MAJOR ARTICLE

First Outbreak of Nosocomial *Legionella* Infection in Term Neonates Caused by a Cold Mist Ultrasonic Humidifier

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Background. To date, all descriptions of legionellosis in neonates have emerged from a small number of isolated case reports in newborns with unusually severe pneumonia. In December 2008, a large outbreak of *Legionella* infection occurred in term neonates in Cyprus, providing new information on the epidemiological and clinical features of Legionellosis in this age group.

Methods. An environmental investigation was performed at a small private hospital where the infected neonates were delivered. The medical records of the infected neonates were retrospectively reviewed to obtain clinical data on presentation, complications, and course of disease.

Results. Nine of the 32 (28%) newborns who were exposed to the contaminated source at the private nursery were infected with *Legionella*. Six subjects had pulmonary infiltrates, but in 3 cases there were no abnormal radiological findings and clinical presentation was mild. In 4 neonates, pulmonary infiltrates at presentation were bilateral and extensive and 3 died, conferring a mortality rate of 50% in subjects with pulmonary infiltrates and an overall mortality of 33.3%. *Legionella pneumophila* serogroup 3 was recovered in neonatal biological samples, although in some patients there was implication of a second strain, serogroup 1. It was determined that the neonates were infected while in the nursery at the private hospital by aerosol produced by a recently installed cold-mist humidifier that was filled with contaminated water.

Conclusions. Use of humidifiers in nursery units must be avoided as the risk of disseminating *Legionella* in neonates is very high. In neonates legionellosis should be suspected when signs of infection first appear and take an unusual course, even when no pulmonary infiltrates appear.

Keywords. legionellosis; neonates; outbreak; respiratory equipment.

Legionella pneumophila, first identified in 1976, is known to cause 2 clinical syndromes: Legionnaires' disease (LD), a syndrome of severe pneumonia, and Pontiac fever, an acute, febrile but self-limited illness [1]. Drinking water systems and respiratory therapy equipment are often colonized with *L. pneumophila* and are commonly implicated in the dissemination of the organism to humans [2–5]. The incidence of *L. pneumophila* infection depends upon the degree of water reservoir contamination, the intensity of the exposure, and the susceptibility of the host [1–3]. Outbreaks of *L. pneumophila* infection are usually preventable, appearing after failure to follow established prevention measures for contamination of water or respiratory therapy systems and bacterial dissemination [6–8].

LD accounts for 2%–9% of pneumonia cases in adults but <1% of cases in children [9–12]. Of note, in 38% of childhood cases, the child is aged <1 year [13].

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To date, only a small number of isolated cases of *L. pneumophila* pneumonia have been reported in neonates [14–18]. Most cases occurred in infants with immunocopromised status, such as prematurity, bronchopulmonary dysplasia, or therapy with systemic corticosteroids, and with a very high fatality rate (70%), whereas no reports of milder cases have been noted [14–18].

In December 2008, a cluster of term neonates delivered at a small private hospital in Cyprus were admitted to the tertiary pediatric hospital in Nicosia, with manifestations and laboratory findings of legionellosis. In this report, we describe the epidemiological and clinical features of the first outbreak of *L. pneumophila* infection in normal-term neonates.

PATIENTS AND METHODS

Outbreak Description

A cold-mist ultrasonic humidifier (Breezy, Hisense Ltd, Israel) was installed in the nursery of a small private hospital in Nicosia, Cyprus, on the 12 December 2008, in order to increase the humidity level. Between 12 December and 29 December 2008 (the date when the nursery eventually ceased operations), 32 neonates were delivered at this private hospital. Eleven term neonates born between 16 December 2008 and 22 December 2008, after being discharged home in good condition, were admitted between 25 December 2008 and 29 December 2008 at the neonatal intensive care unit (NICU) of Archbishop Makarios III Hospital (Figure 1). All neonates were acutely ill, and some had manifestations suspicious for *L. pneumophila* infection.

