

Bus Water Storage Tank as a Reservoir of *Legionella pneumophila*

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ABSTRACT

Health concerns associated with Legionnaires' disease have been identified as an area of the increasing public and professional interest. Any natural water or man-made water systems worldwide might be reservoirs of Legionellae. We presented a sporadic, community-acquired case of Legionnaires' disease caused by *Legionella pneumophila* serogroup 1 in a bus driver who used water for hand and face washing from a bus water storage tank. The history of any other usual place of exposure to Legionellae was negative. The water from the tank was dirty, filled with sediment and leaves, at the temperature of 22 °C. The water was heavily contaminated with *Legionella pneumophila* serogroup 1 isolated from each sample with the concentration of 66,000, 16,000, 42,000, 56,000 and 34,000 CFU/L. The disinfection of the bus water storage tank was made using hyperchlorination with 50 mg/L of free residual chlorine. The control sampling one week after the disinfection yielded negative results. So far, there are no recommendations on regular management or disinfection of water in bus storage tanks, but it seems to be reasonable to assume that passengers as well as bus drivers may be exposed to Legionella and therefore at risk of acquiring the infection. These recommendations should include regular emptying, rinsing and filling the tank with fresh tap water, at least once a week. Finally, we have to be aware that Legionella bacteria are ubiquitous and any potential mode of producing contaminated aerosol should not be overlooked during an epidemiological field investigation and proposed appropriate measures.

Key words: Legionellae, *Legionella pneumophila*, Legionnaires' disease, bus, public transport, water storage tank, hyperchlorination, prevention, Croatia

Introduction

Legionnaires' disease was first recognized in 1976 during an outbreak of pneumonia that had caused 34 deaths at American Legion Convention in Philadelphia, with *Legionella pneumophila* (*L. pneumophila*) subsequently identified as the cause. It is a serious pneumonia infection caused by inhaling *Legionella* species, usually *L. pneumophila* serogroup 1. Many non-pneumonic symptoms may be present, the most frequently diarrhoea and neurological manifestations. The average incubation period is 2–10 days, rarely up to 20 days¹.

Legionella bacteria in different population cause 2–16% community-acquired pneumonia². In the general population, the attack rate is 0.5–5% while case-fatality rate is variable, depending on susceptibility of the ex-

posed persons and may reach up 10–15% in otherwise healthy individuals and 80% in hospital patients, despite the availability of the appropriate antibiotic treatment¹. Recognised risk factors for Legionnaires' disease include older age (>50 years), male sex, smoking habits, and having some underlying disease with or without an associated immunodeficiency^{3,4}. Interhuman transmission has not been documented so far.

It is generally assumed that *Legionella* bacteria need to be inhaled to cause the infection. These pathogens are widespread in the natural aquatic environment such as rivers, lakes, streams, and groundwater as well as in the soil and the compost. However, the infection is more often associated with artificial water system where bacte-

ria *Legionella* may find favourable conditions for growth and multiplication such as stagnant water, water temperature at 20–45 °C, pH 5.5–8.3, the presence of bio films containing algae, amoebae and background bacteria and the presence of sediment, rust, sludge and scale².

Many man-made water systems (cooling towers, hot and cold-water systems, spa pools, humidifiers, evaporative condensers, fountains, sprinklers, respiratory therapy equipment...) were recognized as *Legionella* reservoirs. Potential of creating contaminated aerosol is crucial for a possibility of infection. Aerosol may be generated by spraying the water or by bubbling the air into the water, or by impacting it on solid surfaces^{1,2}.

Legionnaires' disease is mainly sporadic in Croatia, with the occasional outbreaks associated with hotels or industrial sites. The average incidence rate in Croatia is 4/1 million of inhabitants⁵.

