

Humidifier-associated paediatric Legionnaires' disease, Israel, February 2012

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We report a fatal case of community-acquired Legionnaires' disease in an infant aged under six months. Epidemiological and microbiological investigations suggested that a free-standing cold water humidifier using domestic tap water contaminated with *Legionella pneumophila* serogroup 1 served as a vehicle for infection. These findings were corroborated by sequence-based typing (SBT). Humidifier-associated Legionnaires' disease can be prevented by appropriate control measures. This case also illustrates the emerging role of SBT in the investigation of legionellosis.

Case report

In February 2012, an infant under six months of age with an unremarkable gestational and perinatal history was admitted to hospital due to high fever, cough, wheezing, vomiting, gastrointestinal symptoms and loss of appetite of several days' duration. For six weeks before admission, the patient had been treated with inhaled salbutamol and corticosteroids for repeated episodes of shortness of breath and wheezing. Room air humidification had also been advised. Upon admission, the infant was alert and respiratory distress was evident. A lower respiratory tract infection was suspected and a chest X-ray showed bilateral infiltrates. Despite combined standard antimicrobial, corticosteroid and oxygen therapy, the lung infiltrates progressed over the following days and mechanical ventilation was required due to respiratory failure. Antimicrobial therapy was switched to intravenous ceftriaxone and clindamycin and oral azithromycin. Initial tests for bacterial respiratory pathogens were negative; however, on the fifth day in hospital, a urinary antigen test was positive for *Legionella pneumophila* serogroup (sg) 1. A sputum culture and sputum polymerase chain reaction (PCR) test (Seeplex PneumoBacter ACE detection, Seegene, South Korea) obtained that day were both

positive for *L. pneumophila* sg.1. Sputum PCR was also positive for respiratory syncytial virus (RSV). Following diagnosis of Legionnaires' disease (LD), antimicrobial treatment was extended but the patient's condition continued to deteriorate and extracorporeal membrane oxygenation therapy was needed. Despite maximal intensive care and appropriate antimicrobial therapy, the patient succumbed two weeks following admission.

Background

Human *L. pneumophila* infection (legionellosis) typically presents as a self-limiting influenza-like illness (Pontiac fever) or as pneumonia with systemic manifestations (LD) [1]. LD most commonly affects the elderly or individuals with typical risk factors [1]. Paediatric LD is uncommon and accounts for 0.43% of European cases [2]. Most paediatric infections involve immunocompromised children, commonly in a nosocomial setting, while community-acquired infection in an otherwise healthy child is rarely reported [3]. We describe here a fatal case of community-acquired paediatric LD that involved a distinct source of transmission and discuss its public health implications.

Epidemiological investigation

An investigation was carried out, as mandated in all cases of LD, by the regional public health office and the infectious disease and microbiology departments of the hospital where the patient had been admitted. No typical risk factors for LD or possible nosocomial source of infection could be determined. The patient's parents were questioned about possible exposure to water aerosols at home or outdoors and consequently the use of a free-standing cold water humidifier at the patient's home became evident. The humidifier had not been regularly cleaned and had been filled with tap water that had not been pre-boiled or frequently changed.

The investigation did not reveal any epidemiologically linked cases in the patient's household or in the hospital. However, an additional case of paediatric LD (Case 8) had been diagnosed at the same hospital, several weeks before the current case (Table). The finding of two paediatric cases triggered a retrospective review of national surveillance data at the Ministry of Health (January 2010 to July 2012). Seven additional cases (≤ 24 months of age) were identified (Table), none of whom were linked to the current case. Eight of the nine cases were male, seven were likely to have been nosocomial, six had underlying risk factors and the case fatality rate was 33% ($n=3$). Respiratory coinfection was documented in six of the cases. The use of cold water humidifiers was reported for five of the seven cases for whom this information was available.

Microbiological Investigation

Microbiological samples were obtained from several sites of the cold and hot water system at the patient's household. *L. pneumophila* was identified and colony-forming units (cfu) enumerated according to standard methods and phenotypically characterised using the Dresden panel of monoclonal antibodies [4]. Genotype was determined according to the standardised sequence-based typing (SBT) method developed by the former European Working Group for *Legionella* Infections (EWGLI) [5,6] and sequence types (STs) were assigned using the SBT quality tool [7].

The strain recovered from the patient's sputum was identified as *L. pneumophila* sg.1 monoclonal antibody (mAb) subgroup OLDA/Oxford, which typically accounts for almost a third of environmental isolates but only 3.6% of clinical cases [8]. Of seven domestic environmental samples obtained, two grew *L. pneumophila* sg.1: one from the water system was mAb subgroup Allentown/France (300 cfu/L) and one from the humidifier residual water was initially classified as mAb subgroup Philadelphia (30,000 cfu/L).

Initial genotyping revealed that the clinical strain was ST₁, whereas the strain derived from the water system was ST₄₀. It was not possible to obtain the ST of the humidifier-derived strain because of suboptimal sequence quality. When Bionumerics v6.1 (Applied Maths, Belgium) was used to analyse the sequences obtained from the humidifier-derived strain, several double peaks were evident, suggesting the possible presence of mixed STs in the humidifier water, which, in retrospect, gave rise to a false mAb subgroup result, erroneously implicating the presence of the Philadelphia strain in that sample. Following meticulous subculturing and colony picking, both the OLDA/Oxford ST₁ and Allentown/France ST₄₀ strains were isolated and identified in the humidifier sample, thus providing a link between humidifier water contamination and clinical infection.