Humidifier Description

According to the manufacturer's manual, the humidifier should be cleaned once a week with fresh water and a few drops of liquid soap and then rinsed with several changes of clean water. The manufacturer's manual also stated that cold tap water could be used for mist creation; however, tap water from the nursery was used for this purpose. The hospital did not keep maintenance records for the humidifier.

Nursery Unit Description

The nursery was a rectangular ($8\ m^2 \times 5\ m^2$) single room with 8 cots. The humidifier was placed on a bench that was in the middle of one 8-m wall adjacent to the sink where the neonates were bathed. Mothers were in separate rooms, and infants were taken to them for breastfeeding for varying time periods. No records were available for the time the infants spent outside the nursery or the location of each cot in the nursery in relation to the position of the humidifier. Neither a risk assessment nor an examination of any water specimens for *Legionella* was performed by the hospital prior to this outbreak.

Microbiological Investigations

On 29 December 2008, as part of the diagnostic work-up of the ill neonates, urine samples were examined for *L. pneumophila* antigen with a commercially available assay (BinaxNow–Inverness Medical) [19]. After detection of *L. pneumophila* antigen in several urine samples, tracheal secretions from the severely ill patients who were on ventilation were cultured for *Legionella* on selective and nonselective buffered charcoal yeast extract agar with cysteine as described previously [20]. The identity of the putative *Legionella* was confirmed using the Dryspot *Legionella* latex test (OXOID, code DR0800). All samples and all *Legionella* isolates were sent to the Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU), Health Protection Agency, London, United Kingdom, for confirmation and comparison with environmental strains. Clinical (and environmental) isolates were examined by direct immunofluorescence using a *L. pneumophila*–specific conjugated monoclonal antibody (mAb; BioRad). Five colonies were recovered from each sample and examined using RVPBRU rabbit hyperimmune antisera. A single colony of each phenotype from each sample was then confirmed using the Dresden panel of mAbs; the genotype of this isolate was determined using the standard European Working Group for *Legionella* Infections sequence-based typing (ST) method [21]. Serum antibody titers for *L. pneumophila* 1 and 2–14 groups were also measured with an enzyme-linked immunosorbent assay (ELISA) in all suspect cases (EUROIMMUN, Schweiz AG, Medizinische Labordiagnostika). In 6 neonates, antibody titers to respiratory syncytial virus (RSV) and adenoviruses were also sought using ELISA methods.

Case Definition

The European Union case definition for LD requires clinical manifestations of pneumonia and at least 1 of the following laboratory criteria: (1) isolation of *Legionella* spp. from respiratory secretions or any normally sterile site; (2) detection of *L. pneumophila* antigen in urine; or (3) *L. pneumophila* serogroup 1–specific antibody response [22]. As this was a cluster of cases, we extended the case definition to include any case with clinical manifestations of acute illness (not necessarily febrile) that had been exposed to the specific nursery and also had a single elevated titer of immunoglobulin M (IgM) to *L. pneumophila*, as described in criteria for a probable LD case [22].

The medical records of the neonates who fulfilled the case definition criteria were carefully reviewed to obtain the clinical data.

Environmental Investigations

After the first diagnostic work-up of the cluster cases on 29 December 2008, the Surveillance and Control of Communicable Diseases Unit of the Ministry of Health of Cyprus

