

An unusually long-lasting outbreak of community-acquired Legionnaires' disease, 2005–2008, Italy

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SUMMARY

An unusually long-lasting community-acquired outbreak of Legionnaires' disease (LD) occurred in the inhabitants of a town in northern Italy from 2005 to 2008. Overall, 43 cases were diagnosed including five deaths. Hundreds of water samples were collected for *Legionella* isolation but only two clinical samples were obtained. Clinical strains were ST23 as were environmental isolates detected in most *Legionella*-positive patients' homes and those from a public fountain. Although no *Legionella* was found in the municipal water mains, a continuous chlorination was applied in 2008. This action resulted in a halving of cases, although incidence remained tenfold higher than the Italian average incidence until the end of 2013, when it dropped to the expected rate. Retrospective analyses of prevalent wind direction suggested that a hidden cooling tower could have been the main cause of this uncommon outbreak, highlighting the importance of implementation of cooling tower registers in supporting LD investigations.

Key words: Community-acquired pneumonia, disinfection, *Legionella*, public health emerging infections, water (safe).

INTRODUCTION

Since Legionnaires' disease (LD) was first described at the end of the 1970s, many community-acquired outbreaks have occurred and *Legionella pneumophila* serogroup 1 (Lp1) has been found to be the most common causative agent [1]. In Europe, the largest community-acquired outbreak of LD occurred in Spain in 2001 with 449 confirmed cases [2]. In Italy, since the 1990s three outbreaks have been identified: the first outbreak involving 34 cases occurred

in 1995 in Sestri Ponente [3], the second occurred in Rome in 2003 with 15 cases [4, 5], and the latest in Lazise in 2011 with 17 cases [6]. In three of these events, epidemiological and molecular investigations identified a cooling tower as the source of infection, while in the Lazise outbreak the source of most cases was found to be the water distribution system of a campsite. However, globally the occurrence of both sporadic cases and community outbreaks of LD has also been associated with other environmental sources, such as spa pools, water distribution systems of accommodation sites, private homes and ships [7–11].

In this paper, we describe an unusually long-lasting community-acquired outbreak of LD occurring from December 2005 to August 2008 in Cesano Maderno, a

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small industrial town in northern Italy. Epidemiological, microbiological and environmental investigations conducted and control measures implemented to stop the outbreak are reported.

MATERIALS AND METHODS

Setting

Cesano Maderno is a small town (11.5 km²) with 37 400 inhabitants, located in north-west Italy, at 198 m above sea level in the Po valley. It is characterized by the presence of a number of small- and medium-sized factories and commercial companies.

Following the occurrence of the first LD cases, as soon as the existence of an outbreak was recognized, the local health authority formed a joint multidisciplinary outbreak control team consisting of medical doctors, public health professionals, engineers, environmental technicians and microbiologists working at the local level. Epidemiologists and microbiologists of the Italian National Institute of Health (Istituto Superiore di Sanità) coordinated the investigation in Cesano Maderno town and surrounding area.

Epidemiological investigations

A case of LD was defined as a person residing in, or having visited, Cesano Maderno in the 10 days before the onset of disease, according to the EU case definition for LD [12]. Confirmed nosocomial cases or cases associated with travel outside Cesano Maderno for the entire incubation period were not considered.

Case-finding included mandatory notifications, and requests for information to local health authorities, general practitioners and hospitals present in the area. Surveillance was also enhanced, informing physicians of the on-going outbreak and asking them to test all pneumonia cases due to *Legionella*. Standardized questionnaires, concerning health status, type of residence, places visited and routes taken within the district, and usual social activities, were recorded by interviewing the cases.

In addition, at the beginning of the outbreak to ascertain whether further undiagnosed LD cases could have occurred during the outbreak, data regarding hospital discharge records of the main referral hospital were also analysed. To this aim, records reporting the ICD-IX code, identifying 'pulmonary infections without aetiological diagnosis' or 'pulmonary infections due to other Gram-negative pathogens'

occurring during the period 2003–2006, were considered and incidence trend in the 3 years prior to the outbreak was compared to that in 2006.

