ORIGINAL ARTICLE

Is Venice an ideal habitat for *Legionella pneumophila*?

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Key words

Legionella pneumophila • Venice

Summary

Introduction. *Legionella bacterium manifests itself in Legionnaire's disease and Pontiac fever, it is mainly found and transmitted by aerosol produced in cooling towers, water distribution plants and medical equipment, and it affects mainly elder persons in poor health.*
Methods. *The population of Venice Local Health Unit was divided in two areas of study and the incidence of legionellosis in residents of Venice historical centre (Distretto Sanitario 1) and in residents of the mainland and coastal areas (Distretti Sanitari 2, 3, 4) was calculated. The cases were those notified to the Public Health Unit by law, and the population of residents was that of the eligible for health care in the archives of the Local Health Unit. Only cases of legionellosis in residents who had not travelled in the 10 days previous of the onset of disease, and not related to nosocomial clusters were considered. The standardized incidence ratio was then calculated and confidence interval were defined by Poisson distribution.*
Results. *Given the population of the two areas, 59801 in Distretto Sanitario 1 and 237555 in Distretti 2, 3, 4, the raw incidence of disease is respectively 87 per 100000 and 20 per 100000 in time 2002-2010. The standardized incidence ratio for the population of Distretto Sanitario 1 vs the remaining population is 4.3.*
Discussion. *The difference in risk of getting the disease in this two residential areas geographically very close, is probably related to the different buildings' characteristics, old and difficult to maintain in Venice historical centre.*

Introduction

Legionnaire's disease is a pulmonary infection, caused by Legionella bacterium, that manifests itself in two distinct clinical forms, after a common onset of symptoms such as myalgia, headache, anorexia, malaise and subsequent fever, non productive cough, abdominal pain and diarrhea:
Pontiac fever, with an incubation ranging from a few hours to 2-3 days, a flu-like syndrome with recovery in a few days
Legionnaire's disease, with an incubation ranging from 2 to 10 days, a multisystem disease involving an atypical pneumonia with diffused or hotbed consolidation, to one or both pulmonary fields [1].
The disease, especially in its gravest forms, affects mainly persons in poor health: elders, immunocompromised patients, heavy drinkers, heavy smokers, patients with chronic respiratory diseases, diabetes mellitus, renal failure, cancer [2, 3]. Legionella is one of the three most frequent causes of community acquired pneumonia, and accounts for 1-40% of nosocomial pneumonia [4] This bacterium has a variable attack rate ranging from 0.1-5% in general population to 0.4-14% in hospital patients [5-10] and has a similarly variable letality depending on the underlying clinical conditions, ranging from 5 to 25% in immunocompetent patients to 30-50% in patients with a nosocomial infection, even with the best intensive care treatments [11-13].
The World Health Organization classifies legionellosis between the 30 new emerging diseases of the last 20 years.
Legionella is a Gram negative bacterium, asporogenous, aerobic, in which genus 50 species and more than 70 serogroups are found; the large majority of Legionella pneumonia is caused by *Legionella pneumophila* serogroup 1; other species are pathogenic for humans (*L. micdadei, L. bozemanii, L. longbeachae, L. dumoffii*…) but are unusual and found mainly in immunocompromised patients [1, 14].
Temperature is the most important factor in promoting or counteracting the bacterial proliferation: Legionella grows between 25°C and 45°C, with a growth peak between 32°C and 42°C, then the process gradually decreases until it stops at 60°C. This bacterium survives in acid and alkalin environment with pH values ranging from 5.5 to 8.1 [14].
Legionella lives in water pipes, in stagnant water conditions, with sediment and biofilm, it can also grow inside amoebae. Bacterial growth is positively favoured by biofilm, a matrix of organic material used by many bacteria as a defensive mechanism against adverse living conditions such as lack of nourishment and extreme temperature. Biofilm usually forms in stagnant waters, and is made of an hydrated matrix of polyanionic polysaccharide that binds the lipopolysaccharide that forms the cellular wall, creating a protective coating around bacteria. Fragment of biofilm containing microorganisms can detach from the tube walls and colonize other sections

