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The role of air temperature in *Legionella* water contamination and legionellosis incidence rates in southern Italy (2018–2023)

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Key words: Legionnaires' disease, Legionella pneumophila, air temperature, climate change, public health, waterborne disease
Parole chiave: Malattia dei legionari, Legionella pneumophila, temperatura dell'aria, cambiamento climatico, salute pubblica, malattia veicolata dall'acqua

Abstract

Background. *Legionnaires' disease is caused by inhalation or aspiration of small water droplets contaminated with Legionella, commonly found in natural and man-made water systems and in moist soil. Over the past 5 years, notification rates of this disease have almost doubled in the European Union (EU) / European Environmental Agency (EEA), from 1.4 in 2015 to 2.2 cases per 100,000 population in 2019. Some studies show that the greater presence of the microorganism in the water network and the increase in cases of legionellosis could be related to the variations in some environmental factors, such as air temperature, which may influence the water temperature.*
Study design. *Climate change is currently a prominent topic worldwide because of its significant impact on the natural environment. It is responsible for the increase in numerous waterborne pathologies. The purpose of this study was to correlate the air temperature recorded in Apulia region from January 2018 to April 2023 with the presence of Legionella in the water networks of public and private facilities and the incidence rates of legionellosis during the same period.*
Methods. *During the period from January 2018 to April 2023, water samples were collected from facilities involved in legionellosis cases and analyzed for Legionella. During the same period, all the cases notified*

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to the regional epidemiological observatory (OER-Apulia) were included in this study. Statistical analyses were conducted using the Shapiro–Wilk test to determine whether the Legionella load was distributed normally, the Wilcoxon rank sum test to compare the air temperatures (average and range) of the negative and positive samples for Legionella detection, and the multivariate analysis (Poisson regression) to compare the Legionella load with the water sample temperature, average air temperature, and temperature range on the day of sampling. The Wilcoxon test for paired samples was used to compare legionellosis cases between the warmer and colder months.

Results. *Overall, 13,044 water samples were analyzed for Legionella and 460 cases of legionellosis were notified. Legionella was isolated in 20.1% of the samples examined. The difference in the air temperature between negative samples and positive samples was statistically significant (p-value < 0.0001): on days when water samples tested positive for Legionella a higher temperature range was observed than on days when water samples tested negative (p-value = 0.004). Poisson regression showed a direct correlation between Legionella load, water temperature, and average air temperature. The incidence of legionellosis cases in warmer months was higher than in colder months (p-value = 0.03).*

Conclusions. *Our study highlights a significant increase in the load of Legionella in the Apulian water network, and an association between warmer temperatures and legionellosis incidence. In our opinion, further investigations are needed in different contexts and territories to characterize the epidemiology of legionellosis, and to explain its extreme variability in different geographical areas and how these data may be influenced by different risk factors.*

Introduction

Over the past decade, climate change has altered weather patterns and upset the natural balance of the environment. Temperatures are rising and rainfall patterns are becoming more variable, resulting in more frequent, intense, and extreme events. These changes expose the environment to floods, landslides, and damage to buildings, but increase the risks to both humans and animals, including vegetation and food production. A review of the scientific literature indicated that more than 58% of diseases caused by pathogens can be exacerbated by climate hazards, particularly when they affect vulnerable populations (1-3).

The past 5 years have been the warmest of the past decade. Specifically, 2019 was the second warmest year on record, with an average increase in global temperature of ~1.1°C compared with the pre-industrial era (4).

The European Center for Disease Prevention and Control (ECDC) highlights an increase in the number of cases of various infectious diseases caused by bacteria and viruses in recent years (5). Several studies report that the increase in ambient temperature and extreme events can be included among the effects of climate change, playing an important role in the transmission of waterborne diseases, including legionellosis (6-10).

Legionellosis is an infectious disease subject to mandatory notification in class II. It is caused by the inhalation or aspiration of small water droplets contaminated with *Legionella pneumophila* and other *Legionella* spp., which are commonly found in natural and artificial water systems (11-16) and in soil (15). Travel-associated Legionella cases (12.3%) were reported in the United States (17, 18). In the last five years, the notification rates have nearly doubled in the European Union (EU)/European

Environmental Agency (EEA), from 1.4 in 2015 to 2.2 cases per 100 000 population in 2019. Four countries, France, Germany, Italy, and Spain, accounted for 71% of all notified cases (16).