Differences in the hospital discharge records for the considered years were evaluated by applying a χ^2 test using Stata software version 10.0 (Stata Corp LP, USA). A *P* value <0.05 was regarded as significant.

Environmental investigations

As the patients resided in different zones of Cesano Maderno, the entire town was inspected searching for all potential sources of *Legionella* infection. The environmental investigations first focused on large buildings, especially factories and supermarkets, and on patients' homes. The municipal drinking water system, consisting of pipes and wells without any disinfection procedure in place, was also inspected. Moreover, information about works performed on the municipal water system (such as building of new wells, replacement of sections of pipes, disinfection practices, etc.) was requested from the local relevant authorities.

Environmental samples were collected from 10 cooling towers (four located in Cesano Maderno and six in neighbouring towns), from tanks of a wood processing manufacturer, from drinking water taps, wells and tanks of the municipal water system and from public drinking water fountain in Cesano Maderno. In addition, all patients' homes, but one, were sampled as well as 16 control houses, selected for similar heating system, building type and age. Five litres of water were collected from the municipal water network and 1 litre from all the other sampling sites.

The potential sources of infection as well as patients' homes were mapped using the Geographical Information System (Quantum GIS version 1.8.0; www.qgis.org) to detect possible spatial patterns.

Diagnosis of clinical and environmental samples

Legionella urinary antigen was detected by immune-enzymatic test (Biotest, Germany). Isolation of *Legionella* from two clinical samples (one lung tissue and one respiratory secretion) was performed using both glycine vancomycin polymyxin B cycloheximide (GVPC, Oxoid, UK) and buffered charcoal yeast extract (BCYE- α , Oxoid, UK) agar plates.

All water samples were analysed by culture according to ISO 11 731–1:1998 recommendations [13] while the ISO 11 731–2: 2004 standard [14] was used for the municipal water system comparative study.

Active air sampling was performed using a surface air system (SAS) sampler (International PBI, Italy) with a flow rate of 180 l/min and an aspiration volume of 1 m³ of air per sample. The sampler was placed at the emission point of the investigated cooling towers. Presence of *Legionella* was evaluated using replicate organism detection and counting agar plates containing GVPC medium.

Typical *Legionella* colonies from clinical and environmental samples were identified by latex agglutination test (Oxoid, UK) and confirmed by immunofluorescence assay using monoclonal antibodies (Dresden University) directed against the 15 different *Legionella* serogroups.

Molecular typing

Both clinical and environmental strains of Lp1 isolates were assayed for monoclonal subgroup by immunofluorescent indirect assay according to the 'Monoclonal (MAb) Dresden Panel' [15]. They were also typed by amplified fragment length polymorphism (AFLP) and sequence-based typing (SBT), using genomic DNA extracted from single Lp1 colonies with 20% Chelex 100 (Sigma, Germany). Briefly, the colonies were suspended in 1-ml sterile distilled H₂O in a microfuge tube and centrifuged for 1 min at 12 000 *g*. Then the supernatant was removed and DNA extracted by adding 200 μ l 20% Chelex 100 to the pellet followed by boiling for 10 min. AFLP was performed as described elsewhere [16] and the obtained genomic patterns were compared by visual analysis. SBT was performed according to the 'sequence-based typing protocol for epidemiological typing of *Legionella pneumophila*' version 4.2, analysing raw sequence data using the *Legionella* Sequence Quality Tool [17]. Thirty environmental strains, selected for having either the same AFLP profile but different MAb subgroup or different AFLP profile than those of the two clinical strains, were analysed by SBT.

Meteorological data

Meteorological data on air temperatures (minimum, maximum and average daily values), relative humidity (RH), atmospheric events (fog, rain, storm, snow), wind velocity (hourly and maximum daily values) and direction were acquired at two nearby stations in Carate Brianza and Milan Linate airport (at 8.1 and 21.3 km, respectively, from Cesano Maderno) from 4 months before to 4 months after the LD outbreak period (August 2005–December 2008). These

data were correlated with the geographical and temporal distribution of the LD cases in order to explore potential infection sources and promoting factors in the examined area. In particular, wind direction was placed in relation to the geographical distribution of the patients' homes. For every meteorological parameter, the frequency distribution of median values recorded during the LD incubation period (i.e. 2–10 days before each illness onset) was compared with the frequency distribution of the corresponding data acquired in the other days of the investigated period (reference frequency distribution). A χ^2 two-sample test was applied to investigate whether the two frequency distributions differed from one another and a *P* value <0.05 was regarded as significant.

