



Investigation of the presence of *Legionella pneumophila* in water samples from Erzurum and surrounding provinces in Turkey

Ahmet Yilmaz^{1,A-F}, Figen Orhan^{2,A-B,D-F}

¹ Erzurum Provincial Directorate of Health, Erzurum, Turkey

² Department of Medical Laboratory Techniques, Vocational School of Health Services, Ataturk University, Erzurum, Turkey

A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of article

Yilmaz A, Orhan F. Investigation of the presence of *Legionella pneumophila* in water samples from Erzurum and surrounding provinces in Turkey Ann Agric Environ Med. 2021; 28(2): 255–259. doi: 10.26444/aaem/135052

Abstract

Introduction and objective. Legionnaires' disease, which is a waterborne disease leading to pneumonia that can result in death, is one of the major health issues today. The study aims to carry out a three-year retrospective review of routine *Legionella* follow-up analyses in various samples taken mostly from hospitals in the province of Erzurum and nearby provinces, and to discover a the frequency of isolation of the agent and serogroups from each kind of media.

Materials and method. The study included a total of 2,025 water samples taken from hospitals, hotels, Turkish baths and shopping malls in Erzurum, and 13 nearby cities between 2016 and 2018. Samples were filtered by 0.45 µm-diameter membrane filter paper in the Public Health Laboratory of Erzurum and examined for *L. pneumophila* using culture method, according to the criteria set out in ISO 11731-2.

Results. The presence of *L. pneumophila* was found in 65 of the 2,025 water samples taken in hospitals, and in none of 40 samples taken in hotels, Turkish baths and shopping malls. *L. pneumophila* serogroup 2–14 was detected in 46 (70.8%) of 65 samples found positive, whereas *L. pneumophila* serogroup 1 was detected in 18 (27.7%). Furthermore, both the *L. pneumophila* serogroup 2–14 and *L. pneumophila* serogroup 1 were detected simultaneously in one example (1.5%). The first three samples indicate that the highest positivity rates were in hot water taps (11.6%), hot water tanks (6.1%) and shower heads (4.8%).

Conclusions. The Prevalence rat of *L. pneumophila* was found to be quite low compared to other studies conducted in the western regions of Turkey. *Legionella* positivity was found to be higher in the hot water systems of hospitals and related points, compared to the other sample points.

Key words

Legionella pneumophila, hospital, hotel, water samples

INTRODUCTION

Legionnaires' disease was defined for the first time as a result of analyses performed after an outbreak of acute respiratory tract infection in 182 people who attended a meeting of the Pennsylvania American Legion held in Philadelphia in 1976, and caused the death of 29 of the participants. The source of the transmission of the disease were not exactly determined [1]. In the USA, the Center for Disease Control (CDC) revealed that the agent in this outbreak was a gram-negative bacterium and named the agent *Legionella pneumophila* since it causes a pneumonia-like picture [2]. Currently, there are over 60 species of *Legionella* reported; however, the main cause of Legionnaires' disease worldwide is *L. pneumophila* [3, 4]. *Legionella* is transmitted to humans by aspiration of contaminated water and inhalation of contaminated aerosols and/or air conditioning gases [5–7]. Common factors contributing to *Legionella* persistence in water systems include biofilm formation, growth in amoebae, growth in nutrient-poor environments, and disinfectant resistance or tolerance [8, 9]. Two major hotel-related cases

of Legionnaires' disease have been reported in Turkey, the first of which involved 17 cases in a hotel in Kusadasi in 1994, and the other involved 16 cases in a hotel in Istanbul in 1997 [10].

OBJECTIVES

Species of *Legionella* survive for a long time in chlorinated city waters, tank waters and on wet surface, and can be transmitted to humans through such media. Therefore, the aim of this study was to carry out a three-year retrospective review of routine *Legionella* follow-up analyses in various samples taken from hospitals, hotels, Turkish baths and shopping malls in the province of Erzurum and surrounding provinces, and to determine the frequency of isolation of the agent, and serogroups from each kind of environments.

MATERIALS AND METHOD

The study included a total of 2,025 water samples taken from hospitals, hotels, Turkish baths and shopping malls in Erzurum and nearby provinces (Ağrı, Ardahan, Artvin, Bayburt, Bingöl, Erzincan, Giresun, Gümüşhane, Iğdır, Kars,

Address for correspondence: Ahmet YILMAZ, Erzurum Provincial Directorate of Health, Erzurum, Turkey
E-mail: aymet25@hotmail.com

Received: 08.12.2020; accepted: 26.03.2021; first published: 19.04.2021

Muş, Tunceli and Van) between 2016–2018. Analyses were carried out in the Public Health Laboratory of Erzurum, which is also the reference laboratory in the region, by the method described in the relevant circular issued by the Ministry of Health. All samples were examined for *L. pneumophila* using the culture method according to the criteria set out in ISO 11731–2.

