ sampling point	Temperature, °C			Level of contamination, CFU/1L		
	Average	Min	Max	Average	Min	Max
Hot water/ heating unit	50.1	40.1	56.0	1.6×10^3	5.0×10^1	5.0×10^3
Hot water/ apartments	43.7	27.5	52.2	2.1×10^3	1.0×10^2	8.0×10^3
Cold water/ heating units	14.8	4.7	22.3	3.9×10^2	2.0×10^2	5.0×10^2
Cold water/ apartments	16.4	5.0	25.0	4.0×10^2	5.0×10^1	5.0×10^3

occurrence of *L.pneumophila* was observed, while analysis of temperature and level of colonization showed weak correlation.

L.pneumophila was isolated from 5% of sediment samples. However, investigation of sediment samples did not show presence of *Legionella* spp. in 79% of cases when at least one water sample from the same location was *L.pneumophila* positive. Thus, data of the study showed that analysis of sediment samples did not provide valuable information about contamination of water supply systems.

DISCUSSION

During this study *L.pneumophila* was found in 50 out of 94 apartment buildings (53%), which is significantly higher than in other European countries, where occurrence of *Legionella* in water distribution systems varied from 22,6% in Italy (Borella et al. 2004), 26% in Germany (Zietz et al. 2001) to 30% in Finland (Zacheus et al. 1994). The main reasons for these differences could be poor technical condition of apartment buildings and the unfavourable economical situation, which compels the population to spare water and energy. In this case, inhabitants request heating regulators to decrease the temperature of hot water systems leading an increased contamination (Rozentale et al. 2011). Optimum temperature range for proliferation of legionellae is 32-35 °C but they are able to proliferate up to 45 °C (Levesque et al. 2004, Wadovsky et al. 1985). Our data showed that contamination with *L.pneumophila* was observed much more frequently in water at temperatures below 45 °C. Meanwhile, no contamination was detected in samples at temperature 55 °C or higher and this finding agrees with observations from other studies who report that the range 55-60 °C is a critical temperature region above which the proliferation of legionellae in the water supply systems is inhibited (Darelid et al. 2002, Wadowsky et al. 1982).

From all *Legionella* spp. positive samples, 19% represented *L.pneumophila* serogroup 1 and 81% *L.pneumophila* serogroup 2.-14.(15.). These data are consistent with results of other studies. In Poland *L.pneumophila* 2-14 serogroup was isolated from 73% and serogroup 1 from 19.8% of Legionella spp. positive samples (Stojek et.al. 2011), in Italy 75.6% and 22.6% respectively (Borella et.al., 2004).

Statistically significant differences were observed in the distribution of *L.pneumophila* serogroup 1 in samples from various water sources in different districts of Riga. Most cases of serogroup 1 were observed in territories which received treated surface water. Only 7% of *L.pneumophila* serogroup 1 were isolated from samples taken in territories which are provided mostly with underground water. However these cases there may be caused by interblending with surface water, because water supply systems in Riga are not separated. Thus in central part of Riga residents can receive both treated surface water and water from underground sources. Theoretically, it is possible that at any point, water from one or other water sources would also flowed in remote supply networks.

L.pneumophila was isolated from 48% of samples taken in territories which receive underground water. It indicates that underground water which is delivered to consumer without any additional processing, may became a source of *Legionella*. Presence of legionellae in untreated underground water is confirmed also in Portugal (Costa et.al. 2005) and in Canada (Riffard et al. 2001).

Our data showed that temperature of the hot water has a significant influence on *Legionella* contamination in the water system ($p<0.05$). Average water temperature from contaminated systems was 3.2 °C lower than from systems where *Legionella* spp. was not detected. Similar results were presented in other studies (Alary et al. 1991). *L.pneumophila* was found in 44% of hot water samples from heating units and in 52% of hot water samples from private apartments. Our data showed that mean difference of temperature of hot water between heating units and private apartments equals 6.4°C. *L.pneumophila* was found in 5% of cold water samples from heating units and in 17% of cold water samples from private apartments. In all contaminated water supply systems, hot water samples from private apartments were always positive. Thus, including hot water samples from apartments in sampling plans would help to avoid false negative results on water system contamination.

L.pneumophila was isolated from 4% of sediment samples. Investigation of sediment samples did not show presence of *Legionella* spp. in 79% of cases when at least one water sample from the same location was *L.pneumophila* positive. It can be explained with low recovery for this sampling technique. Including of sediment samples in sampling plans raises costs of laboratory investigations and makes sampling procedures more difficult, but does not assure correct information about water supply system contamination levels. However, sediment samples can be used just as additional tool for assessment of the epidemiological situation.

Lack of data obtained in long-term research in Latvia suggests that the real level of contamination of water supply systems may be even higher. The high frequency of *Legionella* contamination in apartment buildings showed that regular preventive actions and controls can be an important part of prevention against legionellosis, which means that appropriate sampling plans and effective sampling strategy is crucial for *L.pneumophila* monitoring.

CONCLUSIONS

Overall, 53% of investigated water supply systems in apartment buildings were contaminated with *L.pneumophila.*

Temperature of hot water may occur an important risk factor for survival and spread of *L.pneumophila*. Only in 3 out of 94 inspected buildings temperature of hot water at the point of consumption was maintained higher than 50 °C, which usually limits growth of *Legionella* spp.

Investigation of sediment samples does not provide full information about presence of *L.pneumophila* in water supply system, thus it can be used just as additional tool for assessment of the epidemiological situation.

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