RESULTS

Descriptive epidemiology

From December 2005 to August 2008, 43 confirmed LD cases in residents of Cesano Maderno were notified, with two peaks, each of five cases, in July and October 2006, respectively, as shown in Figure 1. Mapping of the cases indicated that they were distributed throughout the city (Fig. 2). All cases were hospitalized and five patients died. *Legionella* urinary antigen test was positive for all cases, whereas *Legionella* cultural isolation was only obtained for two patients. Of the two clinical strains the first was isolated in February 2007 and the second in February 2008. The outbreak mainly involved elderly individuals (median age 71 years, range 32–95 years) and the male/female ratio was 1.8:1. Most (75%) of the cases were affected by underlying chronic diseases, such as diabetes, chronic bronchitis, pulmonary emphysema, renal failure, transplant and cancer. Four patients reported they had never left their homes during the disease incubation period. All the patients' homes, both houses (75%) and flats (25%), received drinking water from the municipal network and hot water was produced by gas (71.4%), electric storage (19%) or by instant water heaters (4.8%). In 4.8% of cases people were not able to provide the information. The questionnaires administered to all the patients did not reveal any common habit, places visited or social activity.

Compared to previous years, the analysis of discharge records of the main hospital, where most of the cases were admitted, did not show a significant increase of pneumonia cases without aetiological

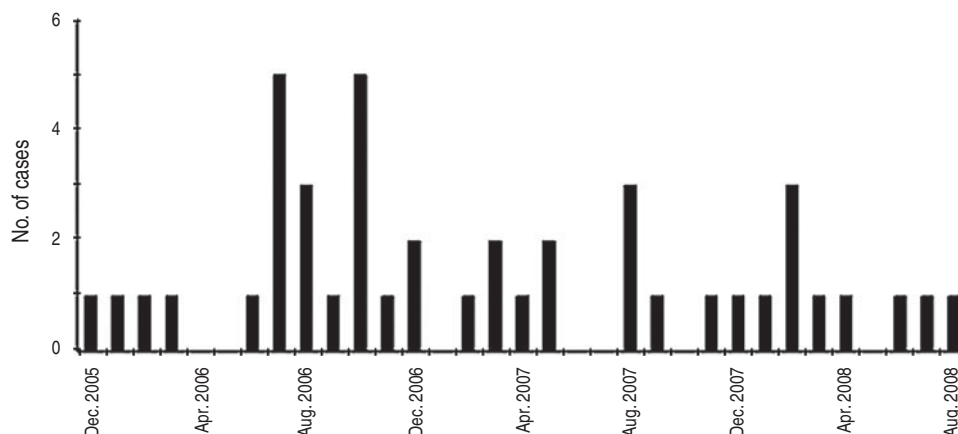


Fig. 1. Legionnaire's disease cases by month of onset in residents in Cesano Maderno, December 2005–August 2008.

diagnosis or due to other Gram-negative pathogens ($P = 0.379$). This suggested that a low probability of undiagnosed and under-reported cases occurred during the outbreak of LD.

Microbiological investigations

Culture examination of the two clinical samples allowed the isolation of Lp1.