The water samples were inoculated both directly and after concentration. Using a sterile 0.45 µm diameter membrane filter paper, 50 ml water samples were filtered. 5ml of sterile water were added to the filter paper which had sediment on it, and vortexed for 30 seconds. 2 ml of this water were taken and 2 ml of acid buffer added. For culturing, 100 µl water was added after 3 minutes of waiting time to both the BCYE (buffered charcoal yeast extract) and the DGVP (dye-glycine-vancomycin-polymyxin B) agar medium. Inoculated plates were incubated at 36 ± 2 °C in an environment of 90% humidity for 10 days. The plates were checked daily for growth after the 3rd day of incubation. Plates with no visible colonies were incubated for the end of the period. Gram staining was performed with suspected *Legionella* cp colonies where the growth was observed within 3–5 days. For the bacillus-shaped bacteria that displayed weak gram-negative staining, 5% sheep blood agar and BCYE agar media was passaged. The passaged samples were incubated at 37 °C for 24–48 hours. Isolates that developed on the BCYE agar, but did not develop on the 5% sheep blood agar, were serologically confirmed by the latex agglutination test (Oxoid), and identified as belonging to the *L. pneumophila* serogroup 1, *L. pneumophila* serogroup 2–14, and *Legionella* species other than pneumophila. ATCC 43111 reference strain was used as a positive control.

Statistical analysis. SPSS 22.0 (SPSS Inc, Chicago, IL, USA) statistical package programme was used for evaluation of all data obtained and statistical analysis of the results. Chi-square test was used to evaluate the isolation and positivity of *L. pneumophila*, and the limit of significance was set at $p < 0.05$. The approval of the Ethics Committee was not needed for the study, only an institutional permit was obtained from the Erzurum Health Directorate on 8 April 2019.

RESULTS

The number of samples taken in different months is shown in Table 1. Positive test results of *L. pneumophila* were found especially at the highest rate in April, May, and June. In November, no positivity was detected in any of the samples studied (Fig. 1). *L. pneumophila* positivity was detected in 65 (3.2%) of the 2,025 samples of water samples taken from hospitals (94.8%), hotels (1.6%), Turkish baths (0.2%) and shopping malls (0.2%). *L. pneumophila* serogroup 2–14 was detected in 46 (70.8%) of 65 samples found positive (Tab. 2, Tab. 3).

Samples were taken from different points, mostly sink taps (41.8%), shower heads (26.9%), cold water tanks (14.7%), and hot water tanks (10.5%) (Tab. 3). Positivity for *L. pneumophila* was found in 65 (3.2%) of the 2,025 water samples. In serological typing performed with latex agglutination test from 65 samples, 46 (70.8%) were identified as *L. pneumophila* serogroup 2–14, and 18 (27.7%) as *L. pneumophila* serogroup-1. Also, in sample (1.5%),

both *L. pneumophila* serogroup 2–14 and *L. pneumophila* serogroup 1 were detected simultaneously.

Only positive results were obtained in samples taken from the hospitals. In 23 of 89 hospitals, *L. pneumophila* positivity was detected. No *L. pneumophila* spp was detected in any samples taken from hotels, public baths, and shopping malls. The highest positivity rates were found in the hot water taps (11.6%), hot water tanks (6.1%), and shower heads (4.8%). No positivity was detected in any of the samples taken from artesian water tanks or cooling towers (Tab. 3). The difference between the groups was statistically significant $p < 0.05$.

Table 1. Distribution of sample numbers by month

Month	Number of samples	Percent
January	66	3.3
February	87	4.3
March	104	5.1
April	263	13.0
May	374	18.5
June	185	9.1
July	104	5.1
August	176	8.6
September	147	7.2
October	275	13.6
November	176	8.7
December	68	3.4

Table 2. Prevalence of *L. pneumophila* in examined facilities

Facility	No. of facilities examined	No. of facilities where positive water samples were found	No. of total samples examined positive/total (percent)
Hospital	89	26	65/1989 (3.3%)
Hotel	3	0	0/32 (0)
Turkish Bath	1	0	0/4 (0)
Shopping Mall	1	0	0/4 (0)
Total	94	26	65/2025 (3.2%)