of the pipe system, continuing to protect the bacteria against chemical biocides and temperature increases, methods usually employed to remove Legionellae from plants [14].
The main reservoir for Legionellae is water, the bacteria is found ubiquitously in the environment at concentration usually too low to cause disease. Water systems and cooling towers in general, and particularly those in historical buildings, are, instead, particularly at risk of contamination, being usually made by old pipes with calcifications and biofilm formation that promote Legionellae growth and make cleaning and disinfection works difficult, already complex because of the type of structures involved. It is being hypothesized that the presence of protozoa, such as Acanthamoeba spp, Naegleria, Hartmanella spp in water pipes, acts as a growth factor for Legionella, protecting bacteria from biocides and thermal disinfection and promoting bacterial replication [15-18].
Transmission of Legionella happens by inhaling contaminated aerosol produced by showers, taps, whirlpool baths, cooling towers, water fountains, irrigation systems, medical devices (dentist's devices, artificial ventilators…), while interhuman transmission has never been described [3,14].
The diagnosis is made by isolating bacteria from respiratory secretions, seroconversion or by detection of urinary antigen. The latter has been diffusely introduced in clinical practice in the '90 and has much simplified and speeded up diagnosis [19]. Since 2001 is available at the microbiology laboratories in Mestre and Venice hospitals.
Legionella detection in environmental matrices is usually done by cultural exam that represents the traditional analysis method; lately, however, new techniques have been introduced, and now molecular biology techniques, such as Real Time PCR, are coming in use [20].
Usually legionellosis cases have a sporadic pattern, while sometimes we can observe clusters of patients with the same disease: in any case is essential, if possible, to detect and consequently clear up the common source of infection [21-23].
In Italy, between 2002 and 2009, 6836 cases of legionellosis have been notified, with a rising trend in the latest years because of a higher awareness of disease in clinicians. The report, however, shows a wide variability in the number of notifications in different regions, because of a different tendency in looking for Legionella and subsequently notifying the disease found in different places. Thus, probably, in wide areas of the country the disease is partially unrecognized and underrated [21].
The aim of this study is to evaluate the difference in incidence of legionellosis, and detecting the possible causes, in the population living in the 2 areas that compose the ULSS 12 (Venice Local Health Unit): the historical area, with ancient buildings (Venice – Distretto Sanitario 1), and the area with prevalence of urban environment in the mainland with buildings of the last few decades (Mestre, Marcon, Quarto d'Altino) associated, because of similar building characteristics, with the insular area of Lido di Venezia and Cavallino-Treporti (Distretti Sanitari 2, 3, 4).

Methods

This is a semiecological study regarding incidence of legionellosis based on data aggregated for geographic area in years 2002-2010. The statistical unit is represented by residence in Venice city centre (Distretto Sanitario 1) versus residence in the rest of ULSS 12 – Venice Local Health Unit (Distretti Sanitari 2, 3, 4).
The population in this study has been defined by data from the electronic registry from ULSS 12 (Venice Local Health Unit), that includes all subjects living in its territory, those with residence in other places but living in the ULSS 12, and excluding those with residence in ULSS 12 but living in other places.
To reduce the error in calculating incidence, because of a modification, even minimal, of the population in time, a mean population made by inhabitants present between 2002 and 2010, subdivided in age groups (< 30, groups of 10 years of age between 30 and 89, > 90) was used. The resulting total mean population consists in 297356 persons, of which 59801 living in the historical centre of Venice (Distretto Sanitario 1) and 237555 in the remaining areas of ULSS 12 – Venice Local Health Unit (Distretti Sanitari 2, 3, 4).
The incidence of the disease has been calculated summing up all legionellosis cases characterized as follows, and using as denominator the mean population of Distretto Sanitario 1 and Distretti Sanitari 2, 3, 4.
In Italy legionellosis cases are subject to notification by law. There is a special informative flux for legionellosis surveillance that requires notification from the doctor who makes the diagnosis to the hospital direction and/or to the Public Health Unit of the relevant ULSS. The latter sees to the appropriate epidemiological and environmental analysis and forwards the notification to the regional Public Health Department, to the Ministry of Health and to the Institute of Health (Document of 4 April 2000 "Guidelines for the prevention and control of legionellosis").
The diagnostic criteria that have to be satisfied are the signs and symptoms of atypical pneumonia followed by laboratory diagnosis with urinary antigen detection, serologic test (single titration or rising antibody titre), immunofluorescence or culture of sputum or other body fluids.
Because in the two hospital of Venice and Mestre the laboratory method to determine the urinary antigen has been introduced in 2001 and is widely used to diagnose Legionella infections, we proceeded with the statistical analysis of notified cases diagnosed by this method from 2002 to 2010 (laboratory kit: BinaxNOW Legionella, Inverness Medical). The aptitude to diagnosis of legionellosis is homogeneous in the two mentioned hospitals, because they share the same medical unit directors and part of the clinical team.

Only cases of disease in persons who lived in ULSS 12 – Venice Health Unit in the 10 days that preceded the onset of symptoms were considered, to exclude those cases that may have been infected in a different territory. Also 4 cases ascribable to the contamination of the water plant of a nursing home have been excluded, being a hospital cluster not having reference to the territory.
To study the relation between legionellosis incidence and zone of residence, the confrontation of indirectly standardized rates (SIR – Standardized Incidence Ratio) with confidence interval defined by Poisson distribution was used.