Over 400 environmental water and air samples were collected and about 140 samples resulted negative for *Legionella* by culture, some of them also by repeated testing. Overall, in 22 (52%) patients' homes *Legionella* was detected at a concentration ranging from 10^2 to 10^5 c.f.u./l. Of these, 19 (86.4%) were positive for Lp1 at 1.2×10^2 to 7.1×10^5 c.f.u./l, two (9.1%) for Lp serogroups 2–15 at 1.4×10^3 to 3.2×10^5 c.f.u./l, and one (4.5%) for *Legionella non-pneumophila* at 3×10^3 to 9×10^5 c.f.u./l. In 4/19 houses positive for Lp1, *Legionella non-pneumophila* was also isolated at 10^3 – 10^5 c.f.u./l. In the remaining 20 houses (48%), although sampling was repeated at least twice, the culture always failed to detect *Legionella*. It is noteworthy that one patient living in one of the negative houses reported he had never left home during the disease incubation period. Water samples were collected in 16 control houses and 14 gave negative results. Of the remaining two houses one was positive for Lp1 at $<2 \times 10^2$ to 8×10^2 c.f.u./l and the other for Lp serogroups 2–15 at 1.3×10^3 to 1×10^4 c.f.u./l (Table 1 and Fig. 2).

Sampling performed at 48 points of the municipal water distribution system in Cesano Maderno gave positive results only in one public drinking water fountain, where Lp1 was isolated at a concentration

of 10^2 c.f.u./l in November 2006 and 7×10^2 c.f.u./l in May 2007.

Seven out of 10 sampled cooling towers were Lp positive (Fig. 2) and four of them were Lp1 and were located in Cesano Maderno (Table 1); the remaining three cooling towers (outside Cesano Maderno) were Lp non-serogroup 1-positive (Fig. 2). In a wood processing factory, equipped with hot-water tanks but without cooling towers, water from a tap was also positive for Lp1 and Lp serogroups 2–15 at $<10^2$ – 10^5 c.f.u./l. A sports centre was found Lp1 positive at $<10^2$ – 10^3 c.f.u./l (Table 1). Air samples collected near the cooling towers and the wood processing factory as well as in the main streets of the town, where most of cases resided, were found negative.

MAB typing

MAB typing showed that Lp1 colonies isolated from two clinical samples were Knoxville for one patient and Philadelphia subgroup for the other one. Overall, 92 Lp1 environmental isolates from patients' homes, municipal water system and factories' cooling towers were typed as Philadelphia (45.7%), Knoxville (30.4%), Olda (15.2%), Oxford (4.3%), France/Allentown (2.2%), and Benidorm (2.2%). In four patients' homes, as well as in the public drinking fountain, water samples were contaminated with Lp1 (both Knoxville and Philadelphia subgroups). Lp1 Olda strains were found in three houses and one cooling tower, while Lp1 Oxford strains were found only in two cooling towers. The two Lp1 France/Allentown strains were found in two houses' water systems together with Knoxville subgroup. The only Benidorm subgroup strain was found in the sports centre together with Knoxville subgroup (Table 2).

