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May car washing represent a risk for Legionella infection?

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Key words: Legionella pneumophila, pneumonia, Legionnaires' disease, car wash

Parole chiave: Legionella pneumophila, polmonite, legionellosi, autolavaggio

Abstract

Background. *Legionella* is a ubiquitous Gram-negative bacterium naturally found in aquatic environments. It can pose a health problem when it grows and spreads in man-made water systems. *Legionella pneumophila* is the most common cause of Legionnaires' disease nowadays, a community-acquired pneumonia with pulmonary symptoms and chest radiography no different from any other form of infectious pneumonia. *Legionella* monitoring is important for public health reasons, including the identification of unusual environmental sources of *Legionella*.

Methods. We report two cases of Legionnaires' disease associated with two different car wash installations in the province of Vicenza, in the Veneto region, northeastern Italy. Patients were not employees of the car wash installations, but users of the service. In both cases, *Legionella* antigen was detected in urine using the Alere BinaxNOW® *Legionella* Urinary Antigen, and *Legionella* antibodies were detected in serum using SERION ELISA classic *Legionella pneumophila* 1-7 IgG and IgM. Water samples were also analyzed as part of the surveillance program for *Legionella* prevention and control in compliance with the Italian guidelines.

Results. Both patients had clinical symptoms and chest radiography compatible with pneumonia, and only one of them had diabetes as a risk factor. *Legionella* urinary antigen and serological test on serum samples were positive for *Legionella* in both patients, even if much slighter in the case A due to the retrospective serological investigation performed a year later the episode and after the second clinical case occurred in the same district. The environmental investigations highlighted two different car wash plants as potential source of infection. A certified company using shock hyperchlorination was asked to disinfect the two plants and, subsequently, control samples resulted negative for *Legionella pneumophila*.

Conclusions. Any water source producing aerosols should be considered at risk for the transmission of *Legionella* bacteria, including car wash installations frequently used by a large number of customers and where poor maintenance probably creates favorable conditions for *Legionella* overgrowth and spreading. Additional research is needed to ascertain optimal strategies for *Legionella* monitoring and control, but environmental surveillance, paying careful attention to possible unconventional sources, should remain an important component of any Legionnaires' disease prevention program. Additionally, all available diagnostic methods would be recommended for the confirmation of all cases even in the event of non-serogroup 1 *Legionella pneumophila* infection, probably underestimated at this time.

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Introduction

Legionella is a Gram-negative bacterium found naturally in aquatic environments, such as springs, streams and lakes. It can pose a health problem when it grows and spreads in man-made water systems, such as cooling towers, evaporative condensers, water plumbing in homes, hotels, hospitals and nursing homes, decorative fountains, swimming pools, spas, etc. (1). The *Legionella* genus comprises over 60 species with 70 distinct serogroups. *Legionella pneumophila* (Lp) is the most common cause behind cases of Legionnaires' disease diagnosed nowadays. This species currently includes 16 different serotypes, with *Legionella pneumophila* serogroup 1 (*Lp1*) the most prevalent disease-causing variant (responsible for 81% of human infections in Europe) (2).

People are exposed to *Legionella* on inhalation or aspiration of airborne water droplets containing the bacteria, or particles derived by drying. Airborne water droplets can form when water is sprayed, when air bubbles are entrained through a body of water, or when water hits a solid surface. The danger of these water droplets is inversely proportional to their size. Smokers, and individuals with chronic lung disease or diabetes or a weakened immune system are at greater risk of *Legionella* infection and most cases occur in the elderly and predominantly in men (3). People infected with *Legionella* can develop two different conditions: Legionnaires' disease and Pontiac fever. Legionnaires' disease usually presents as community-acquired pneumonia (CAP), characterized by acute pneumonia with a low attack rate (0.1-5% of the general population; 0.4-14% in hospitals), and an incubation time of 2-10 days (4). Although there is no typical syndrome and the radiographic evidence is indistinguishable from that of other forms of pneumonia, some laboratory abnormalities can lead to the diagnosis of

Legionnaires' disease (hyponatremia, mildly or moderately elevated serum transaminases; hypophosphatemia, very high serum ferritin or procalcitonin levels and microscopic hematuria) (5). Pontiac fever, on the other hand, is an acute, self-limiting, influenza-like disease, with no pneumonia.

