

# Biological risk of *Legionella pneumophila* in irrigation systems

## Riesgo biológico por *Legionella pneumophila* en sistemas de riego

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### ABSTRACT

**Objective** The goal of this study is to determine the risk of exposure to *Legionella pneumophila* in hotel golf courses located in the province of Málaga (Spain).

**Method** Spray irrigation systems were analyzed as sources for spreading the *Legionella bacterium*. Spanish legislation requires that irrigation systems be monitored for their water quality as well as for reasons related to health and hygiene. Based on an observational study and non-parametric tests (Goodman-Kruskal Tau and uncertainty coefficient), this study states the regulatory enforcement among the systems and contributed to announce *Legionella* prevention. The quality criteria for recycled water, waste water treatment plant and well water were analyzed in relationship to the hotels' categories.

**Results** Deficiencies were found in the preventive maintenance of irrigation systems, but no relationship exists between the type of water and the risk detected.

**Conclusions** The study suggests that aerosolized water used in golf course watering systems could pose risk to the population by exposing them to *Legionella*.

**Key Words:** Legionellosis; golf; *Legionella pneumophila*; water quality (source: MeSH, NLM).

### RESUMEN

**Objetivo** El propósito de este trabajo es determinar el riesgo de exposición a *Legionella pneumophila* en campos de golf de hoteles ubicados en la provincia de Málaga (España).

**Método** Se realizó un estudio de los sistemas de riego por aspersión como fuentes de dispersión de la bacteria *Legionella*. El análisis de las condiciones higiénico-sanitarias de las instalaciones y la calidad del agua es una actividad de obligado cumplimiento contemplada en la legislación sanitaria española. Mediante un estudio observacional y el empleo de pruebas no paramétricas (Tau de Goodman-Kruskal y coeficiente de incertidumbre) se expresó el cumplimiento de la reglamentación en los sistemas estudiados y la prevención de *Legionella*. Los criterios de calidad para el agua reciclada, procedente de la estación depuradora de agua residual y de pozo, fueron analizados en relación con la categoría de los hoteles.

**Resultados** Se encontraron deficiencias en el mantenimiento preventivo de los sistemas de riego, pero no hubo relación entre el tipo de agua y el riesgo identificado.

**Conclusiones** El estudio sugiere que el agua aerosolizada proveniente de los sistemas de riego de los campos de golf presenta un riesgo de infección por *Legionella* para la población expuesta.

**Palabras Clave:** Legionellosis; golf; *Legionella pneumophila*; calidad del agua (fuente: DeCS, BIREME).

Legionnaires' disease refers to the development of an illness caused by the bacterium *Legionella pneumophila* and others of the same genus. The first outbreak of pneumonia due to *Legionella* spp. occurred in Philadelphia in 1976 and since then there have been numerous outbreaks. Recent years have seen an increase in the incidence of Legionnaires' disease in several European countries,

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including Spain (1). Between 1999 and 2004 the world experienced four particularly relevant outbreaks (Spain, the Netherlands, the United Kingdom and Australia), with a total of 1,000 cases and 40 deaths (2). In 1996 Spain made it a requirement (notifiable disease) to report the existence of Legionnaires' disease (3) and implemented specific health prevention and control measures at the national level (4).

*Legionella* spp. is ubiquitous (5,6) and although its natural ecological niche is surface water, it can also be found in a wide variety of settings (7-10) without necessarily provoking a sporadic case or outbreak. Therefore, there are numerous sources of transmission (11,12), with many outbreaks originating in water supply systems (13-15). For the disease to evolve, the bacterium needs a dispersion mechanism (16-18), such as aerosolization via mist droplets (19-21), which can later be inhaled. If the bacteria are present when the size of the mist droplets approaches 5 µm they can be inhaled into the lungs, posing an imminent risk for contracting the disease. Installations with irrigation systems that use sprinkling devices, for example those found on golf courses, are one likely source for the proliferation and dispersion of *Legionella*.