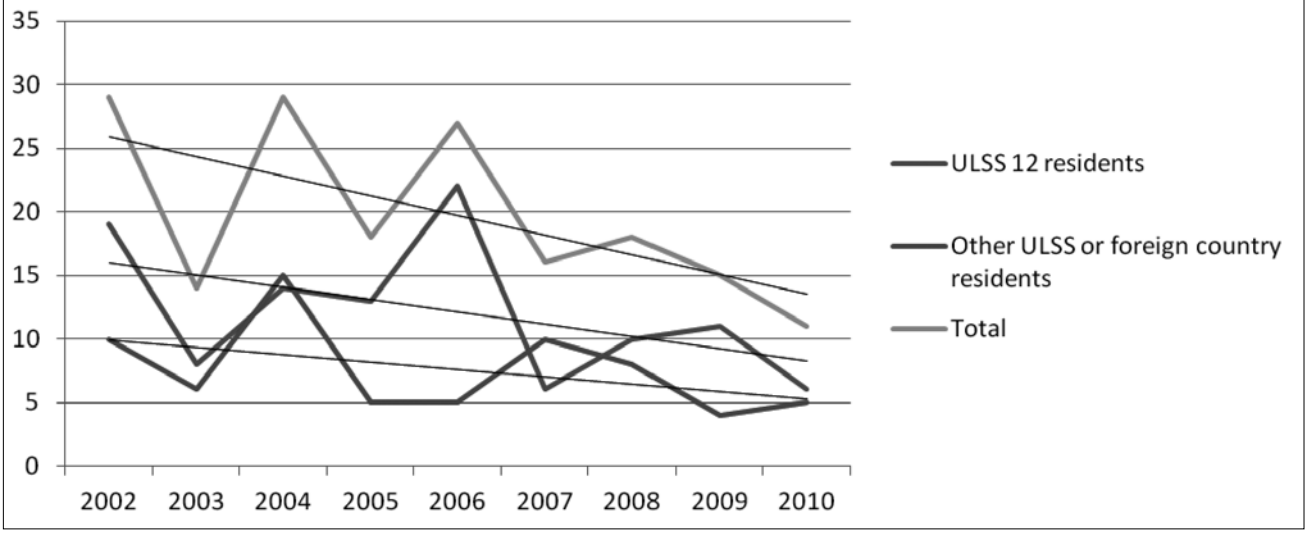
Results

From 2002 to 2010, 111 cases of legionellosis have been notified in persons with residence in ULSS 12 – Venice Health Unit area, in addition to 43 cases of diseases in tourists from foreign countries or other Italian regions and who stayed in hotels or camping grounds in ULSS 12 – Venice Health Unit. Of the 111 residents 9 stayed in hotels outside the area in this study in the 10 days preceding the onset of diseases and 5 were hospital infections, therefore they have been excluded from the study because of the possibility that their infection was acquired in circumstances different from those in study (Fig. 1).
Therefore 97 cases of legionellosis in residents in ULSS 12 – Venice Health Unit who developed the disease 'in loco' are left for the study, 52 living in Venice historical town (Distretto Sanitario 1) and 45 in the remaining territory (Distretti Sanitari 2, 3, 4). Given the population of the two areas, 59801 in Distretto Sanitario 1 and 237555 in Distretti 2, 3, 4, the incidence of disease is respectively 87 per 100000 and 20 per 100000.
The male/female rate is 4.1 (78 males and 19 females), the average age is 62 (range 37-88), females are older (mean age 73) than males (mean age 60). We observe a difference in age between cases living in Venice (Distretto Sanitario 1) and those in the rest of ULSS 12 – Venice Local Health Unit (Distretti 2, 3, 4): in the first group the average age is 66 and in the other is 58.
Smokers or former smokers are 75 (77%), non smokers are 17 (17%) and for 5 cases (5%) the information is not available. In particular in Venice, smokers are 41 out of 52 cases (79%) and in the other group 34 out of 45 cases (76%).
The persons who affirm to drink more than 0.5 l of wine or equivalent of other alcoholic drinks every day are 24 (25%), those who do not drink that amount are 58 (60%) and for 15 persons (15%) he information is not available. In Venice Distretto Sanitario 1 the number of person who drink the specified quantity is 9 on 52 cases (17%) while in the rest of ULSS 12 – Venice Local Health Unit it is 15 on 45 cases (33%).
The prevalence of chronic diseases predisposing to legionellosis in the population of cases is 34%, with a more elevated rate in Distretto Sanitario 1 than in Distretti Sanitari 2, 3, 4, 40% vs 27%.
The raw rates of legionellosis is 87 cases per 100000 in Venice historical town and 20 per 100000 in the rest of the territory with a relative risk of 4.4 (2002-2010).
Since the population of Distretto Sanitario 1 is slightly older than the population of Distretti Sanitari 2, 3, 4, we performed the indirect standardization obtaining a SIR of 4.3 (CI 95% 3.3-5.6) for the Venice population versus the population of the remaining part of ULSS 12 – Venice Local Health Unit.