Legionnaires' disease is notifiable in all European countries, but probably goes underreported due to misdiagnoses or failure to notify cases to the health authorities (6). In Italy, a total of 1,497 cases of legionellosis were notified to the National Surveillance System in 2014, 1,456 of which were classified as confirmed cases, and 41 as probable cases according to the European case definition accepted in 2012 (7). Overall, the notifications were 25.1 per million (incidence rate), meaning a slight increase over the previous year (22.6 per million); the incidence ranged, however, from 39.4 per million in Northern Italy to 6.7 per million in the South of the country. Patients with legionellosis were male in 70.2% of cases, and 55.4% had comorbidities. Approximately 17.0% of the individuals affected reported a situation that exposed them to the risk of *Legionella* infection in the 10 days before the onset of symptoms: out of 1,497 cases reported, 151 patients (10.1%) had been travelling, 62 (4.1%) had been admitted to hospital, 38 (2.5%) were attending day care centers, and 5 (0.3%) had other risk factors (prisons or other closed communities). The urinary antigen test was the most often used diagnostic test (95.2%). *Legionella pneumophila* serogroup 1 was responsible for almost all cases diagnosed by culture, though *Legionella* serogroup 7 was found too. The case-fatality ratio was 30.8% for hospital-acquired cases and 10.1% for community-acquired cases (8).

Case presentation

We report two cases of Legionnaires' disease associated with two different car wash installations in the province of Vicenza,

in the Veneto region, northeastern Italy. Patients were not employees of the car wash installations, but users of the service, and only one of them has diabetes as a health risk.

Methods

Prompt laboratory tests are crucial in diagnosing Legionnaires' disease and choosing antibiotics active against *Legionella* spp. Urinary antigen testing is the diagnostic tool used to confirm *Lp* in 82.0% of cases identified in Europe (9). *Legionella*-specific antigens can be detected in patients' urine soon (2-3 days) after their clinical symptoms have become apparent and may be excreted for periods ranging from several days to 10 months - and even during antibiotic treatment (10). Urinary antigen tests are available in two main formats: an enzyme immunoassay (EIA) and a rapid immunochromatographic test (ICT) in a card-based format. In both our cases, *Legionella* antigen was detected in urine using the Alere BinaxNOW *Legionella* Urinary Antigen Card. This method has been approved only for the detection of *Lp1*, but cross-reactivity for non-serogroup 1 *Lp* has also been described (11, 12). Serological testing for antibodies against *Legionella* is a tool that can be useful for retrospective epidemiological investigations. A positive result on a single serum sample (> 256) has a presumptive diagnostic value, the sensitivity of serological testing in routine use being 80-90%, and its specificity >90%. In both our cases, *Legionella* antibodies were detected in serum using SERION ELISA classic *Legionella pneumophila* 1-7 IgG and IgM, a qualitative and quantitative immunoassay for detecting human antibodies in serum or plasma against *Legionella pneumophila* serotypes 1 to 7. In accordance with the National Guidelines for the Prevention and Control of Legionellosis in Italy (13), isolated cases of Legionnaires' disease are investigated as part of a surveillance

and notification protocol that includes sample collection and analysis to identify environmental sources of contamination, and related risk assessments. Under the Veneto surveillance program for *Legionella* prevention and control, environmental samples are usually analyzed by the Regional Agency for Environmental Prevention and Protection (ARPAV). The environmental investigations prompted by our two cases led to *Lp* being detected in water samples in compliance with the Italian guidelines (13). After disinfection, water sampling and microbiological analysis were performed by a certified laboratory according to the specifications of the International Standard document for the isolation of *Legionella* and estimation of their number in water samples (14); samples containing 100 CFU/L or more are considered positive.