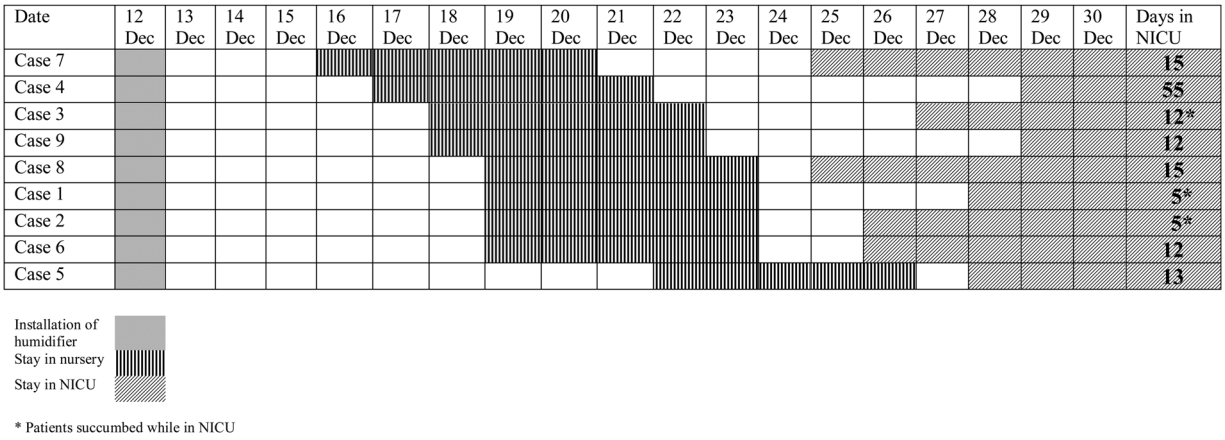

Figure 1. Timeline of *Legionella pneumophila* infection outbreak in neonates in December 2008. Abbreviation, NICU, neonatal intensive care unit.

performed environmental investigations at the private hospital. Culture samples were obtained from the hospital's water main supply and reservoir system, hot and cold water taps of the nursery, and the humidifier.

Microbiological processing of the environmental samples was based on ISO 11731:1998 [23]. In brief, water samples were cultured in 3 ways: untreated, heat treatment, and with acid treatment. Subsequently presumptive *Legionella* colonies were subcultured and further characterized as described above. In addition, blood samples were obtained from the medical and nursing personnel of the maternity unit for serological investigations (ELISA) including *L. pneumophila.*

RESULTS

Clinical Data

Of the 11 acutely ill neonates who were admitted to the NICU, 9 met the case definition (Table 1), thus yielding a confirmed *Legionella* infection in 9/32 (28%) newborns who were delivered at the private hospital between 12 December 2008 and 29 December 2008. Median age of patients on admission to the NICU was 7 days (range, 6–12 days) and all were born at term (Figure 1). The most common clinical manifestations at presentation were lethargy (78%), abnormal temperature (67%), food denial (56%), pallor (56%), and respiratory distress (56%; Table 2). Less frequently reported manifestations included poor peripheral circulation (44.4%), hypoxia (33.3%), apnea (33.3%), and gastrointestinal symptoms (22.2%). At presentation the majority had elevated C reactive protein (62.5%) and white blood cell count (55.5%). On admission, 6 neonates had pulmonary infiltrates on chest X-ray (CXR), but in 3 cases there were no abnormal radiological findings. The patients with no pulmonary infiltrates had milder presentation, demonstrating mainly lethargy (100%) and food denial (67%; Table 2). None of the 3 neonates with normal radiographs had respiratory symptoms at presentation, but 2 subsequently required nasal continuous positive airway pressure (NCPAP) support for 12 hours and for 3 days, respectively, because of apnoeic episodes and an incident of mild respiratory distress. The pulmonary shadows in the 6 neonates included patchy and or interstitial infiltrates. In 4 neonates, pulmonary infiltrates at presentation were bilateral and more extensive and all had to be intubated and ventilated invasively. In the 6 infants with pulmonary infiltrates, the most common complications were persistent tachycardia (67%), hypotension (50%), jaundice (33%), persistent hyponatremia (50%), hypokalemia (50%), and thrombocytopenia (33%), suggesting a multisystem involvement that is probably more common in neonates than in older patients (Table 3). Three of the ventilated neonates developed multiorgan failure, adult respiratory distress syndrome, and intractable severe hypoxia, despite using very high ventilatory settings (peak inspiratory pressure >40 cm H_2O, positive end-expiratory pressure >5 cm H_2O, FiO_2 = 100%). As there were no inhaled nitric oxide or extracorporeal membrane oxygenation treatment modalities in Cyprus, transfer of 2 of the infants to a unit in a neighboring country was considered but eventually deferred due to the infants' unstable condition. All 3 infants died after 4–12 days of ventilation, resulting in a mortality rate of 50% in the subjects with pulmonary infiltrates and an overall mortality rate of 33.3% in our neonatal Legionellosis cohort (Table 3). The course of the fourth neonate with extensive pulmonary infiltrates was complicated by pneumothorax and appearance of cavitation lesions, as confirmed on chest computed tomography on day 23 of hospitalization (Figure 2). Cavitary lesions gradually improved and the patient was weaned off invasive ventilation after 32 days.