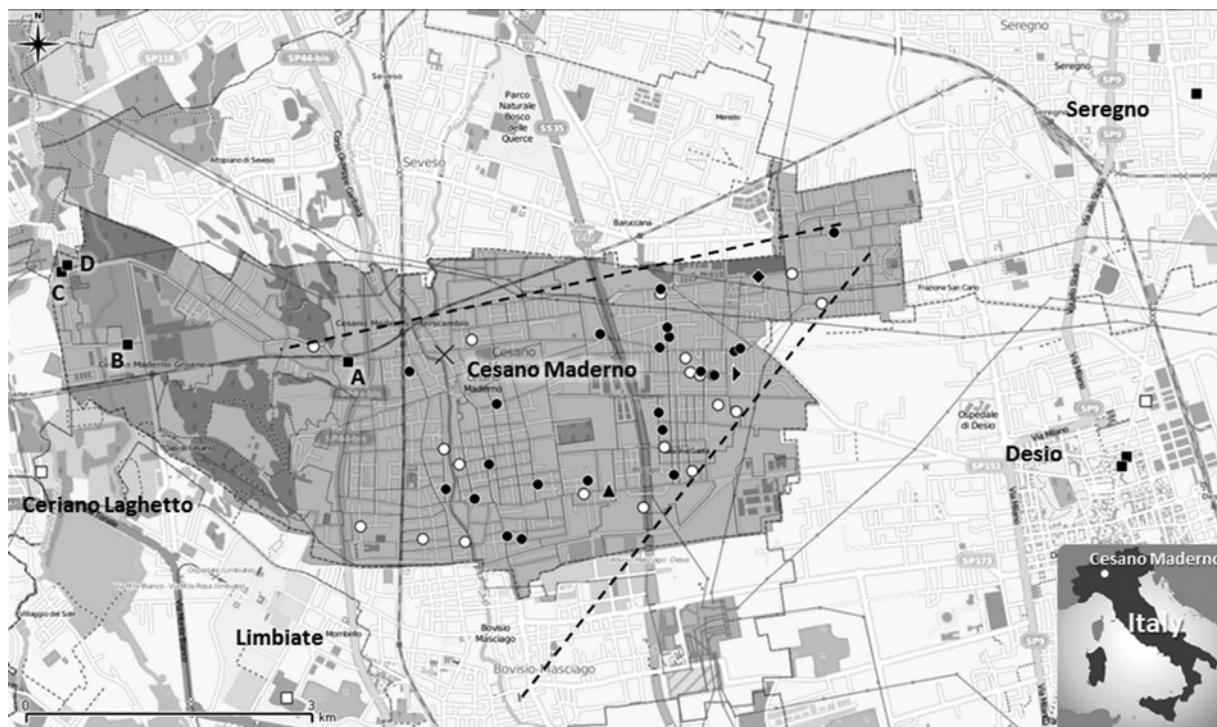


Fig. 2. Geographical distribution of the examined sites. ●, Home contaminated by *Legionella*; ○, home not contaminated by *Legionella*; ×, not-sampled home; ◆, sports centre; ►, public drinking water fountain; ■, cooling tower contaminated by *Legionella*; □, cooling tower not contaminated by *Legionella*; ▲, wood processing factory. Dashed lines indicate the boundaries of the funnel-shaped area.

Table 1. *Legionella* contamination in the investigated sites in Cesano Maderno

| Sampling site | No. positive sites/ no. sampled sites (%) | % Lp1-positive sites (c.f.u./ml range) | % Lp1 non-positive sites (c.f.u./ml range) | % L-np-positive sites (c.f.u./ml range) | No. positive sites for mixed contamination |
|--------------------------|---|--|---|--|--|
| Patients' homes | 22/42 (52) | 86.4* (1.2×10^2 – 7.1×10^5) | 9.1* (1.4×10^3 – 3.2×10^5) | 4.5* (3×10^3 – 9×10^5) | 4 |
| Control houses | 2/16 (13) | 50 (2×10^2 – 8×10^2) | 50 (1.3×10^3 – 1×10^4) | n.d. | 0 |
| Cooling towers | 4/10 (40) | 75* (1×10^5 – 1.6×10^5) | 50* (1×10^2 – 1.6×10^5) | 50* (1.2×10^3 – 1×10^4) | 2 |
| Other buildings† | 3/3 (100) | 6* (1×10^3 – 2×10^5) | 66* (2.3×10^3 – 2×10^5) | n.d. | 2 |
| Public drinking fountain | 1/1 | 100 (1×10^2 – 7×10^2) | n.d. | n.d. | 0 |
| Sports centre | ¼ | 100 ($<1 \times 10^2$ – 2×10^3) | n.d. | n.d. | 0 |

Lp1, *Legionella pneumophila* serogroup 1; L-np, *Legionella non-pneumophila*; n.d., not detected.

* Different species and serogroups were often present simultaneously in the analysed samples.

† Wood processing factory, supermarket and health centre.

AFLP and SBT analyses

The two Lp1 clinical and 92 environmental strains were typed by the AFLP method and 14 different

genomic profiles were identified. The two clinical and 11 environmental strains, nine from patients' houses and two from the public drinking water