To date, molecular studies based on genome sequences have made it possible to identify 66 species of *Legionella* (19). Although *Legionella pneumophila* serogroup (sg) 1 is considered the main causative agent of the disease, an increasing number of cases are attributed to other species (20-22), some of which are more prevalent in water supplies (23).

Temperature plays a key role in the survival of this microorganism. *Legionella* thrives at a temperature range of 25°C to 45°C, with an optimum temperature of 37°C, but can also survive temperatures between 5.7°C and 63°C (24-26). Its presence has also been detected in the waters of Antarctic lakes, which record a temperature of ~0°C (27). For this reason, it is important to investigate all of the environmental parameters that can affect the presence of *Legionella* in water (28).

The aim of this study was to correlate air temperature measured in the Apulia region (southern Italy) from January 2018 to April 2023 with the presence of *Legionella* in the water networks of public and private facilities and the incidence rates of legionellosis during this same period.

Methods

In 1998 the Government of Apulia Region established a regional epidemiological observatory (OER-Apulia) at the Department of Biomedical Sciences and Human Oncology, now the Interdisciplinary Department of Medicine, University of Bari Aldo Moro, Italy. The OER has the task of monitoring and carrying out surveillance of all infectious diseases, including legionellosis (29).

From 2000 to date, environmental and clinical monitoring on the presence of *Legionella* in water networks and on cases of legionellosis was considered by the

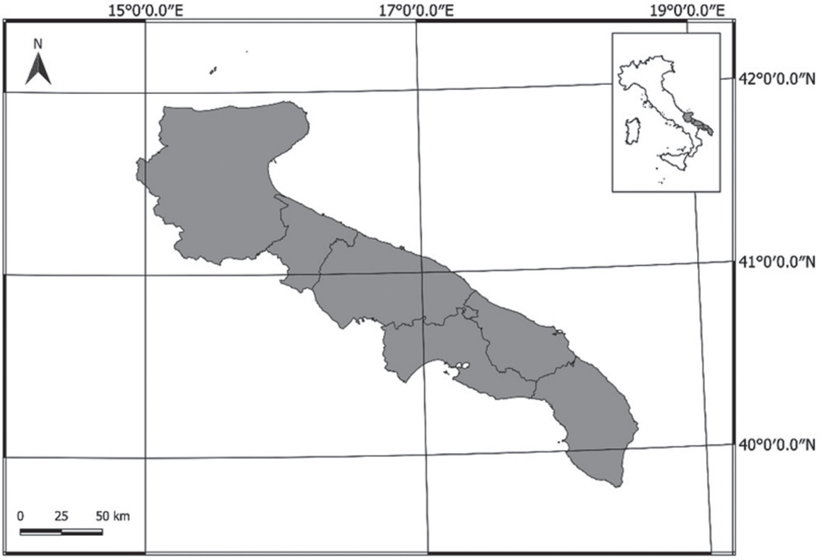

Figure 1 – Geographical position of Apulia region (south-eastern Italy) according to the WGS84 reference system

Laboratory of Environmental Hygiene of the Interdisciplinary Department of Medicine (20, 25, 30-34). A stump library was set up to store environmental and clinical isolates, and data were stored in a database that allows for real-time information delivery regarding the cases notified and the distribution and degree of contamination in public and private water systems.

All of the data from the analysis of water samples collected during the period from January 2018 to April 2023 according to the ISO 11731:2017 (35) and reported in previous research (23), and all the cases of legionellosis notified to the OER Apulia during the same period were included in this study.

Apulia climatic characteristics

Apulia is a region geographically located in south-eastern Italy, between 41.5° and 39.7° north latitude, falling within the subtropical belt that extends between 30° north and 50° south latitude (Figure 1). Winters are generally mild and moderately rainy, summers are hot with little rain, and rainfall is abundant during autumn. In Apulia, the warmer months are generally May–October, the coldest months are January–April and November–December (36).