When the first diagnostic results became available, patients were started on 10 mg/kg/day of azithromycin intravenously

Table 1. Diagnostic Laboratory Investigations and Radiological Findings in 9 Neonates With *Legionella* Infection

Case No.	Pulmonary Infiltrates on CXR	*Legionella* Antigen in Urine	IgM to *L. pneumophila* Serogroup 1	IgM to *L. pneumophila* Serogroups 2–14	Culture of Tracheal Secretions			RSV IgG Antibodies	RSV IgM Antibodies	Adenovirus IgM Antibodies
					No. of Colonies Examined	Serogroup (subgroup)	Genotype (ST)			
1	Yes	Yes	No	Yes	1	3	ST93	No	No	No
2	Yes	Yes	No	Yes	Not done	. . .	. . .	. . .	. . .	. . .
3[a]	Yes	Yes	Yes	Yes	14	3	ST93	Yes	No	No
					1	1(Oxford/ OLDA)	ST1			
4	Yes	Yes	Yes	Yes	12	3	ST 93	No	No	No
5	Yes	Yes	No	No	Not done	. . .	. . .	Yes	No	No
6	Yes	Yes	No	No	Not done	. . .	. . .	Yes	No	No
7	No	No	No	Yes	Not done	. . .	. . .	. . .	. . .	. . .
8	No	Yes	No	No	Not done	. . .	. . .	. . .	. . .	. . .
9	No	Yes	No	No	Not done	. . .	. . .	Yes	No	No

Abbreviations: CXR, chest X-ray; IgG, immunoglobulin G; IgM, immunoglobulin M; RSV,respiratory syncytial virus; ST, sequence-based typing.

[a] Lung biopsy culture from patient 3 grew 4 colonies with *L. pneumophila* Dresden serogroup 3mAb positive ST93, and 1 colony with *L. pneumophila* serogroup 1, monoclonal antibody subgroup "Oxford/OLDA" ST1.

(median, 1 day after onset of symptoms; range, 0–5 days) and 10 mg/kg/day of rifampicin (median, 2 days after onset of symptoms; range, 0–7 days). One ventilated neonate died on the day the initial diagnostic results became available and did not receive azithromycin (Table 4).

Table 2. Clinical Manifestations and Acute Phase Reactants on Admission to the Neonatal Intensive Care Unit of the 9 Neonates With *Legionella* Infection

Manifestation	All Subjects (n = 9)	Subjects with Pulmonary Infiltrates (n = 6)	Subjects With No Pulmonary Infiltrates (n = 3)
Lethargy	7 (77.8)	4 (66.6)	3 (100)
Abnormal temperature[a]	6 (66.6)[a]	5 (83.3)[a]	1 (33.3)
Food denial	5 (55.6)	3 (50.0)	2 (66.6)
Pallor	5 (55.6)	4 (66.6)	1 (33.3)
Poor peripheral circulation	4 (44.4)	4 (66.6)	0 (0)
Respiratory distress	5 (55.6)	5 (83.3)	0 (0)
Hypoxia	3 (33.3)	2 (33.3)	1 (33.3)
Apnea	3 (33.3)	2 (33.3)	1 (33.3)
Gastrointestinal symptoms	2 (22.2)	2 (33.3)	0 (0)
White blood cell count>10 000/ mm^3[b]	4 (44.4)[b]	4(66.6)[b]	0(0)
Elevated CRP[c]	5 (62.5)[c]	4 (80.0)[c]	1 (33.3)

Abbreviation: CRP, C-reactive protein.