Croatia does not have specific legislation focused on Legionnaires' disease prevention and control. However, preventive and anti-epidemic measures have been incorporated into the Act on Protection from Communicable Diseases⁶. Legionnaires' disease is subject to mandatory reporting to the Epidemiology Service. This notification is accompanied by a supplemental questionnaire with the additional data on each patient and potential source of exposure. Routine practice is to perform epidemiological investigation. If epidemiologist finds it necessary, the water is sampled and anti-epidemic measures have to be carried out³. The primary aim of these measures is to find and disinfect the source of infection with *Legionella* as well as to prevent the infection of other people exposed to that source⁵.

Case Report

A 35-year-old man was admitted to the Infectious Diseases Clinical Department of the University Hospital Centre with a 5-day history of high fever (39.8 °C), chills, malaise, chest pain, cough, muscle aches, headache and diarrhoea. He had used cefuroxim for five days. He was a former intravenous drug addict with chronic hepatitis C with sustained virological response. The patient was treated for hypertension and he was a cigarette smoker.

Physical examination revealed that his temperature was 40 °C. He had hyperaemic throat, weaker breathing sound in bilateral bases of the lung and diffuse bilateral bronchial breath sounds. Tachypnoea and tachycardia were present. The rest of the patient's examination showed no abnormalities.

Blood tests results on the sixth day of the disease revealed serious bacterial infection with elevated C-reactive protein (>320 mg/L), erythrocyte sedimentation rate (105 mm/h) with leukocytosis (13.0x10⁹/L) and differential blood count with 80% of neutrophils. Several other blood tests included blood glucose (7.2 mmol/L), serum creatinine (114 µmol/L), bilirubin (21.1 µmol/L), AST (126 IU/L), ALT (56 IU/L), LDH (586 IU/L), CK (1977 IU/L), sodium (131 mmol/L), potassium (3.2 mmol/L),

and chlorine (92 mmol/L). Analysis of the urinary sediment revealed proteinuria (1.0 gr/L), aseptic leukocyturia (4 L), hematuria (3 E) and positive nitrite. The patient blood culture was sterile.

Chest X-ray revealed inhomogeneous confluent shadows in lower, middle and upper zones of the left lung suggesting extensive bronchopneumonic infiltration.

On the 13th day of the infection, MSCT angiography showed areas of pulmonary thromboembolism in the right lower lobe with massive consolidation in the left lower lobe and both upper lobes. Left pleural effusion was also noted.

Legionella antigen was detected in the urine. ELISA test on *L. pneumophila* serogroups 1–7 on the 8th day was negative while on the 23rd day IgM and IgG were positive. Other bacterial serologies (*Coxiella burnetii*, *Mycoplasma pneumoniae*) were negative except for positive *Chlamidia pneumoniae* IgG antibody level. Anti-HIV test was negative.

The patient's respiratory signs and chest X-ray revealed significant improvement with the antibiotics. The patient had been treated with moxifloksacin for 12 days. Rifampin was added 2nd–6th hospital day and azitromycin 9th–13th hospital day. Ibuprofen, enoxaparin, potassium chloride in sodium chloride injection, infusion of crystalline solution and oxygen mask were also prescribed. Three weeks after the discharge from the hospital at the control examination clinical, laboratory and chest X-ray results were normal.

The patient was a sporadic case. He was employed as a touristic bus driver. During the incubation period he had two short travels, but with no hotel accommodation or hospital admission. History of any other usual place of exposure to *legionella* (cooling towers, Jacuzzi's, decorative fountains, dental intervention, gardening...) was negative. Domestic hot water tank was used on daily basis with water so hot that it was impossible to use it without mixing it with cold water. None of the family members was sick with respiratory symptoms. However, the patient mentioned hand and face washing with water from the bus water storage tank, while his work colleague, who drove the same bus with him and had no symptoms, was not accustomed to washing his hands or face using that water.

The bus water storage tank was originally used for passengers' hand washing in the toilet, though the toilet had not been in use for several months. This water was occasionally used for washing the bus, but not during the incubation period. It was a long, narrow, plastic tank of approximately 60 L of water (Figure 1) that could run through rubber hose with regulator jet (Figure 2).