Table 2. Molecular typing of clinical and environmental *Legionella pneumophila* serogroup 1 isolates

| Strain origin | ST | MAb | AFLP type |
|----------------------------|-----|--------------|-----------|
| Clinical 1 | 23 | Knoxville | 3C |
| Clinical 2 | 23 | Philadelphia | 3C |
| Patient's house 1 | 23 | Philadelphia | 3C |
| Patient's house 1 | 146 | Olda | 42 |
| Patient's house 2 | 23 | Philadelphia | 40 |
| Patient's house 2 | 23 | Knoxville | 41 |
| Patient's house 3 | 23 | Philadelphia | 40 |
| Patient's house 3 | 23 | Knoxville | 40 |
| Patient's house 4 | 23 | Philadelphia | 3C |
| Patient's house 5 | 23 | Philadelphia | 3 F |
| Patient's house 6 | 23 | Knoxville | 3 F |
| Patient's house 7 | 23 | Knoxville | 3 F |
| Patient's house 8 | 23 | Knoxville | 3C |
| Patient's house 9 | 23 | Knoxville | 3C |
| Patient's house 10 | 23 | Knoxville | 3C |
| Patient's house 11 | 23 | Knoxville | 3E |
| Patient's house 12 | 23 | Philadelphia | 3C |
| Patient's house 13 | 23 | Philadelphia | 3C |
| Patient's house 14 | 23 | Philadelphia | 3 F |
| Patient's house 15 | 146 | Olda | 3 F |
| Patient's house 16 | 23 | Philadelphia | 35 |
| Patient's house 17 | 23 | Knoxville | 3C |
| Patient's house 18 | 23 | Knoxville | 3C |
| Cooling tower A | 1 | Oxford | n.d. |
| Cooling tower B | 1 | Olda | 39 |
| Cooling tower C | 1 | Philadelphia | 38 |
| Cooling tower D | 1 | Oxford | n.d. |
| Wood processing factory | 23 | Philadelphia | 3 F |
| Public drinking fountain 1 | 23 | Philadelphia | 3C |
| Public drinking fountain 1 | 23 | Knoxville | 3C |
| Sports centre | 42 | Knoxville | 33 |
| Sports centre | 42 | Benidorm | 33 |

MAb, Monoclonal antibody; AFLP, amplified fragment length polymorphism, n.d., not determined.

fountain, showed the same genomic profile. All the other environmental strains, indicated by the same number but different letters (i.e. 3E, 3 F), differed for a maximum of two bands, while the remaining isolates differed for at least three bands (Table 2).

SBT showed that the two clinical strains were ST23, a type frequently observed in community- and travel-associated cases occurring in Italy [18].

Overall, 30 environmental *Legionella* strains were typed: 21 from 18 patients' homes (for one patient's home no Lp1 strains were provided by the Regional Reference Laboratory), four from three cooling towers, two from the sports centre, two from the public drinking fountain, and one from a tap of the wood

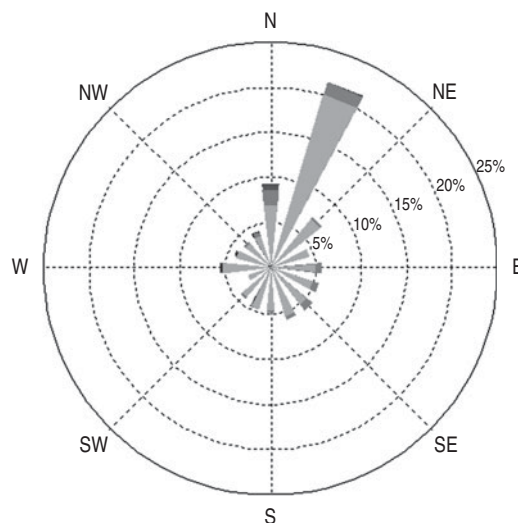


Fig. 3. Wind rose plot obtained from hourly data on wind directions (directions from which the wind was blowing) and velocities acquired between 2 and 10 days before every confirmed case of Legionnaire's disease. Hourly wind velocities (m/s): ■, 0.0–0.3; ■, 0.4–2.0; ■, 2.1–4.0; ■, >4.0.

processing factory's tank. All the Lp1 (both Philadelphia and Knoxville subgroups), from patients' homes, from the public drinking water fountain and from the taps of the wood processing factory's tank were ST23. The Lp1 Olda subgroups found in two houses were ST146. Four isolates from three cooling towers were all identified as ST1. Both Lp1 Knoxville and Benidorm found in the sports centre were ST42 (Table 2).