[a] All patients had fever except for 1 who had hypothermia.

[b] One subject had leucopenia.

[c] Not available for 1 subject.

Microbiological Investigations

The initial diagnostic investigations in Cyprus provided evidence of the cause of the outbreak: (1) *L. pneumophila* antigen

Table 3. Complications and Course of Disease in 9 Neonates With *Legionella* Infection

Complication	All Subjects (n = 9)	Subjects With Pulmonary Infiltrates (n = 6)	Subjects With No Pulmonary Infiltrates (n = 3)
ARDS	3 (33.3)	3 (50.0)	0 (0)
Multi-organ failure	3 (33.3)	3 (50.0)	0 (0)
Hypotension	3 (33.3)	3 (50.0)	0 (0)
Jaundice	3 (33.3)	2 (33.3)	1 (33.3)
Persistent tachycardia	4 (44.4)	4 (66.6)	0 (0)
Anemia	1 (11.1)	1 (16.6)	0 (0)
Thrombocytopenia	2 (22.2)	2 (33.3)	0 (0)
Hyponatremia	4 (44.4)	3 (50.0)	1 (33.3)
Hypokalemia	4 (44.4)	3 (50.0)	1 (33.3)
Invasive ventilation	4 (44.4)	4 (66.6)	0 (0)
Nasal continuous positive airways pressure	3 (33.3)	1 (16.6)[a]	2 (66.6)
Mortality	3 (33.3)	3 (50.0)	0 (0)

Abbreviation: ARDS, adult respiratory distress syndrome.

[a] After weaning off of invasive ventilation, 1 subject required nasal continuous positive airways pressure support.

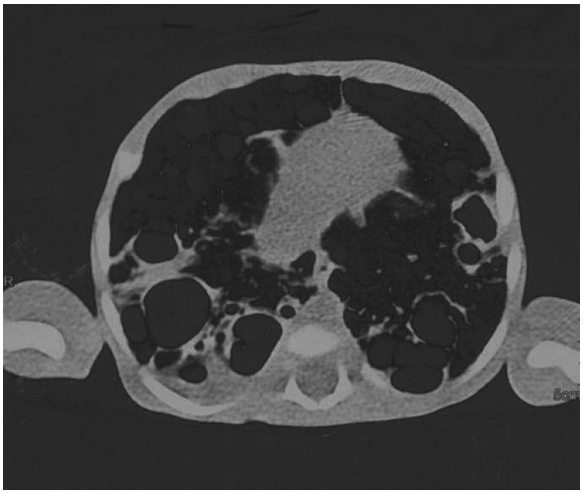

Figure 2. Cavitation lesions on chest computed tomography on day 23 of hospitalization in the neonate with extensive pulmonary infiltrates who survived.

was detected in the urine of 8 neonates, (2) *L. pneumophila* strain belonging in serogroup 2–14 was cultured from 6 tracheal secretion samples obtained from 3 ventilated neonates, and (3) *L. pneumophila* strain belonging in serogroup 2–14 was isolated from a postmortem lung biopsy (Table 1). In the culture of the postmortem sample, there was also evidence of a second strain: *L. pneumophila* serogroup 1. Further investigation at RVPBRU by direct immunofluorescence demonstrated larger quantities of *L. pneumophila* (>10^4 per mL) in all 6 tracheal secretions samples. Examination of multiple colonies from each sample confirmed that the 3 neonates were infected with *L. pneumophila* serogroup 3 DNA-sequence type ST93. In addition, culture of the postmortem lung biopsy confirmed a dual infection with *L. pneumophila* serogroup 3 ST93 and *L. pneumophila* serogroup 1, mAB subgroup "Oxford/OLDA" ST1. Serology studies confirmed the presence of elevated IgM antibodies to *L. pneumophila* 1 in 2 neonates and elevated IgM antibodies to *L. pneumophila* serogroups 2–14 in 4 neonates (Table 1).