It was placed in the bottom of the bus, partly near the engine. That storage tank was not regularly emptied and rinsed, only occasionally filled with tap water at various points during the bus ride. Water from the tank was dirty, filled with sediment and leaves, at the temperature of 22 °C (Figure 3).



Fig. 1. Long, narrow, plastic bus water storage tank.



Fig. 2. Rubber house of bus water storage tank with regulator jet.



Fig. 3. Regulator jet and dirty water from bus water storage tank.

Five samples of water were collected in 1 litre sterile plastic containers with biocide added.

For the isolation and count of *Legionella* spp. the laboratory technique ISO 11731-2 (07/2008) was implemented. Additionally, the Total Mesophilic count (HRN EN ISO 6222), Total coliforms (HRN EN ISO 9308-1), and *Pseudomonas aeruginosa* (HRN EN ISO 16266) were isolated in concentration of more than 100, 1 and 1 CFU/L, respectively. The bus water samples were exam-

ined for *Legionella* spp. after 72 h at 36 °C on Glycine, Vancomycin, Polymyxin B, Cycloheximide bacteriological agar (GVPC) agar (Biomérieux), subcultivated on buffered charcoal yeast extract agar with L-cystein (BCYE) and buffered charcoal yeast extract agar without L-cystein (BCY) agar (Biomérieux) for 48 h at 36 °C.

The confirmation tests were performed by *L. pneumophila* latex kit (Biomérieux). It was shown that water was heavily contaminated with *L. pneumophila* serogroup 1, which was isolated from each sample in the concentration of 66,000, 16,000, 42,000, 56,000 and 34,000 CFU/L (Figure 4).

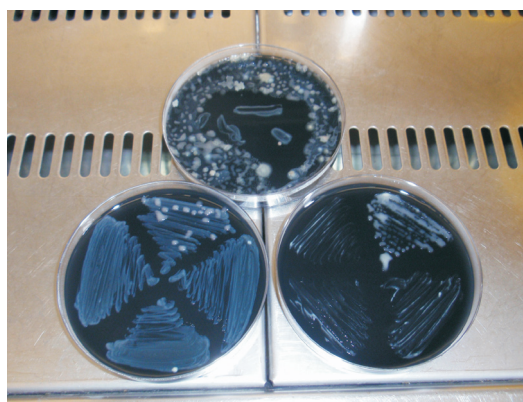


Fig. 4. Unusual growth of heterogeneous bacterial flora on highly selective GVPC agar, absence on BCY agar and presence of *Legionella pneumophila* serogroup 1 on BCYE agar.

The disinfection of the bus water storage tank was made by hyperchlorination with 50 mg/L of free residual chlorine. The control sampling one week after the disinfection yielded negative results. The drivers and a responsible person were educated to empty, rinse and fill the storage tank with fresh tap water regularly, at least once a week.

Discussion and Conclusions

Although exposure to contaminated cold water was already associated with Legionnaires' disease, to the knowledge of these authors, this is the first Croatian case of the isolated *Legionella* bacteria in the bus water storage tank. Unfortunately, we were not able to match isolated *L. pneumophila* from the water samples with the patient's specimens, since cultivation was not performed with the patient's samples. Accordingly, we could not have associated this disease exclusively to exposure to the water from the bus storage tank. However, many results pointed to this storage tank as the most likely reservoir in this particular case. There was stagnant, dirty, and almost tepid water which was heavily contaminated with *L. pneumophila* serogroup 1. On the other hand, pneumonia caused by *L. pneumophila* serogroup 1 was clinically, radiologically, by detection of *Legionella* antigen from the urine and serologically confirmed with no history of an exposure to other known reservoirs of

Legionellae. There was also a possibility of creating an infected aerosol and specific exposure which was not present in case of the patient's colleague.