Analysis of meteorological data

Prevailing wind direction in the examined area was North-Northeast (Fig. 3) during all the investigated period with a very low hourly wind velocity (mainly in the range 0.3–2.0 m/s). It was perfectly aligned with the main axis of the funnel-shaped geographical distribution of the patients' homes (Fig. 2). Frequency distribution of the maximum daily wind velocities recorded during the LD incubation period was statistically different ($P < 0.05$) from the corresponding reference frequency distribution (Fig. 4) due to a significant increase of the percentage of the values in the range 3.0–4.0 m/s, which corresponds to a light breeze according to the Beaufort scale.

During all the investigated period, the average daily air temperature was in the range 0–30 °C (with a mode of 25 °C) while the maximum daily air temperature

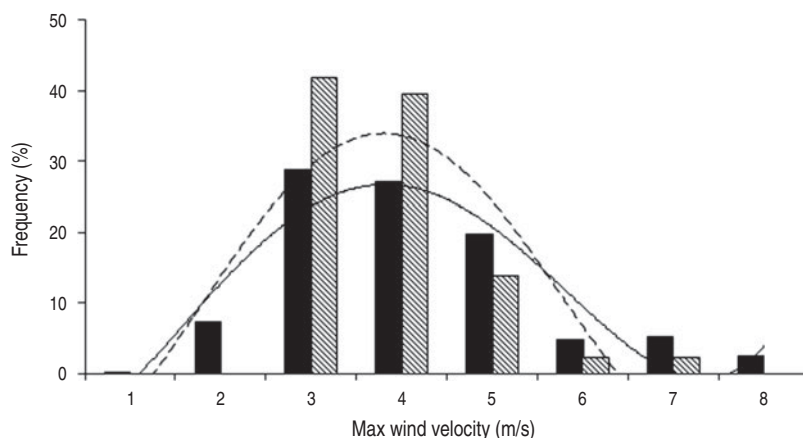


Fig. 4. Frequency distributions of maximum daily wind velocities. ▨, Median of the data acquired between 2 and 10 days before each confirmed case of Legionnaire's disease (values interpolated with a continuous line); ■, data acquired on the other days of the investigated period (values interpolated with a dashed line).

was in the range 5–40 °C (with a mode of 30 °C). A statistically significant difference ($P < 0.05$) in the frequency distribution of the minimum daily air temperature was observed between the LD incubation period of every case and the other days of the investigated period due to a shift of the first towards higher temperatures (20–25 °C). Similarly, the two frequency distributions of air humidity differed substantially between them ($P < 0.05$) as a consequence of the significant incidence of medium-high humidity values (mainly 65% relative humidity) during the LD incubation period. Overall, these findings are consistent with the ecology of *Legionella*, whose airborne survival and transmission is affected by various environmental factors, chief among them being warm air temperature and medium-high relative humidity [19–21].

Unlike the results of a previous report on the influence of rainfall on LD occurrence [22], data acquired in this case study have shown that the LD incubation period was characterized by scant rainfall events. This meteorological condition together with thermal inversion, which usually affects the Po valley, has probably increased the permanence of the supposed aerosol plume in the atmosphere above the area.

Recommendations, implemented control measures and follow-up of the outbreak

During environmental and microbiological investigations, the local health authority and the National Institute of Health released recommendations according to Italian and European *Legionella* guidelines [23, 24] on how to reduce the risk of *Legionella* contamination in household water distribution systems.

Implementation of control measures and disinfection practices were requested for the cooling towers found positive for *Legionella* and in June 2008 a continuous disinfection (0.2 mg/l of residual chlorine) was applied as preventive action throughout the whole municipal water network. In addition, all the general practitioners of the city were alerted to pay more attention to patients reporting symptoms of pneumonia and influenza-like diseases. These recommendations and the chlorination applied to the municipal water network slightly reduced the number of cases in the subsequent years (eight in 2009, seven in 2010, six in 2011, seven in 2012) but the incidence of LD in Cesano Maderno remained tenfold higher than the average incidence in Italy up to 2012. It was only in 2013 that, due to unknown reasons, only three cases were notified, returning the LD average incidence to the expected value.