Four neonates had elevated IgG antibodies, probably of maternal origin, whereas IgM antibodies for RSV and adenoviruses were negative (Table 1). Cultures of biological materials obtained on admission to the NICU did not yield growth of other bacteria.

Of the 17 private hospital personnel who were present in the nursery, serology studies detected high levels of IgM antibodies to *L. pneumophila* serogroup 1 in 13 (76%). On direct

Table 4. Respiratory Complications, Management, and Course of Disease in 6 Neonates With Pulmonary Infiltrates at Presentation

Case No.	Type of Pulmonary Infiltrate	Respiratory Complications	Respiratory Support	Initiation of Azithromycin Intravenously After Onset of Symptoms (d)	Duration of Ventilatory Support/ Hospitalization (d)	Outcome
1	Bilateral patchy and interstitial infiltrates	ARDS	IPPV (PIP>40 cm H_2O, PEEP>5 cm H_2O, FiO_2 = 100%)	1	5[a]	Death
2	Bilateral interstitial infiltrates	ARDS	IPPV (PIP>40 cm H_2O, PEEP≤5 cm H_2O, FiO_2 = 100%)	. . .[b]	4[a]	Death
3	Bilateral patchy and interstitial infiltrates	ARDS, pleural effusion	IPPV (PIP>40 cm H_2O, PEEP>5 cm H_2O, FiO_2 = 100%) Trial of HFO	2	12[a]	Death
4	Bilateral patchy and interstitial infiltrates	Cavitation-pneumatoceles, pneumothorax	IPPV (PIP≤40 cm H_2O, PEEP≤5 cm H_2O, FiO_2 = 100%) NCPAP post extubation	1	32/55	Survival, respiratory morbidity
5	Bilateral interstitial infiltrates	None	None	2	0/13	Survival, no respiratory morbidity
6	Unilateral interstitial infiltrates	None	None	1	0/12	Survival, no respiratory morbidity

Abbreviations: ARDS, adult respiratory distress syndrome; HFO, high frequency oscillation; IPPV, intermittent positive pressure ventilation; NCPAP,nasal continuous positive airways pressure; PEEP, positive end expiratory pressurre; PIP, peak inspiratory pressure.

[a] Duration of mechanical ventilation until death.

[b] Patient died on the day the first diagnostic tests became available.

Table 5. Microbiology Results of Environmental Samples Obtained From the Neonatal Unit of the Private Hospital

Sample Description		*Legionella* Species (CFU/L)	Serogroup of *L. pneumophila* (%)[a]	Monoclonal Antibodies [b]	Genotype (sequence-based typing)[c]
Humidifier in neonatal unit	Water from feeding tank	5.5×10^7	1: 20	Oxford/OLDA	Isolate 1: ST 1
					Isolate 2: NT
			2–14: 80	Dresden Sg3	Isolate 1: ST 93
					Isolate 2: NT
	Water from humidifier base	2.9×10^8	1: 40	Oxford/OLDA	Isolates: NT
			2–14: 60	Dresden Sg3	Isolate 1: ST 93
					Isolate 2: NT
	Swab from exit nozzle	Detected	1: 40	Oxford/OLDA	Isolates: NT
			2–14: 60	Dresden Sg3	Isolate 1: ST 93
					Isolate 2: NT
	Biofilm from the humidifier's base	Detected	1: 10	Oxford/OLDA	Isolate: NT
			2–14: 90	Dresden Sg3	Isolate 1: ST 93
					Isolate 2: NT
Water facilities in neonatal unit	Coldwater tap (postflush) from neonatal bathing sink (water temperature,[b]22.5°C)	1.2×10^2	1: 83	Oxford/OLDA	Isolate 1: ST 1
					Isolate 2: NT
			2–14: 17	Dresden Sg3	Isolate 1: ST 93
					Isolate 2: NT
	Hot-water tap (postflush) from neonatal bathing sink (water temperature,[b]41°C)	4.1×10^5	2–14: 100	Dresden Sg3	Isolate 1: ST 93
					Isolate 2: NT
	Hot-water (pre-flush) from washing hand sink	2.1×10^5	2–14: 100	Dresden Sg3	Isolate 1: ST 93
					Isolates 2-4: NT
	Hot-water (postflush) from washing hand sink (water temperature,[b]50°C)	2.3×10^5	2–14: 100		
	Cold-water (preflush) from washing hand sink	50	1: 60	Oxford/OLDA	Isolate: NT
			2–14: 40	Dresden Sg3	Isolate 1: ST 93
					Isolate 2: NT
	Cold-water (postflush) from washing hand sink (water temperature,[b]21°C)	30	1: 33	Oxford/OLDA	Isolate: NT
			2–14: 67	Dresden Sg3	Isolate 1: ST 93
					Isolate 2: NT
Swabs from the air-conditioning grid in neonatal unit	Swab from the back of the grid	Not detected	. . .	. . .	. . .
	Swab from the front of the grid	Not detected	. . .	. . .	. . .