Table 3. Prevalence of *L. pneumophila* at specific sampling points in hospitals

Sampling point	No. of sampling points examined	No. of sampling points where positive water samples were found	No. of water samples examined positive/total (percent)
Lavatory faucets	848	16	16/848 (1.9%)
Shower heads	546	26	26/546 (4.8%)
Cold water tank	299	4	4/299 (1.3%)
Hot water tank	213	13	13/213 (6.1%)
Mains water inlet	51	1	1/51 (2.0%)
Hot water tap	43	5	5/43 (11.6%)
Artesian water tank	14	0	0/14 (0%)
Cooling tower	11	0	0/11 (0%)
Total	2025	65	65/2025(3.2%)

Our study was carried out on water samples taken from 14 provinces in eastern Turkey (Fig. 2). When considering the distribution of positive *L. pneumophila* test results by

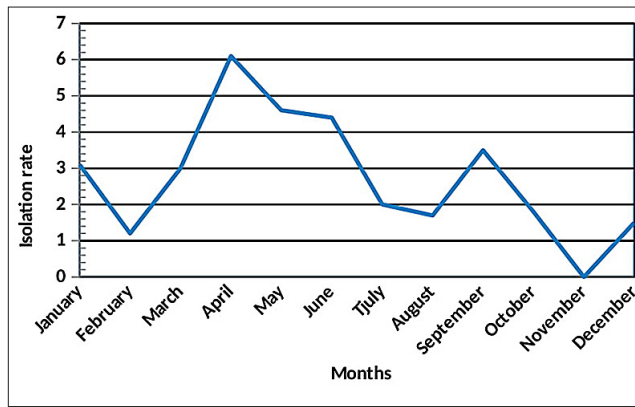


Figure 1. Comparison of *L. pneumophila* positive results by months

DISCUSSION

Legionnaires' disease is important for preventive medicine because it is a waterborne disease that leads to severe pneumonia which can result in death. Prevention of *Legionella*'s settlement and proliferation in water-related areas and water systems constitutes the basic protection strategy for Legionnaires' disease [1]. While bacteria cause no health problems in most healthy individuals, they can cause very serious diseases in some individuals [11]. The Center for Disease Control (CDC) has reported that people over 50 years of age, smokers, people with chronic lung disease, and people with a weakened immune system, are at serious risk for Legionnaires' disease [12]. It is reported that the incidence of Legionnaires' disease in the USA has