The rational use of water is particularly important in guaranteeing the sustainability of water resources. It encompasses the care of turf cover on golf courses, mainly *Agrostis stolonifera* and *Cynodon dactylon* (22), and attention to problems related to the use of waste water for irrigation purposes.

At the European level, Directive 91/271 addresses the reuse of waste water in coastal areas (23) while at the regional level the Andalusian Environmental Plan (24) includes the sustainable use of natural resources. Regarding the water cycle, the Andalusian Plan encourages the reuse of urban waste water for other purposes. It promotes the creation of infrastructure networks adapted to the distribution and consumption of treated waste water for use in golf courses, tourist complexes and agricultural irrigation. Water available for irrigation purposes from waste water treatment plants, or other sources expressly covered under the specific regulation that governs golf courses, will have automatic systems designed to optimize consumption. Under no circumstances is treated wastewater to be mixed or diluted with drinking water for irrigation purposes. While some wastewater is unsuitable for agricultural use (due to risk of food contamination) it can be suitable for golf course irrigation, even if it has a determined concentration of salts, and particularly for watering certain ornamental plants. Waste water originating from domestic consumption (recycled) must be treated to comply with established quality levels before it can be used to irrigate golf courses (4).

The risk posed by length of exposure to *Legionella* acquires particular relevance in golf course areas such as tees and greens where prolonged watering by sprinkling systems is required. In contrast fairways, and to an even greater degree the rough, pose a lower risk because they require much less water.

Sprinkler-based irrigation systems, one source where *Legionella pneumophila* can develop, are specifically covered under Spanish health legislation. The research problem is the lack of knowledge of the existing sanitary legislation implementation at the risk facilities described. To avoid the bacterium propagation, the law requires that water quality control and disinfection methods used in maintenance activities be reported. The goal of this study is to determine the degree of compliance with health protocols designed to prevent the disease by using Spanish regulation on preventive maintenance as indicators of bacterial development.

## METHODS

An observational study was conducted on sprinkler irrigation systems (n=31) located on hotel golf courses along Malaga's Coast of the Sun (Malaga, a province in Spain's Andalusian Region). Information was collected between 2004 and 2009.

All the measures were recollected through a survey designed for the study, directed to the hotels, and carried out in the province. The instrument was duly processed and validated by a questionnaire (pilot survey) to ensure the absence of faults in the elaboration. The evaluation of the pretest was based, among others, on the design terms and variable coding. A discussion group was considered to express people understanding in relation to the survey. In the previous evaluation, the non-response ratios have not been verified. The final survey (self-administered) included 61 items and a cover letter, tolerating acceptable levels of coverage errors (among other factors to be done by email). We considered dichotomous and multiple response questions that avoid orientation to a specific response (absence of bias). The survey easily reached all hotel categories and installations that not were close, with a relatively low cost. Afterwards, a telephone contact and a visit to the facilities were done, to contrast the information provided by the hotels. An advantage was the possibility to access to the entire province under study.

The buildings have been contributed by the Government of Andalusia (Spain) through different regional ministries (Education and Science, Tourism and Health) and the Provincial Delegation of Malaga. An analysis was defined based on categorical variables and measures of association for the proportional reduction

of error. Directional association measures such as the Goodman-Kruskal Tau (25) and the uncertainty coefficient were used for this study.

The first suggests that by predicting to which category of the variable “health-hygiene conditions (% according to the R.D. 865)” (26) certain waters pertain, a random assignation can be made by using the probability of belonging to each category as a reference; the same would apply for the variable “type of water”. This reduces the probability of predictions and erroneous classification, calculating dependence for both variables. Similarly, the uncertainty coefficient shows the proportional reduction of error by using the values of one variable to predict the values of the other, taking solely into account the symmetrical version, since there was no reason to make a distinction between the dependent and independent variable. For both measures typical asymptotic errors were calculated without assuming independence among the variables.