Abbreviations: NT, not typable; Sg, serogroup; ST, sequence-based typing.

[a] The percentages given for each serogroup are estimated based on latex agglutination of several colonies from the primary isolation plate.

[b] For cold-water postflush sampling, water was allowed to run for 2 min before temperature was recorded and sample was taken. For hot-water postflush sampling, water was allowed to run for 1 min before temperature was recorded and sample was taken.

[c] The monoclonal antibody subgroup and genotype (ST) are based on full characterization of single-colony picks submitted to the Respiratory and Vaccine Preventable Bacteria Reference Unit.

questioning, no one reported any febrile or respiratory manifestations between 12 December 2008 and 30 December 2008. Serology studies were not performed in the mothers of the infected infants, but none had any manifestation suggestive of an infection postpartum and up to the discharge of their infants from the NICU.

Epidemiological Data

L. pneumophila were isolated from the following locations: (1) cold and hot water taps and bathing sink in the nursery room, (2) swabs from the nozzle and base of the humidifier, and (3) water from the filling tank and base of the humidifier (Table 5). No *L. pneumophila* were recovered from the hospital's water main supply and main water reservoir of the private hospital. Forty-four isolates were phenotypically characterized and shown to be *L. pneumophila* serogroup 3 or *L. pneumophila* serogroup 1. Representative isolates from each sampling site were also genotyped, and only 2 strains were identified: *L. pneumophila* serogroup 3 ST93 and *L. pneumophila* serogroup 1 1mAb subgroup "Oxford /OLDA" ST1, the same strains that were recovered from the neonates.

DISCUSSION

We report the first outbreak of *L. pneumophila* infection in term neonates that provides new information on the clinical spectrum of Legionellosis in this age group. To date, all descriptions of Legionellosis in the neonatal period were obtained from a small number of isolated case reports with unusually severe pneumonia that prompted diagnostic work-up for Legionellosis [15]. We were able to detect, for the first time, the infection in neonates with atypical symptoms such as lethargy, food denial, and fever in the context of the specific epidemiological cluster that prompted investigation of milder manifestations that in other circumstances might have been misdiagnosed. Detection of severe LD in adults was previously reported to help in the recognition of clusters of milder cases of Pontiac fever [24], but this is the first time this is reported in neonates.

The recognition of these less severe infections brings the mortality rate of neonatal Legionellosis to 50% in the subjects with pulmonary infiltrates and 33.3% in all of those infected. Three of the 4 (75%) neonates who developed severe pneumonia died, and this is comparable to the 70% mortality from sporadic cases of severe LD in newborns [15]. The fourth infant with severe pneumonia survived and developed extensive lung cavitation that seems to be a consistent complication of *Legionella* pneumonia in infants [14, 25]. Although Pontiac fever is considered to be self-limiting, we treated the subjects with mild clinical symptoms as we did not know the prognosis if they were not given specific antibiotic treatment. In children and neonates with *L. pneumophila* pneumonia, survival has been shown to depend directly on administration of macrolides [13, 18]; however, no data exist on neonates with limited or no pulmonary infiltrates.