DISCUSSION

This study describes an unusual community-acquired LD outbreak, characterized by 43 cases including five deaths, distributed throughout a period of 3 years with two major peaks in July and October 2006, followed by a period of high LD incidence lasting up to 2012.

Although an accurate epidemiological, environmental and microbiological investigation was conducted, no common source of infection which could explain such a long-lasting distribution of cases spread throughout the area of Cesano Maderno was identified.

Legionella contamination was found in 52% of the internal water distribution systems of the patients'

homes. This high percentage of *Legionella* contamination never described before, is in contrast with the high presence of instant heaters (71%) known to be less prone to favour colonization of water systems [25] and is probably explained by the extra attention given to the search for the bacterium with repeated samplings.

Molecular typing strongly suggested a clonal relation between the two clinical isolates and most of the analysed Lp strains (i.e. those found in 18 household water systems, in the public drinking water fountain and in the wood processing factory). MAb typing revealed that clinical isolates were from two different subgroups, Philadelphia and Knoxville both from the MAb 3/1 positive group, while the genomic (AFLP) and allelic (SBT) profiles showed that they were identical and matched many environmental strains described previously. Since phenotypic differences between strains are possible, when a correlation between strains must be defined, genetic identity has a greater discriminatory power especially when it is confirmed by two typing methods.

Although according to molecular typing the houses and the water mains could be considered a probable source of infection, the samples collected in the municipal network of Cesano Maderno repeatedly failed to provide evidence of *Legionella* presence. In addition, no plumbing work on the water mains of Cesano Maderno, which might suggest an increased risk of LD acquisition [26] had taken place.

Even though house contamination by *Legionella* is widely demonstrated [11, 26–28], the occurrence of a so high number of cases linked to house contamination is not common [29, 30]. Notwithstanding, it is not possible to exclude that the poor water system maintenance in some of the houses may have acted as source of infection, especially for those patients who, because of health problems, never left their homes during the incubation period.

The modest wind velocity only suggested that an aerosol plume released intermittently by a hidden cooling tower might have contaminated the area. The surprising fit of the prevalent wind direction with the main axis of the funnel-shaped geographical distribution of the homes of the cases, made by a retrospective comparative examination, has suggested that the source of microbiological contamination was presumably localized in the north-east part of Cesano Maderno. The direction of the light breeze, the thermal inversion and the scant rainfall events, resulting from the analysis of the meteorological data, could all be responsible for the cases within 4–5 km of the

supposed contamination source. This hypothesis, as well as the outcomes so far examined, is fully compatible with previous studies [31–33].

However, we were not able to demonstrate this hypothesis because only four cooling towers were found to be contaminated by Lp1 but with different sequence types compared to the two clinical strains. As a matter of fact, one of the weaknesses of this study was that in spite of the high number of cases only two clinical isolates were available, due to the large diagnostic use of the urinary antigen test that in Italy has almost completely replaced all other diagnostic tests.

Another critical aspect was the lack of a cooling tower register, as their notification by owners is not mandatory in Italy. The thorough environmental investigations conducted in Cesano Maderno and in the neighbouring towns allowed identification of the presence of 10 cooling towers, most of them subjected to proper and regular maintenance so that *Legionella* was found in low concentrations in the collected water samples. However, the possibility of having missed some cooling towers cannot be ruled out. Indeed, these devices may be hidden by thick vegetation or located below the road surface as we experienced during the last two outbreak investigations conducted in Italy [4].

Although since June 2008 a continuous disinfection treatment has been implemented in the municipal water supply, the incidence of LD in Cesano Maderno remained high in the following years. This finding underlines that in all likelihood, this intervention, albeit appropriate, was not able to halt the outbreak because one or more undetected sources were still active.

It was only in 2013 that the frequency of cases suddenly decreased to the expected background level. We might speculate that this effect was caused by the shutdown of the supposed contaminated cooling tower following the closure of several factories or companies affected by the recent worldwide economic crisis.

In conclusion, this investigation highlights, once more, how the identification of a source of a LD outbreak can be difficult and how the presence of a cooling tower register and the availability of as many as possible clinical isolates for comparison with environmental isolates, may facilitate this task.

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DECLARATION OF INTEREST

None.

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