Results

Case A

A 56-year-old Italian male, a carpenter with a medical history of hypertension and diabetes mellitus type 2, fell ill with fever (39.5°C), associated with dry cough and vomiting, on June 23, 2015. Chest radiography on June 26 showed an area of consolidation in the apical-dorsal segment of the left upper lobe, and pneumonia was diagnosed. On the same day, the patient was admitted to an acute care hospital for further tests and treatment was started with intravenous azithromycin (500 mg daily) and intravenous ceftriaxone (2 g for two days). Physical examination was unremarkable except for hepatomegaly. Blood tests on admission revealed: white blood cell (WBC) count 13,030 cells/ μ L (normal range = 4,500-11,000 cells/ μ L), with 89% neutrophils (normal range = 39-78 %); serum C-reactive protein 38.74 mg/dL (normal value < 0.5 mg/dL); serum sodium 133 mmol/L (normal range = 135-145 mmol/L); slightly

elevated serum transaminases, with aspartate aminotransferase (AST) 48 IU/L (normal range = 5-34 IU/L), and gamma-glutamyl transpeptidase (GGT) 77 IU/L (normal range = 12-64 IU/L); glycated hemoglobin 6.9% (normal value <5.9%); serum procalcitonin 2.67 µg/L (normal value < 0.50 µg/L). Laboratory tests on urine revealed mild microscopic hematuria, red blood cells (RBC) 50/µL (normal value < 20/µL); and no proteinuria. Blood culture was negative for aerobic or anaerobic microorganisms in all three samples taken before starting the antibiotic therapy. Cultures obtained on throat swab, sputum and urine were negative for pathogenic bacteria or mycetes. Sputum culture was also negative for mycobacteria. The QuantiFERON-TB Gold test was negative. On June 27, 2015, pneumococcal urinary antigen was negative, while *Legionella* urinary antigen was positive and oral levofloxacin (750 mg daily) was added to azithromycin. No culturing for *Legionellae* was performed on clinical samples. The patient responded well to the antibiotics and made a good recovery. He was discharged from hospital on July 3, 2015 with a confirmed diagnosis of pneumonia caused by *Legionella*, diabetes mellitus type 2 treated with diet, and hypertension (treated with lisinopril 20 mg and amlodipine 10 mg daily). Oral azithromycin (500 mg daily) and levofloxacin (500 mg daily) were administered for another 8 days at home. Chest X-ray and laboratory tests repeated on July 23, 2015, were unremarkable. Meanwhile, investigations to identify the source of infection established that there was no aerosolized water exposure in the workplace. The patient had not been to hospital or the dentist within 10 days prior to the onset of his symptoms. He had no recent history of travelling or visiting swimming pools or wellness centers. Two potential sources of infection were identified and investigated: the patient's home and a bay at a self-serve car wash installation where the

patient had washed his car with a hand-held hose on June 20, 2015, three days before the onset of symptoms. Five water samples were taken from the patient's home, three from the bathroom (shower and taps) and two from the kitchen (hot and cold taps); and two samples were obtained from the high-pressure washer gun at the car wash installation (hot and cold water). All samples from the patient's home were negative. The hot water sample from the high-pressure water hose at the car wash installation was found positive for *Legionella pneumophila* serogroup 2-14 (2,300 CFU/L), while the cold water sample was negative. The car wash installation was disinfected by a certified company using shock hyperchlorination on July 29, 2015, after which a control sample was negative for *Legionella pneumophila* (< 50 CFU/L). To complete the epidemiological investigation, a blood test was performed on August 23, 2016, when the *Lp* IgG titer in the patient's serum was 59 U/mL (negative <50 U/mL).

Case B

A 59-year-old Italian male, a priest with no health risk factors, was admitted to an acute care hospital after suffering a syncope associated with hyperpyrexia (40.0°C) on May 17, 2016. He was treated with oral amoxicillin/clavulanate (1 g twice daily). Blood tests on admission showed: WBC 11,450 cells/µL (normal range = 4,500-11,000 cells/ µL); C-reactive protein 15.72 mg/dL (normal value < 0.5 mg/dL); sodium 134 mmol/L (normal range = 135-145 mmol/L); procalcitonin 1.76 µg/L (normal value < 0.50 µg/L). A first chest X-ray on May 17 was negative. Blood cultures were negative for any bacterial pathogens in all samples. Urine culture was negative for both bacteria and mycetes. Pneumococcal urinary antigen was negative on May 20; and anti-*Mycoplasma pneumoniae* antibodies were negative. *Legionella* urinary antigen was positive on May 21, 2016, and the patient started treatment with intravenous