The climatic data for the study period were obtained from the meteorological stations present in the region (36), within a maximum distance of 30 km from each sampling point. We considered the average air temperature and the temperature range (difference between the maximum and minimum daily temperature) detected on the day of sampling.

Statistical analysis

The Shapiro–Wilk normality test was used to determine whether the load of *Legionella* spp. was distributed normally and consequently whether a parametric statistical test (normal distribution) or non-parametric statistical test (non-normal distribution) should be used. The Wilcoxon rank sum test was used to compare the air temperatures (average and range) of the group of samples that tested negative for *Legionella* spp. and those that tested positive for *Legionella* spp. Multivariate analysis (Poisson regression) was used to compare the *Legionella* spp. load with the water sample temperature, average air temperature, and temperature range on the day of sampling.

To quantify the effects of each independent variable on *Legionella* spp. load, we computed the influences ($e\beta-1$), which virtually corresponded to the relative risks (7).

The Wilcoxon test for paired samples was used to compare legionellosis cases between warmer months (May–October) and colder months (January–April and November–December). Tests were considered statistically significant when the p-value was <0.05. Statistical analyses were performed using R software version 3.6.1 (Brandon Greenwell, Cincinnati, Ohio).

Results

In the period from January 2018 to April 2023, 460 cases of legionellosis were notified in the Apulia Region and 13,044 water samples from 110 facilities were analyzed for the presence of *Legionella*. Overall, 2,621 water samples tested positive (20.1%). The Shapiro–Wilk test on the *Legionella* load revealed that the distribution was not normal (W = 0.0076115, p-value < 0.001). Therefore, non-parametric statistical tests were used for statistical analyses.

The difference in the air temperature between negative samples (mean: 15.8°C, median: 14.9°C) and positive samples (mean: 17.2°C, median: 16.8°C) was statistically significant (Wilcoxon rank sum test with continuity correction W = 11245744, p-value < 0.0001). A statistically

Table 1 – Poisson regression model of the Legionella load (cfu/L) – Final model

Sampling day	β	(eβ-1) = RR (%)	p-value
Intercept	7.02		<0.0001*
Water temperature	0.15224	16.4	<0.0001*
Average air temperature	0.14318	15.4	<0.0001*

RR: relative risk; *, $p < 0.05$

significant difference was also detected in the temperature range on the day of sampling (negative samples: mean = 6°C, median = 5.8°C; positive samples: mean = 6.1°C, median = 6.2°C): when water samples tested positive for *Legionella* a higher temperature range was observed than on days when water samples tested negative (Wilcoxon rank sum test with continuity correction W = 12413893, p-value = 0.004).

Poisson regression (Table 1) showed a direct correlation between *Legionella* load, water temperature, and average air temperature, but no statistically significant difference was found in the temperature range on the day of sampling (p-value > 0.05).

Figure 2 shows the correlation between *Legionella* average load (ordinate) and median air temperature (abscissa) for each quarter of each year during the period from January 2018 to April 2023. Each quarter is indicated by a different color. The trend line reveals that higher air temperatures correspond to higher loads of *Legionella.* The highest temperatures and highest average loads of *Legionella* in water samples occurred in the third quarter (shown in red).

Of the 460 cases of legionellosis notified during the period from January 2018 to April 2023, 286 (62.2%) occurred in the warmer months and 174 (37.8%) occurred in the colder months, and this difference was statistically significant (warmer months – mean: 48 cases per month – median: 52 cases per month; colder months – mean: 29 cases per month– median 27 cases per month