The original DNA extract used for PCR-based diagnosis was re-tested post-mortem. An additional untested

sputum sample that had been frozen was also tested and was culture-negative. Both samples were positive for *L. pneumophila* based on a quantitative PCR assay that targets the *mip* gene (cycle threshold values of 18 and 21, respectively, indicating a high level of the gene target in the tested samples) [9]. Both samples were also tested by direct nested SBT [10]: both were ST₁.

Public health and control measures

After elucidating the role of free-standing cold water humidifiers as possible vehicles for LD, the identified risk was communicated by the Public Health Services to healthcare professionals as well as to the public via national media (television news and Internet). The Public Health Services issued guidance to the public regarding the regular maintenance of domestic water systems and the safe and appropriate use of cold water humidifiers. This included the mandatory use of sterile or chilled pre-boiled water with daily water changes, and regular weekly and seasonal instrument cleaning.

Additionally, the Public Health Services have approached the Standards Institution of Israel and new regulations mandating hazard labelling of cold water humidifiers and inclusion of package inserts with user manuals aimed at legionellosis prevention are being set up.

Discussion

As paediatric LD is highly unusual, especially in the first months of life, it may be easily missed by clinicians [3]. Paediatric LD may be either community acquired (e.g. neonatal LD following water birth [11]) or hospital acquired, and may be associated with respiratory coinfection [12,13]. In our case report and case series, such coinfection chiefly involved RSV or adenovirus. In the case reported here, *L. pneumophila* was most likely transmitted at home via the proliferation of the pathogen in stagnant water within a cold water humidifier followed by the generation of *Legionella*-contaminated aerosols by this device. The isolation of *L. pneumophila* sg.1 strains with an identical ST from both the humidifier in the patient's home and respiratory specimens has implicated the humidifier as the source of infection and suggest that humidifiers may serve as competent vehicles for community-acquired LD. The role of humidifiers in additional paediatric LD cases in Israel is intriguing (Table) and should be investigated prospectively.

Viral or bacterial coinfections, as described above, have been reported in LD [12-14]. In the current case, and perhaps in others in our series, respiratory coinfection may have played a role in contracting LD. Specifically, we hypothesise that the prolonged episode of respiratory infection caused by RSV in the current case, who lacked typical underlying risk factors for LD, facilitated *Legionella* infection, by frequent use of the humidifier as well as increased susceptibility to the infection through alteration of local immunity and an enhanced inflammatory response.

TABLE

Paediatric cases of Legionnaires' disease, Israel, January 2010–July 2012 (n=9)

| Case number | Year | Age group (months) | Outcome | Risk factors | Setting | Intensive care | Diagnostic method | Coinfection | Environmental findings | History of cold water humidifier use |
|-------------------|------|--------------------|--------------------|----------------------------|-------------------------|----------------|--------------------------|---------------------------------|------------------------|--------------------------------------|
| 1 | 2010 | 19–24 | Alive | Chronic lung disease | Nosocomial | No | Urinary Ag | RSV | sg.1, sg.3 | Yes |
| 2 | 2010 | 7–12 | Alive | None | Community | No | Urinary Ag | Adenovirus | None | Yes |
| 3 | 2011 | 7–12 | Death ^a | Immunocompromised | Nosocomial | Yes | Urinary Ag | None | sg.3 | Unknown |
| 4 | 2011 | 1–6 | Alive | Surgery | Nosocomial ^b | No | Urinary Ag, PCR | None | None | Yes |
| 5 | 2011 | 19–24 | Alive | Immunocompromised | Nosocomial ^b | Yes | Urinary Ag, PCR | None | None | No |
| 6 | 2012 | 13–18 | Alive | Immunocompromised, surgery | Nosocomial | Yes | Urinary Ag | RSV | sg.3 | Yes |
| 7 | 2012 | 7–12 | Death ^a | Immunocompromised | Nosocomial | No | PCR | <i>Streptococcus pneumoniae</i> | sg.3 | No |
| 8 | 2012 | 1–6 | Alive | None | Nosocomial ^b | No | Urinary Ag | RSV and adenovirus | No data | Unknown |
| 9 Current case | 2012 | 1–6 | Death ^a | None | Community | Yes | Urinary Ag, culture, PCR | RSV | sg.1 | Yes |

Ag: antigen; PCR: polymerase chain reaction; RSV: respiratory syncytial virus; sg: serogroup.

^a Attributed to Legionnaires' disease.

^b Most likely nosocomial but epidemiological investigation inconclusive.

In 2008, humidifiers were implicated as a possible vehicle of LD in a cluster in a nursery in Cyprus: 11 neonates fell ill and the case fatality rate was 27% [14]. According to the preliminary report, possibly more than one strain of *L. pneumophila* was implicated [15]. It is noteworthy that evidence supporting a clear association between LD and humidifier use in that nursery has not been published to date; to the best of our knowledge, our report is the first to confirm this potential source of infection.

Newly manufactured devices with water sonication may improve humidification efficiency but may also generate potentially contaminated aerosols. This may explain the accumulation of humidifier-associated LD in recent years, as is evident from our report and the cluster in Cyprus. In light of the increased availability of new-generation humidifiers, enhanced vigilance and a low index of suspicion should be exercised. Education of healthcare professionals, parents and caregivers regarding correct device use is crucial. Bearing in mind that humidifier-associated LD has been recognised in different countries and settings, these control measures should be complemented by appropriate public health actions, such as national or international regulations that mandate hazard labelling of cold water humidifiers. Lastly, the role of humidifiers in the transmission of *Legionella* should be prospectively studied.

This case also illustrates the challenges associated with linking clinical and environmental *Legionella* isolates, in light of recognised limitations of culture-based methods. Thus SBT emerges as a powerful tool not only for delineating the molecular epidemiology of legionellosis, but also for outbreak investigation.

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