ceftriaxone (2 g/day for 7 days) and intravenous levofloxacin (500 mg daily for 6 day), followed by oral levofloxacin (750 mg/day for 5 days). No culturing for *Legionellae* was performed on clinical samples. A second chest radiography on May 23 showed an area of consolidation in the basal posterior segment of the left lower lobe, and the diagnosis of *Legionella*-induced pneumonia was confirmed. During the hospital stay, the patient's serum transaminases were elevated, with AST 40 IU/L (normal range = 5-34 IU/L), and ALT 146 IU/L (normal value < 55 U/L). Cytomegalovirus (CMV) IgM titers were negative and IgG titers were positive. Antiviral capsid antigen (VCA) and anti-Epstein-Barr nuclear antigen (EBNA) IgG titers were positive, and VCA IgM titers were negative. On neurological examination, the episode of loss of consciousness was classified as a vaso-vagal syncope and no treatment or diagnostic study was recommended. Laboratory tests and repeat chest X-ray on May 30, 2016, were unremarkable, and the patient was discharged on May 31 with a confirmed diagnosis of pneumonia caused by *Legionella pneumophila*. He continued treatment with oral levofloxacin (750 mg daily) for another two days at home. Blood tests on July 29, 2016, showed a *Lp* IgG titer of 276 U/mL (negative <50 U/mL). During the 10 days preceding the onset of his symptoms, the patient had neither been in hospital or to the dentist, nor to any swimming pools or wellness centers. He had been to Rome on a religious pilgrimage. The place where he stayed in Rome was promptly identified and investigated as the potential source of infection. Twelve samples of hot water (from 24.9°C to 44.4°C) were obtained from taps and showers in three different bathrooms and bedrooms on three different floors. Three samples of hot water (32.2°C to 39.4°C) were taken from the tap in the kitchen and from the tap and shower in the cook's bathroom. All fifteen samples obtained in Rome were negative for

Legionella pneumophila. Back home, one potential source of infection was identified and investigated, i.e. a washing bay at a self-serve car wash installation where the patient had washed his car within 10 days before falling ill. Four samples were taken from two different bays at the car wash installation: two from the self-serve car washing program with hot water and two from the one with water treated by reverse osmosis. Both samples from the latter were negative, while both samples of hot water coming from the high-pressure washer guns in the two different bays were found positive for *Legionella pneumophila* serogroup 2-14 (2,000 CFU/L and 1,700 CFU/L, respectively). A certified company disinfected the car wash using shock hyperchlorination on June 13, 2016, after which all four control samples resulted negative for *Legionella pneumophila* (< 50 CFU/L).

Discussion and Conclusions

Car wash systems used to clean the outside (and sometimes also the inside) of motor vehicles can be classified as: self-serve, fully-automated or full-service (where attendants wash the vehicle). In fully-automated systems, cars are driven onto a pad where they are automatically washed by brushes mounted on a moving frame. In self-serve installations, the vehicle is parked in a covered bay equipped with brushes and a high-pressure spray gun. Customers insert coins in a machine and run the washing program of their choice. The process generally includes a first washing step with detergents, followed by a rinsing phase. Some car wash installations offer the opportunity to use water that has undergone a reverse osmosis treatment to remove its mineral content so that the vehicle will dry without leaving any spots. The water delivered to the spray guns can also be heated to a chosen temperature, up to a maximum of about 65