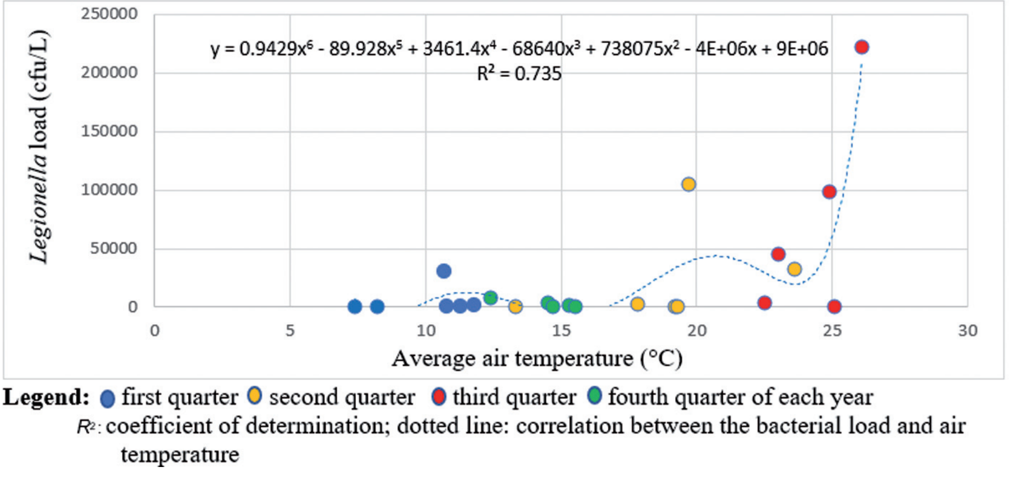

Figure 2 - *Legionella* load in water samples and the mean air temperature by quarter for each year (January 2018 – April 2023)

- Wilcoxon test for paired samples V= 23.5, p-value = 0.03).

Discussion

In recent years, climate change has been recognized as a global phenomenon with far-reaching impacts on various aspects of life, including public health (10). The increase in temperature, combined with inconsistent increases in other factors (e.g., humidity, cumulative rainfall, extreme events) are modifying the environmental ecological balance. Furthermore, seawater intrusion is negatively affecting the coastal aquifer, which is exploited for drinking purposes, thereby potentially impacting in a severe way the distribution of anomalous microbial species and gastrointestinal disease due to the presence of fecal-oral pathogens (8, 37-39).

In terms of waterborne microorganisms, *Legionella*, which is responsible for legionellosis, is known to thrive in aquatic environments and can pose a significant health risk. It is highly sensitive to warmer temperatures, which provide an ideal environment for bacterial multiplication in both natural water sources and artificial water systems (40). According to previous studies (12, 41, 42), the increase in legionellosis cases during the warmest months could be due to an increase in seasonal temperatures causing the proliferation of *Legionella* in the cold water supply of a building and, consequently, a greater risk of disease.

Understanding whether there is a correlation between *Legionella* load and environmental temperature is critical in order to identify potential epidemiological trends, predict outbreaks, and implement effective prevention strategies for legionellosis, especially in high-risk settings such as healthcare facilities. Some studies report seasonal cases of legionellosis associated not only with longer summer periods but also with higher temperatures in spring and autumn (16, 43).

Our study also highlighted a correlation between the average load of *Legionella* and the average air temperature for each quarter of the period examined. Higher air temperatures corresponded to a greater load of *Legionella*, especially in the third quarter (July–September), which represents the hottest period in Apulia, followed by the fourth quarter (October–December). Similarly, the cases of legionellosis notified in the study period occurred more frequently in the warmer months than in the colder months (62.2% vs 37.8%), and this difference was statistically significant.

Although some authors have examined other climatic factors that can influence the presence of *Legionella* in aquatic environments (e.g., solar ultraviolet, rainfall, humidity, flood waters) (9, 41, 44), including wastewater treatment plants and the filtering capacity of the subsoil that conveys the groundwater to wells intended for irrigation (45-48), the association between warmer weather and the incidence of legionellosis is well documented (6, 8, 10, 12, 48).

Another risk factor that does not find common consensus among researchers is the scarce use of the water network. Some authors (49) reported that, during the COVID-19 pandemic, the lockdown has drastically reduced the demand for water in many buildings, without causing an increase in the *Legionella* load. On the contrary, other authors (50) revealed that the water network closed for three months because of COVID-19 emergency showed a higher *L. pneumophila* contamination after lockdown period.