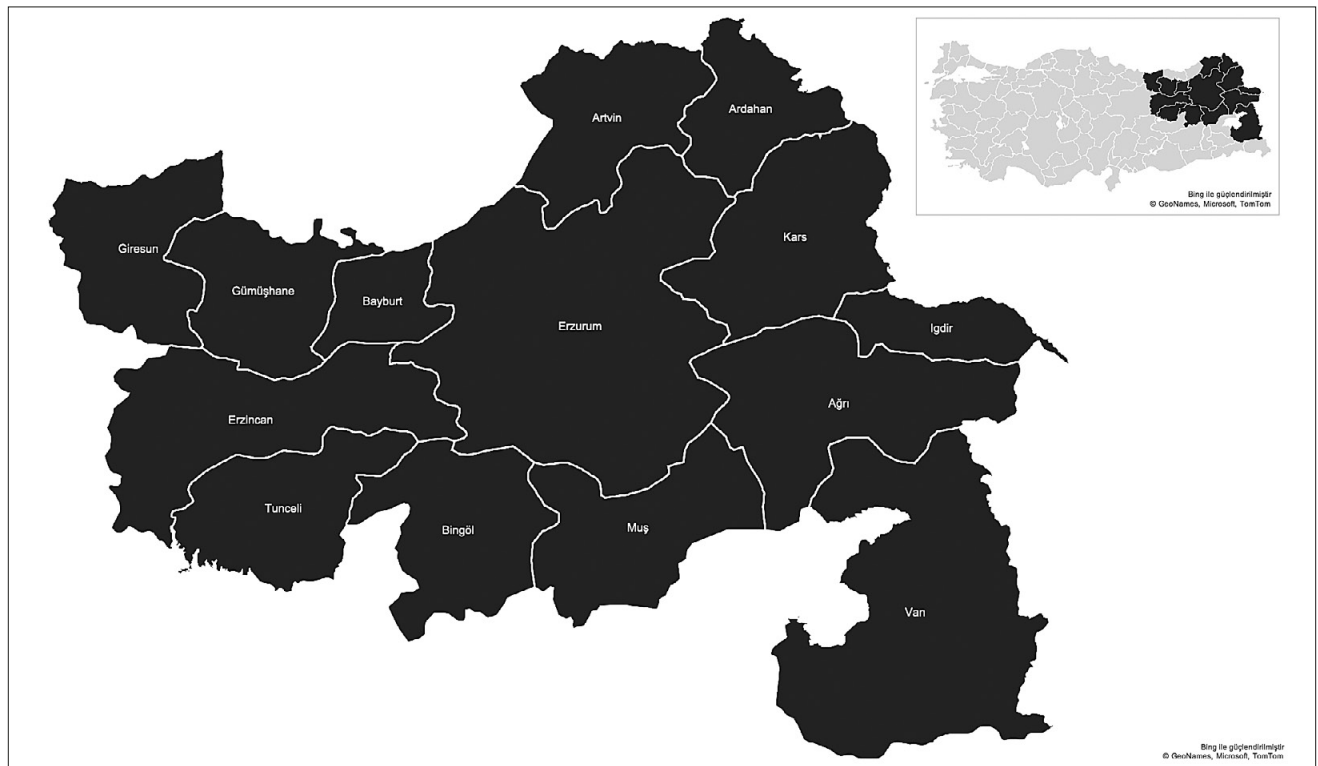


Figure 2. The provinces in eastern Turkey included in the study

provinces, the highest positivity rates were detected in the provinces of Tunceli (23.5%), Gümüşhane (11.6%), and Erzurum (4.4%). No positivity was detected in any samples studied found in Ardahan, Bayburt, Giresun, Iğdir, and Van provinces (Fig. 3).

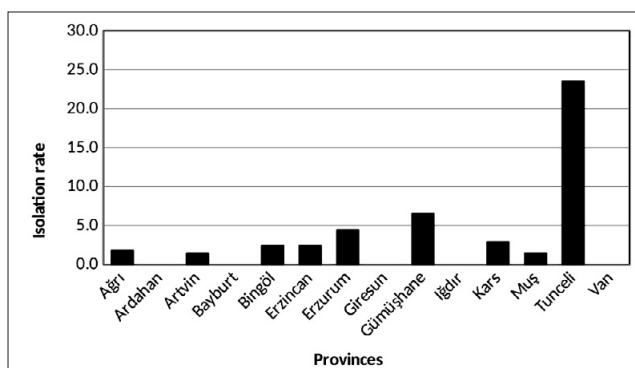


Figure 3. Distribution of *L. pneumophila* positive results by provinces

increased four times since 2000, and the incidence in Europe has increased approximately three times since 1995 [13]. In 2016, 7,069 cases were reported in 30 European countries, of which 6,560 (92.8%) were verified [14]. The disease is divided into three categories which are reported as follows: 1) approximately 70% of *L. pneumophila* cases are community-acquired, 2) 20% travel-related, and 3) 10% nosocomial [13].

Some studies show that 12% – 70% of the water systems in hospitals involve *Legionella* bacteria colonization [15]. Stagnation of water at the usage points, in other words, at the end points, increases the colonization of *Legionella*. There are studies showing that stagnation in places such as sensor lavatory faucets, lavatory drainage pipes, ice machines, and decorative fountains are associated with nosocomial infection [16].

When evaluated at the national level, it is reported that the number of cases of Legionnaires' disease is the highest in countries such as Italy, France, Spain and Turkey [17]. In a 15-year study by Lagana et al. [18] conducted in a hospital in Italy,

812 (60%) of 1,346 water samples were *Legionella* positive. 588 (72%) of the positive samples were *L. pneumophila* serogroup 2–14, and 147 (18%) were *L. pneumophila* serogroup 1, and 119 (15%) were non-*L. pneumophila*. Borella et al. [19] reported *Legionella* positivity in 60.5% of 119 samples taken from hot water systems in 40 hotels in Italy. Chochlakakis et al. [20] reported *Legionella* colonization in 108 (6.9%) samples in hot and cold water distribution systems in 1,495 water samples from 124 hotels in Greece. Yakunin et al. [21] demonstrated that 60% of the examined hotels were colonized with *L. pneumophila*, of which 470 (17%) were *Legionella*-positive from 2,830 water specimens taken from 168 hotels in Israel.