°C. The water used at car wash services may be collected in tanks and recycled, or used only once and drained away. When assessing the risk of contracting Legionnaires' disease from a car wash installation, there are numerous factors influencing the growth of *Legionella* that need to be considered. First, there is the exposure to aerosols during the washing cycle (especially when using warm water). Second, there are issues with water temperature and water stagnation. *Legionella* bacteria generally proliferate where temperatures range between 20° C and 60° C, and the optimal range for their growth is 32-42° C. They have even been isolated in hot water systems reaching temperatures as high as 66° C, but they are destroyed by temperatures exceeding 70° C (15). Third, there is particular potential for contamination in car wash systems where the water is recycled. Soaps, dirt, oils and sediments provide nutrients that support the growth of bacteria and protozoa, and the latter are an important vector for the survival and growth of *Legionella*. Additionally, there is the no less important problem of biofilm developing on hand-held hoses and storage tanks at a car wash installation. Many microorganisms (including *Legionellae*) form biofilms to facilitate nutrient and gaseous exchange and for protection against biocides and periodic increases in temperature (16). Both our patients used a self-serve covered car wash bay, chose a program with reverse osmosis technology, and used heated water to rinse their cars. Some countries have published specific guidelines and protocols for managing the risk of Legionnaires' disease at vehicle washing installations. The Water Management Society's recommendations (first published in June 2008, and revised in January 2014) include checks on water quality and microbial load, to include testing for *Legionella*, and routine cleaning and disinfection of car wash systems (17). The Australian Car Wash Association drew up a document for *Legionella* control at car

wash plants after the world's first outbreak of Legionnaires' disease reported at a car wash in Hoppers Crossing, Victoria (18), that led to 7 people being admitted to hospital with a diagnosis of Legionnaires' disease. In addition to this specific document, a Public Health and Wellbeing Act, and a Public Health and Wellbeing Regulation were introduced in 2008 and 2009, respectively. These official documents establish general requirements for water delivery systems to manage the risks related to *Legionella*, such as: water should not be stored at temperatures between 30° C and 60° C, instant water heating systems should be used instead; rubber hosing should be replaced with poly tubing, metal tubing, or copper tubing; and systems should be regularly disinfected with a chlorine-based disinfectant (19, 20). In Italy, guidelines for the prevention and control of legionellosis were updated in May 2015 (13), and the new document considers personnel responsible for cleaning car wash installations at risk of legionellosis, but not their customers. It also includes no specific recommendations concerning measures to adopt at car washes to avoid the risk of contamination by *Legionella*. Only sanitary water installations, air conditioning systems, cooling towers, spas, swimming pools, and hot tubs are considered as common sources of infection. In the presence of isolated cases, however, it is recommended that greater attention be paid when similar cases are repeatedly reported. Correlating environmental and clinical *Legionella* strains is only recommended in the event of a cluster, not for isolated cases of disease. Apart from the outbreak in Australia in 2008, we were only able to find one other report of Legionnaires' disease associated with a car wash installation in the Netherlands (21), involving an 81-year-old male with a cardiac pacemaker and a history of cardiovascular disease. A previous study cited in the same article reported the results of investigations into sources of *Legionella* conducted in the

Netherlands between 2002 and 2010, and car wash installations had been found positive for *Legionella non-pneumophila* in two of 11 investigations (22).

In conclusion, any water source that can produce aerosols should be considered a potential source of *Legionella* transmission, including car wash installations, and the risk of legionellosis applies to the car wash users as well as to the cleaning staff. In Italy, such sources of infection responsible for sporadic cases have rarely been investigated and identified to date. A limitation of our study that needs to be acknowledged lies in that the first patient (case A) underwent serological testing only a year after being diagnosed with Legionnaires' disease. This further retrospective investigation was thought necessary once a second event associated with the use of a car wash has been reported in the same district. This serological analysis enabled to confirm the diagnosis. In fact, *Lp* IgG titer was low, but not negative, probably due to the clearance of specific antibodies during the year elapsed since he became infected. Another limitation of our study, however, lies in that *Legionella* species were not cultured from our two patients, so it was impossible to correlate the clonality of the environmental *Legionellae* and the biological samples. Currently, in Veneto there is no standardized sampling path to the Reference Laboratory for genotyping and association between environmental and biological *Legionella*'s strains. Additionally, in hospitals urinary antigen is the only test used for the diagnosis of Legionellosis. In the presence of even only isolated cases attributable to installations such as car washes, which are routinely used by large numbers of customers, a genome sequencing comparison should always be warranted in order to promptly establish the relationship between clinical and environmental isolates. The methods available for diagnosing legionellosis are not particularly sensitive and 100% specific (23),