Discussion and conclusions

Having standardized for age, that is one of the most important risk factors for legionellosis and considering distribution of other risk factors nearly the same in the two populations, the study shows a higher risk, statistically significant, of developing legionellosis in the population of Venice historical centre compared with the population of the other areas of ULSS 12 – Venice Local Health Unit (SIR 4.3; CI 95% 3.3-5.6).
The explanation of such a result can be reasonably attributed to the different conformation and construction time of water plants and cooling towers in the buildings in Venice and in the rest of ULSS 12. In fact, there is a big difference in terms of quality and age of the building heritage in the territory of ULSS 12: the historical area of Venice city centre (Distretto Sanitario 1) is characterized by the presence of historical buildings in which it is not always possible to perform maintenance and renovation works on water plants and cooling towers, due to numerous architectural, structural and landscape restraints, and for which it is extremely difficult, if not impossible, to obtain the exact plant plans. The mainland and coastal areas (Distretti Sanitari 2, 3, 4) have a more recent building and infrastructural stock, in which it is easier to intervene because there are not the abovementioned restraints. Because in the historical centre the edifices are older, cooling towers and plumbing systems have been most likely added after the original building. Therefore, being old and not perfectly assembled, the plants may have been prone to deposit that can be removed with difficulty from out of reach points.
Moreover, after a survey done by the ULSS 12 Servizio Igiene e Sanità Pubblica (Public Health Unit) in 2006, 39 cooling towers have been found in Venice (Distretto Sanitario 1) while only 5 were located in the mainland and coastal areas (Distretti 2, 3, 4).
This peculiarity is crucial in explaining this phenomenon, and this has been highlighted in 2006 when there has been a cluster of legionellosis with 8 cases in Venice (Distretto Sanitario 1) from August 6th to August 9th and no cases in the other areas of ULSS 12. At that point the following has been done: first the geographic location of the houses of the patients were defined and then the customary routes followed by them were identified, to detect overlaps and indentify a common infection source. From this survey, more potential sources of infection, geographically close, were found, and, after sampling of environmental matrix (water and air from cooling towers) they have been found contaminated. In particular on 13 sampled sites, 7 were positive (August-September 2006).

Fig. 1. Trend in legionellosis cases in residents, residents who travelled and tourists who have been in touristic structures in ULSS 12 – years 2002-2010.



This event has been an occasion to enforce a continuing monitoring on structures identified by scientific literature as the main source of legionellosis. From 2006 on, the environmental monitoring was potentiated, both after cases of legionellosis and with periodic monitoring of hotels, campings, swimming pools, nursing homes and cooling towers throughout the territory.

The particular growth and diffusion pattern of Legionella, which is at the same time ubiquitously, but not necessarily found in adequate concentration when sampled, (6 positive samples on 85 total sampled sites from 2002 to 2010 in the plumbing systems in the structures attended by cases), induced to focus the surveillance and prevention program organized by the Servizio Igiene e Sanità Pubblica (Public Health Unit) on development and update of self-monitoring plans by the various structures' managers. These plans are based on risk analysis that, by law (Document of 4 April 2000 "Guidelines for the prevention and control of legionellosis", Conference of 13 January 2005 for relation between the State, the Region and the Autonomous Provinces of Trento and Bolzano 'Guidelines markings information on legionellosis for managers of tourist facilities and spa") needs to be done every two years or when there is a case of legionellosis, and the plan encompasses the analysis of updated plumbing and cooling plants, identifying critical points and inspecting the structure and its critical points, besides the institutional surveillance activity done by the health authority.

Thanks to this surveillance system, the contaminated sites have been very few, 12 on 101 samples on various cooling towers and a slight decrease on the overall incidence of disease in city residents and tourists was observed.

Concluding, the ULSS 12 Servizio Igiene e Sanità Pubblica (Public Health Unit) has been sampling environmental matrices and checking the self monitoring plans of the various structures, thus inverting the trend of incidence of legionellosis in its territory.

The small proportion of positive samples found in the environment, even when cases of disease are observed, bears out the methodological layout represented by producing and updating the self monitoring plans rather than implementing the sampling activity.

The difference in risk of getting the disease found in two residential areas geographically very close, but very different in terms of types, characteristics and building age, raises the question on how to effectively reduce Legionella proliferation in old buildings in which maintenance and clearance works are very difficult. It could be very interesting to verify whether this difference is observed also in other historical cities with similar structural characteristics and also to determine the realistic 'best practice' for Legionella clearance in this particular kind of buildings.

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