A few studies concluded that drivers were at higher risk than general population of sporadic cases of Legionnaires' disease^{7–10}. In England and Wales, professional drivers are five times more commonly represented among community acquired sporadic cases than expected. It was found that driving through industrial areas (OR 7.2) and driving in a vehicle with windshield wiper fluid without added screen wash (OR 47.2) increased the risk of Legionnaires' disease among professional drivers in comparison with the control from the general population⁷. Dutch study identified being a driver by profession as independent risk factors for Legionnaires' disease⁸. One study explained disproportionately high number of cases among transportation industry workers with hypothesis that *Legionella* bacteria could be acquired from the air conditioning evaporators of motor cars. The air-conditioning systems of motor cars were sampled and half of them were positive for *L. pneumophila* serogroups 1–6¹⁰. The second study of the same research group pointed to driving through rain puddles on roads as the way of means of introducing contaminated aerosol into the car as this pathogen had been isolated from these puddles¹¹. Turkish study concluded that long distance bus drivers were chronically exposed to *Legionella* bacteria, since

15.2% were found to be seropositive to *L. pneumophila*⁹. Further investigation should be carried out in order to better establish the association between exposure during travel in public transport and legionellosis, including the occupational risk for drivers.

This study contributed to the notion that bus drivers as well as passengers may also be exposed to *Legionella* bacteria when using water from bus storage tanks if these are not properly managed.

A range of preventive measures for Legionnaires' disease risk reduction in Croatia that can be applied to hotels and other touristic accommodation sites¹², public buildings and institutions¹³, boats and yachts¹⁴ have been recommended. So far, there are no recommendations on regular management or disinfection of water in bus storage tanks, but it seems reasonable to assume that passengers as well as bus drivers may be exposed to *Legionella* and therefore at risk of acquiring the infection. These recommendations should include regular emptying, rinsing and filling the tank with fresh tap water, at least once a week.

Finally, we have to be aware that *Legionella* bacteria are ubiquitous and any potential mode of producing contaminated aerosol should not be overlooked during an epidemiological field investigation and proposed appropriate measures.

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AUTOBUSNI SPREMNIK VODE KAO REZERVOAR BAKTERIJE *LEGIONELLA PNEUMOPHILA*

S A Ž E T A K

Legionarska bolest je područje rastućeg javnozdravstvenog i profesionalnog interesa. Voda u prirodi kao i vodeni sustavi koje je stvorio čovjek, mogu biti rezervoari legionela. Ovdje prikazujemo sporadičan slučaj Legionarske bolesti stečene u zajednici uzrokovane bakterijom *Legionella pneumophila* serogrupa 1. Anamneza na izloženost uobičajenim rezervoarima legionela bila je negativna. Pacijent je bio zaposlen kao vozač autobusa te je koristio vodu iz autobusnog spremnika vode za pranje ruku i lica. Voda iz autobusnog spremnika je bila prljava, puna sedimenta i lišća, temperature 22 °C. Pokazalo se da je voda vrlo kontaminirana bakterijom *Legionella pneumophila* serogrupe 1 koja je u uzorcima vode iz autobusnog spremnika izolirana u koncentraciji od 66000, 16000, 42000, 56000 i 34000 CFU/L. Napravljena je hiperklorinacija vode u autobusnom spremniku s 50 mg/L slobodnog rezidualnog klora, nakon čega su rezultati kontrolnog uzorkovanja nakon tjedan dana bili negativni. Do sada nema posebnih preporuka za redovito održavanje ili dezinfekciju vode u autobusnim spremnicima vode, ali se čini razumnim pretpostaviti da putnici ili vozači mogu biti u autobusu izloženi legionelama te biti u riziku od obolijevanja. Stoga bi takve preporuke trebale obuhvatiti redovito pražnjenje, ispiranje i punjenje svježom vodovodnom vodom barem jednom tjedno. Naposljetku moramo biti svjesni da su legionele ubikvitarne i da se niti jedan mogući izvor kontaminiranog aerosola ne smije preskočiti tijekom epidemiološkog izvida i mogućih intervencija.