The most frequent manifestations of neonatal legionellosis are atypical and include lethargy, abnormal temperature, food denial, and respiratory distress. Even in infants with pulmonary infiltrates, it is difficult to distinguish LD from other types of pneumonia by chest radiography pattern [13]. Thus it is critical to suspect the disease early in any neonate with atypical signs of infection who has had contact with respiratory therapy devices with other infants with pulmonary infiltrates and a severe course of disease. The urine antigen test is a valuable diagnostic modality to have in place, and a positive result should prompt initiation of appropriate treatment in the expectation of further diagnostic results. Furthermore, in the context of a cluster of cases with suspected symptoms and common epidemiological link, an extended case definition should be used to increase sensitivity when selecting patients for treatment [22].

In previous reports, *L. pneumophila* serogroup 1 was the predominant serogroup implicated in neonatal legionellosis, with serogroups 6 and 8 detected less frequently [14, 16, 17, 26]. We report, for first time, isolation of *L. pneumophila* serogroup 3 ST93 in tracheal samples from 3 neonates. This strain has not been reported frequently (44/6858 isolates in the International SBT database) [27], but it is noteworthy that it has been reported in several European countries and is most often associated with nosocomial infection. Although beyond the scope of this study, it would be interesting to investigate the virulence traits of ST93. However, in 1 neonate, a second strain of *L. pneumophila* serogroup 1 mAb "Oxford/OLDA" ST1, was also detected in the postmortem lung biopsy, and in 8 of the 9 neonates we detected urinary antigen using the BinaxNow *L. pneumophila* serogroup 1 kit. Although cross-reactivity of the urinary assay to antigens of other serogroups (including serogroup 3) is well established [28, 29], it is possible that both serogroups were involved in the infection of several, or even all, neonates. *L. pneumophila* serogroup 1, 1mAb subgroup "Oxford /OLDA" ST1, and *L. pneumophila* serogroup 3 ST93 were also isolated from the environmental samples. This strongly supports the causal relationship of the private nursery with the outbreak, although is not certain which of the 2 strains caused the clinical picture.

The neonates were probably infected by aerosol produced by the recently installed cold-mist humidifier that was filled with contaminated water from the nursery's water taps. The intensity of the exposure was such that the pathogens infected at least 28% of the neonates who were delivered during that period. The majority of staff also showed evidence of exposure, and although the specificity of serological tests has been questioned [30, 31], none of the exposed adults developed clinically evident manifestations, probably because they were more

immunocompetent. A similar case of a humidifier-associated, community-acquired fatal LD was recently reported in Israel in an infant age 6 months [32]. A freestanding cold-water humidifier using domestic tap water served as the vehicle for that infection. In Cyprus, apart from this outbreak, over a 7-year period (2006–2012), an additional 15 cases of LD were confirmed. All were sporadic, community-acquired cases in adults (median age, 66 years; range, 36–84 years) with no reported association with humidifiers.

Colonization of potable hot and cold water systems in hospitals with *Legionella* species is not infrequent, and temperatures <50°C favor the growth of the pathogen [33, 34]. According to current recommendations for the prevention of healthcare-associated pneumonia [8], "in facilities with hemopoietic stem-cell- and/or solid-organ–transplantation programs, periodic culturing for *Legionella* in water samples from the transplant unit(s) can be performed as part of a comprehensive strategy to prevent Legionnaires' disease in transplant recipients." In light of this report, we believe that term neonates should also be included in the high-risk group for Legionellosis. The 2003 Centers for Disease Control and Prevention guidelines advise against the use of large-volume, room-air humidifiers that create aerosols in hospitals unless they can be sterilized or subjected to high-level disinfection on a daily basis and filled only with sterile water [8, 35, 36]. Unfortunately, in this instance, the humidifier manufacturer stated that tap water could be used for mist creation. We have no data from Cyprus on the use of humidifiers in nurseries and whether they use sterile or tap water. In any case, following this outbreak, we suggest that the use of humidifiers in nurseries be avoided as the risk of disseminating *Legionella* to large numbers of neonates and personnel is particularly high.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

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