The variable proposed was “enforcement with R.D. 865”, a categorical variable defined by 40 items staking only two values, “selected” or “not selected,” with the first level being considered a fit. A binomial test was used to confirm the goodness of fit hypothesis (compliance or non-compliance), assuming that 38 % of the critical installations comply with the national regulation (16); the question to be answered is whether that percentage is maintained in sprinkler-based irrigation systems. In other words, to test the hypothesis that the observed proportion of compliance with national regulation fits the theoretical proportion of a binomial distribution.

Thus, taking random samples of size  $n$  and defining the variable  $x$  = “number of selections in the  $n$  extractions” in each sample, a distributed random variable was obtained if the proportion of fits ( $\pi$ ) remains the same in each extraction, according to the model of binomial probability,

with the parameters  $n$ =“number of extractions” and  $\pi$ =“proportion of selection”. Binomial distribution probabilities were used to obtain the exact probability related to each one of the values for the variable  $X$ . Results indicate that when the value of  $n$  increases, the distribution of  $X$  approaches normal distribution. Applying the continuity correction by adding (when  $X$  is less than  $n\pi$ ) or subtracting (if  $X$  is greater than  $n\pi$ ) 0.5 points to  $X$  so that the contrast isn’t so conspicuous, obtains the variable  $Z$ . The result is a distribution model of normal probability, which is used to discover the probabilities associated with the values of the  $Z$  statistic and the approximate ones associated with  $X$  (27).

Since the values of a dichotomous variable define the dichotomy, it is necessary to test the hypothesis that the proportion observed in the first category (selected) is similar to the proposed theoretical proportion (0.38) (16). This proportional contrast enables the population value proposed in the null hypothesis to be defined, assuming that the dichotomous variable fits the binomial probability distribution model with  $\pi=0.38$  and taking the one corresponding to the first valid case as a dichotomous reference category. It appears to be one-sided, given the value of  $\pi$ , and critical value is obtained by calculating the probability of finding a number of cases greater than/equal or less than/equal to the category of reference (“selected” at the legislation compliance) (4). SPSS software (Copyright SPSS Inc., 1989-2006. Windows. Version 15.0.1. 22 Nov. 2006) was used for data analysis.

## RESULTS

Relationship between type of water irrigation and hotel category, and the last with the enforcement level are expressed in Tables 1 and 2.

**Table 1.** Relationship between type of water irrigation and hotel category (number of hotels)

		Type of water			Total
		Recycled water	Waste water treatment plant	Well water	
Hotel category	Three-star hotel	6	0	7	13
	Four-star hotel	10	3	1	14
	Five-star hotel	1	1	0	2
	Luxury five-star hotel	1	1	0	2
	Total	18	5	8	31

**Table 2.** Relationship between enforcement level and hotel category (number of hotels)

		Health/hygiene conditions (%) <sup>a</sup>					Total
		1-25	26-50	51-75	76-99	100 <sup>b</sup>	
Hotel category	Three-star hotel	2	1	5	2	3	13
	Four-star hotel	1	0	1	3	9	14
	Five-star hotel	0	1	0	1	0	2
	Luxury five-star hotel	0	0	1	0	1	2
	Total	3	2	7	6	13	31

<sup>a</sup> Attending to R.D. 865; <sup>b</sup> Total compliance of health/hygiene conditions.

Talking about the directional association measures, Tau takes values between 0 and 1, with “0” indicating no reduction of classification error and “1” a global reduction. In our study, the predictor variable does not contribute to reducing classification error because it is close to 0; consequently, the theoretical independent variable does not allow to predict which category of the dependent variable the classified cases pertain (Table 3). Additionally, the approximate signification or critical value that lends relative determination to the independence hypothesis is shown for the nominal data’s association measures.

The last analysis is based on the values obtained from Tables 1 and 2, in addition to the test performed in Table 4. The observed proportion of selected cases is 0.42 and the test proportion is 0.38 (16). Since the test value differs from 0.5 and the observed proportion of the reference category (“selected”) is greater than the test value, the contrast is one-sided on the upper tail, presenting as a critical value the probability of obtaining a number of cases  $\geq 13$  when  $n=31$  and  $p=0.38$ . Besides the sample size is  $>25$ , the proposed option is based on a normal approximation.