Many hospital-related and hotel-related outbreaks of Legionnaires' disease were reported in Turkey. In a study by Erdoğan et al. [10] on an outbreak of *Legionella* that occurred in a newly-opened hotel in Alanya in 2009, *Legionella* was isolated from 11 out of 13 water samples taken, and all the positive samples were defined as *L. pneumophila* serogroup 1 in typing. Akkaya et al. [22] detected *Legionella* in 8 (6.7%) of the 120 samples taken from schools, hospitals, hotels and houses in Kayseri, Turkey, in 2008. Özen et al. [23], in their study involving a total of 403 samples collected from 56 different hotels in Antalya, reported that *L. pneumophila* was isolated in 37.5% of the hotels and 10.1% of water samples. Özen also reported that 85% of the samples were *L. pneumophila* serogroup 2–14 positive, and 15% of the samples were *L. pneumophila* serogroup 1 positive. Ignak et al. [24] detected *Legionella* colonization in 7% of 100 samples taken from various departments of Istanbul Medical Faculty Hospital (three of which were *L. pneumophila* serogroup 1, and four were *Legionella*). In the presented study, *L. pneumophila* colonization was detected in 65 (3.2%) of 2,025 samples, mostly taken from the water systems of hospitals. *L. pneumophila* positivity rate was found to be quite low. This is believed to be the result of the fact that the optimal reproductive temperature of legionella spp is 35 °C, and because the Erzurum province is the coldest region in Turkey the average temperature of mains water is quite low; therefore, the growth of *Legionella* is negatively affected. *L. pneumophila* serogroup 1 is reported as the species responsible for the majority of clinical cases in Europe and America [25]. In this study, the presence of serogroup 1 was found in 29.2% of positive samples while the most common was serogroup 2–14.

Due to reasons such as the characteristics of buildings, structure of the water system, organic content of the water, seasonal conditions and geographical factors, the isolation rate of *Legionella* bacteria varies from study to study [26]. In Israel, Sharaby et al. [27] reported in their study that the prevalence of *Legionella* in the water system and the resulting infection risk are seasonal, and twice as high in the summer months compared to the wintertime. In the current study, the highest number of cases was observed in April and May on an average of three years. No conclusion could be drawn regarding the reason for higher positivity in the spring.

Prevalence of *L. pneumophila* is reported to be higher in hot water systems that feed faucets and showers in health facilities, compared to the other species of *Legionella* [28, 29]. This opinion is also supported by the fact that in the current study, the three points with the highest positivity among the points from which samples were taken, were hot water faucets (11.6%), hot water tanks (6.1%) and shower heads (4.8%), respectively.

This study was conducted using data from 14 provinces in eastern Turkey, and when comparison of the positivity rates of the samples taken from these provinces, the three provinces with the highest positivity rate were found to be Tunceli (23.5%), Gümüşhane (11.6%), and Erzurum (4.4%). No *Legionella* bacteria were found in water samples taken from the provinces of Ardahan, Bayburt, Giresun, Iğdır and Van. Due to the retrospective nature of the study, no conclusions could be formed for the causes of this difference. The element iron is important for the reproduction and survival of *Legionella* because of its involvement as a co-factor in bacterial enzymes [10]. However, no information could be obtained about the iron content of the water samples taken from the provinces, the structure of the water systems or the age of the buildings. This was one of the limiting factors of the current study.

A Legionnaires' disease control programme has been carried out in Turkey since 1996 [11]. In considering the studies to determine the presence of *Legionella* in Turkey, the studies conducted in the western of our country are especially noteworthy. As no other study could be found for *Legionella* in Erzurum and nearby cities, the authors of the presented study believe it to be important for a number of reasons: it includes data obtained from the provinces in eastern Turkey with a cold climate, and it involves the biggest number of samples to date with respect to *Legionella*.

CONCLUSIONS

A summary of results of this study indicate that they differ from studies conducted by other researchers in different geographical regions of Turkey. The positivity rate of *L. pneumophila* is quite low compared to the other studies conducted the western part of the country. Since most of the water samples that involved growth of *Legionella* were taken from a hospital, the study emphasizes the importance of continuous observation, especially in water samples from hospitals to prevent the risk of hospital-induced legionellosis. The incidence rate of *L. pneumophila* serogroup 2–14 was higher than the incidence rate of serogroup 1. *Legionella* positivity was found to be higher in hot water systems of hospitals and related points, compared to the other sampling points. Furthermore, it is believed that the data obtained in this study can serve as important epidemiological data for *Legionella* surveillance control in Turkey. In addition, the results also indicate that further studies, such as molecular identification methods, are needed to identify the types of *Legionella* isolates and obtain more epidemiological data in Turkey.

Acknowledgements

The authors would like to thank to Dr. Gursel Bedir, Savas Calık, and the staff of the public health laboratory for their contributions to the study.

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