however. The more numerous the diagnostic methods adopted, the greater the likelihood of a reliable diagnosis of legionellosis, particularly in the case of infection by *Lp* non-serogroup 1. As stated in the Italian guidelines, risk assessment should ascertain whether existing control measures suffice and are implemented effectively. If not, additional measures should be identified. To prevent the growth of *Legionella* species at car wash installations, for instance, the temperature of the hot water should be raised to a high enough temperature (>60 °C), the temperature of the cold water must not exceed 20° C and the whole system should undergo regular, appropriate maintenance, which - in the case in point - entails disinfection and controlling microbiological growth.

Additional research is needed to determine optimal strategies for *Legionella* monitoring and control, but environmental surveillance - paying careful attention to possible unconventional sources - should remain an important part of any Legionnaires' disease prevention program. The importance of water quality management (adequate chlorine residues, pH, and temperature), including control on biofilm formation, cannot be overemphasized in any community setting.

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Consent

Written informed consent to the publication of these case reports was obtained from both patients.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships between the authors and other people or organizations that could inappropriately bias conduct and findings of this study.

Riassunto

Il servizio di autolavaggio può rappresentare un rischio legionellosi per l'utente?

Introduzione. Le Legionelle sono batteri Gram-negativi ubiquitari, che, a seguito di disseminazione e amplificazione negli ambienti acquatici artificiali, possono costituire una potenziale condizione di rischio per la salute umana. La malattia dei Legionari si presenta come una polmonite acquisita in comunità con sintomi polmonari e radiografici non diversi da qualsiasi altra forma di polmonite infettiva.

Metodi. Vengono presentati due casi di legionellosi associati all'utilizzo di due differenti autolavaggi in provincia di Vicenza, in Veneto (nord-est Italia). I pazienti non erano dipendenti degli impianti di lavaggio auto, ma utenti del servizio. In entrambi i casi, la diagnosi si è basata sulla determinazione qualitativa dell'antigene urinario specifico utilizzando il Kit commerciale Alere BinaxNOW® Legionella Urinary Antigen e sulla ricerca degli anticorpi specifici nel siero attraverso il Kit diagnostico SERION ELISA classic *Legionella pneumophila* 1-7 IgG e IgM. Inoltre, sono stati analizzati i campioni d'acqua in accordo a quanto indicato nelle Linee guida per la prevenzione e il controllo della legionellosi in Italia.

Risultati. Entrambi i pazienti presentavano sintomi clinici e radiografici compatibili con un quadro di polmonite e solo uno ha il diabete come fattore di rischio. Entrambi sono risultati positivi alla ricerca dell'antigene urinario e al test sierologico per la presenza di anticorpi anti-Legionella nel sangue, anche se il titolo anticorpale nel primo paziente è risultato basso poiché indagato un anno dopo l'episodio e a seguito del secondo caso clinico verificatosi nella stessa area.

L'indagine ambientale ha identificato due diversi autolavaggi come verosimile fonte di infezione. Gli autolavaggi sono stati successivamente sottoposti a bonifica attraverso un trattamento di iperclorazione shock e i successivi controlli dei campioni d'acqua sono risultati negativi.

Conclusioni. Qualsiasi sistema idrico in grado di produrre aerosol può essere considerato una potenziale fonte di trasmissione di Legionella, compresi gli autolavaggi, dove affluiscono annualmente un gran numero di clienti e la scarsa manutenzione può creare le condizioni favorevoli per la crescita e la diffusione di Legionella. Sono necessarie ulteriori ricerche per determinare le strategie ottimali per il monitoraggio e il controllo della Legionella, ma la sorveglianza ambientale, con particolare attenzione alle possibili fonti non convenzionali dovrebbe rimanere una componente fondamentale del programma di prevenzione della legionellosi. Inoltre, sarebbe auspicabile avvalersi di tutti i metodi diagnostici

disponibili per la conferma di tutti i casi di infezione anche diversi dal sierogruppo 1, probabilmente al momento sottostimati.

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