Finally, Figure 1 shows the relationship that exists between type of water and health/hygiene conditions.

**Table 3.** Directional measures

		Value	Typical asymptotic error <sup>a</sup>	Approximate signification
Goodman-Kruskal Tau	Type of water dependent	0.18	0.10	0.18 <sup>b</sup>
	Health/hygiene conditions dependent	0.13	0.07	0.03 <sup>b</sup>
Uncertainty coefficient	Symmetrical	0.18	0.07	0.10 <sup>c</sup>

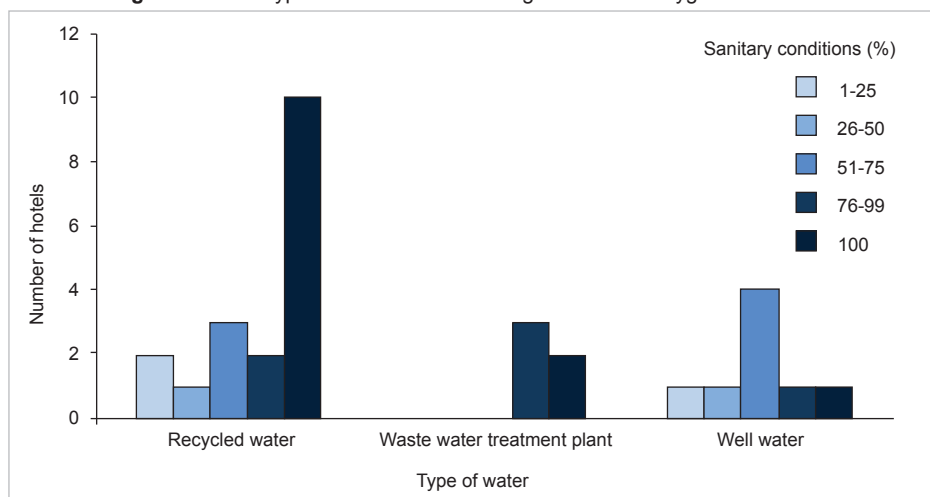
<sup>a</sup> Assuming the alternative hypothesis; <sup>b</sup> Based on chi-square approximation; <sup>c</sup> Probability of chi-square likelihood ratio.

**Table 4.** Enforcement level in irrigation systems on golf courses

	Category	N	Observed proportion	Test proportion	Asymptotic significance (one-sided)
Compliance with R.D. 865	Selected	13	0.42	0.38 <sup>a</sup>	0.01 <sup>b</sup>
	Not selected	18	0.58		
	Total	31	1.00		

<sup>a</sup> National legislation enforcement of Legionnaires’ disease critical facilities in southern Spain; <sup>b</sup> Based on the approximation Z.

**Figure 1.** Water type classification according to the health/hygiene conditions



## DISCUSSION

Prevention and control of Legionnaires’ disease are priority issues in public health. The monitoring is carried out through epidemiological surveillance and compliance with existing health legislations. In Spain, since 1996 is notifiable disease and the regulation of

the biological agent (*Legionella* spp.) through legislation enacted in 2003 (4).

The bacterium, which normally stayed in water, can develop in different environments and types of waters. In our study, we detected that the proportion of systems that use recycled water was higher for the total number of cases studied, with the potential hazard of Legionnaires’

disease being the same in all of them. In fact, this type of water system is more apt to be found on golf courses than systems which obtain water from wells or waste water treatment facilities. When water is got from waste water treatment plants, purification occurs at a secondary level, through digested sludge, which later must be treated specially for use in sprinkling irrigation. Reusing treated water for irrigation purposes on golf courses requires a tertiary treatment that includes filtering and preventive disinfection against microorganisms (28). Water reuse can help avoid the over exploitation of aquifers, improve biological treatment, and effluent purification that is eventually discharged into the sea.