Conclusion

Further studies are needed to characterize the epidemiology of legionellosis, understand its extreme variability in different geographic areas, and determine how these data may be influenced by the numerous risk factors that distinguish this disease from other waterborne diseases. Furthermore, understanding the correlation between *Legionella* load, climate change and other risk factors may lead to the development of proactive control and prevention strategies. Strengthening surveillance systems on legionellosis cases and monitoring the load of *Legionella* in water systems will become an essential means of minimizing the risk of contamination and providing timely and effective intervention.

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Riassunto

Ruolo della temperatura dell'aria sulla contaminazione dell'acqua da Legionella e tassi di incidenza della legionellosi nel sud Italia (2018-2023)

Introduzione. La legionellosi è causata dall'inalazione o dall'aspirazione di piccole goccioline d'acqua contaminate da *Legionella*, comunemente presenti nei sistemi idrici naturali e artificiali e nel suolo umido. Negli ultimi cinque anni, i tassi di notifica di questa malattia sono quasi raddoppiati nell'UE/AEE, passando da 1,4 nel 2015 a 2,2 casi per 100.000 abitanti nel 2019. Alcuni studi evidenziano che una maggior presenza del microrganismo nella rete idrica e l'aumento dei casi di legionellosi potrebbero essere correlati alla variazione di alcuni fattori ambientali, registrata negli ultimi anni, come la temperatura dell'aria.

Disegno dello studio. Oggi il cambiamento climatico è l'argomento più discusso in tutto il mondo, soprattutto perché responsabile di impatti diversi sull'ambiente naturale, compreso l'aumento di numerose patologie veicolate dall'acqua. Tra i vari fattori che influenzano la sopravvivenza e l'adattamento dei microrganismi alle nuove condizioni ambientali, la temperatura è uno dei temi più dibattuti.

Lo scopo di questo studio è correlare la temperatura dell'aria registrata in Puglia da gennaio 2018 ad aprile 2023 con la presenza di *Legionella* nelle reti idriche di strutture pubbliche e private e i tassi di incidenza della legionellosi nello stesso periodo.

Metodi. Sono stati esaminati campioni di acqua provenienti dalle strutture coinvolte nei casi per la ricerca di *Legionella*. L'analisi statistica ha utilizzato il test di Shapiro-Wilk per capire se la carica di *Legionella* seguisse una distribuzione normale, il test di Wilcoxon rank-sum per confrontare le temperature dell'aria rilevate nei giorni in cui i campioni sono risultati negativi con quelli positivi, e l'analisi multivariata (regressione di Poisson) per confrontare la carica di *Legionella* spp con la temperatura del campione di acqua, la temperatura media dell'aria e l'intervallo di temperatura nel giorno del campionamento.

Il test di Wilcoxon per campioni appaiati è stato utilizzato per confrontare i casi di legionellosi tra i mesi più caldi e quelli più freddi.

Risultati. Sono stati analizzati 13.044 campioni di acqua e notificati 460 casi di legionellosi. *Legionella* è stata isolata nel 20,1% dei campioni. La differenza nella temperatura dell'aria tra campioni negativi e campioni positivi è risultata statisticamente significativa (p-value < 0,0001): nei giorni in cui i campioni di acqua sono risultati positivi per *Legionella* è stato osservato un intervallo di temperatura più elevato rispetto ai giorni in cui i campioni sono risultati negativi (p-value = 0,004). La regressione di Poisson ha mostrato una correlazione diretta tra carica di *Legionella*, temperatura dell'acqua e temperatura media dell'aria. L'incidenza dei casi di legionellosi nei mesi più caldi è stata maggiore rispetto ai mesi più freddi (p-value = 0,03).

Conclusioni. Il nostro studio evidenzia un aumento significativo della carica di *Legionella* nella rete idrica pugliese e un'associazione tra temperature più calde e incidenza di legionellosi. A nostro avviso, sono necessarie ulteriori indagini in diversi contesti e territori per caratterizzare l'epidemiologia della legionellosi e per comprendere la sua estrema variabilità nelle diverse aree geografiche e come questi dati possono essere influenzati da diversi fattori di rischio.

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