Also, preservation of typical Mediterranean tree species (carobs, oaks, pines, olives, etc.) can help reduce irrigation demands. The use of *Agrostis stolonifera* (on greens) in golf courses requires a great deal of water and fertilizer. In addition to requiring regular aeration and topdressing with sand to improve overall soil quality, it is also quite sensitive to fungal diseases; it could be substituted for another species of grass with less demanding requirements. *Cynodon dactylon* (used on fairways) supports irrigation with low quality water, drought or heat (29).

In the study a variable relationship exists between the hotel's category and the corresponding risk facility. 64 % of the four-star hotels complied with the health legislation, followed by 50 % of the luxury five-star hotels and 23 % of the three-star hotels. Five-star hotels were not found to be in full compliance with health regulation for preventing Legionnaires' disease. The majority of their health/hygiene operations did not include any established procedures for general disinfection (4,30), nor did they meet the minimum cleaning standards required under law.

Similarly, a conservative behavior could be observed regarding the type of preventive hygiene maintenance employed. Water temperature controls were inexistent and insufficient maintenance was detected in 58 % of the sprinkler irrigation systems studied. Overall, an interpretation based on several legally-required activities and their minimal frequency revealed that compliance in these installations is deficient. The practical absence of any health and hygiene maintenance programs adapted to the different systems' characteristics is worthy of note. Hydraulic functioning schemes were not available and there was no systematic inspection of the installations' components. Periodic maintenance programs should be implemented on these installations that include structural and functional improvements, as well as controls of the physicochemical and microbiological quality of the water, to ensure they pose no risk to the public's health.

Regarding the different types of water used in irrigation and the degree of compliance with health and hygiene

requirements, the two variables were statistically independent and, given the uncertainty coefficient's value, when one variable is known the error of predicting the other's values was reduced by 18.1 %. No relationship exists between the type of water studied and the risk detected (% of compliance), therefore all irrigation installations should be considered as possible sources for biological development and subject to the same consideration for treatment. This suggestion is substantiated by classifying water used in risk installations according to its degree of compliance. Data showed the distribution of distinct percentage intervals among the different systems, demonstrating that recycled water fulfilled more highly than other types of water. This implies promoting improvements in all cases that do not show the maximum value (100 %), including water from wells or waste treatment facilities.

Since the critical value was considerable ( $>0.05$  in almost all measures), the null hypothesis of independence could be accepted with regard to Spanish health regulation on spray irrigation systems, over the total number of risk systems; leading to the conclusion that the two variables described are unrelated. In other words, given the level's value, the null hypothesis of goodness fit ( $p\text{-value} < 0.38$ ) was rejected, so that the true population proportion was higher than 0.38. This means that in the area under study, enforcement with regulation was higher in the spray irrigation systems described than in other risk facilities.

The above is a positive factor, considering that water in these systems can be pumped outwards in aerosol form and once the pulsing mechanism ceases those tiny droplets of water can be dispersed over a distance by the wind, with the possible health risk that would carry. Depending on the existing weather conditions and the location of the sprayers, the water droplets can travel considerable distances. That is why it is so important for irrigation systems to be strategically located, as far away as possible from places where people gather (social clubs, bars, sports facilities, etc.), so the aerosolized water can be dispersed as much as possible by the wind (lowering the concentration of bacteria) and reducing potential risk. Controlling the direction of aerosolized water droplets makes it harder for the bacteria to spread, as does their landing on the ground. The use of natural barriers and night-time irrigation can be workable approaches to prevent exposure and, in the case of irrigation, to help save water (by less insolation).

Water used to irrigate golf courses deal with a risk to exposed persons. As *Legionella* bacterium in the water is feasible to be identified in a wide range of hotels with watering systems and in different types of water; all the irrigation facilities included in golf links should be considered as sources of origin in the development and spread

of the bacterium. Besides the equipment should apply a specific preventive hygienic maintenance, that does not affect the prevailing species and the soil environmental conditions by the scattering of treated water. Considering the above, and in order a better prevention of the bacterium development, we can conclude that hotels in the South of Spain must improve irrigation systems maintenance in golf courses ♣

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