



## Sanitation workers from Portugal: Is there evidence of *Leptospira* spp?

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### ABSTRACT

Leptospire is a spirochete of the *Leptospira* genus. Infection in humans occurs by penetration into the mucous membranes, or into the skin (small wounds or abrasions). Humans are infected when they contact with urine of rodents, the main reservoirs. We aimed to evaluate the presence of anti-*Leptospira* spp antibodies and leptospiral DNA in sanitation workers (occupational group with increased risk) from Lisbon and Tagus Valley Region (Portugal).

Blood samples were collected from 347 sanitation workers, being applied a questionnaire to analyze exposure to rodents and preventive measures. The samples were screened by MACROLepto-test, for the presence of antibodies against pathogenic leptospire. "Positive" and "Non conclusive" samples were then tested with Microscopic Agglutination Test (MAT). Two nested-PCR protocols (primers LeptoA-LeptoB and *lipL32*) were applied for *Leptospira* spp DNA detection.

It was not observed anti-*Leptospira* spp antibodies in the worker's samples. However, it was detected non-pathogenic leptospire in a serum sample. Furthermore, 77% had previously seen rodents in the workplace and 94% always used Personal Protective Equipment (PPE).

Despite the regular presence of rodents in their workplace, the use of PPE and hygiene measures seemed to be able to prevent the workers contact with this infectious agent.

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### Introduction

Leptospirosis is an infectious zoonotic disease, whose global importance has been increasing over time [1]. Rodents, specially rats and mice, remains the most common reservoirs [2]. The transmission to humans occurs mainly through direct contact with urine, blood or tissues of rodents or other infected animal, per inlet port, which can be an abrasion on the skin or the mucous [2,3].

The contact with these spirochetes can be detected by the titers of anti-*Leptospira* spp antibodies, which remain months in the infected person, occurring a variable titer decline over time [1].

There are occupational groups considered at risk to develop leptospirosis [3]. In 1994, a study was carried out in an urban region of south Brazil, where was sought evidence for the presence of anti-*Leptospira* spp antibodies in sanitation workers [4]. Although this study has shown significant results, there is no record that a similar study has ever been conducted in mainland Portugal [4,5].

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In this study, we aimed to search the presence of anti-*Leptospira* spp antibodies and to detect leptospiral DNA in sanitation workers from Lisbon and Tagus Valley region (Portugal), as well as to describe workplace characteristics and workers behavior.

### Materials and methods

This was a cross-sectional study carried out in Lisbon and Tagus Valley Region (Portugal). This area comprises 52 City Halls, distributed over 12,204 km<sup>2</sup>.

During the selection process, 38 City Halls, as well as 30 private companies with roles of sewage cleaning, disinfestation or Wastewater Treatment Plant (WWTP) were contacted. The study was developed with 10 entities, located in 17 cities of this region, between February and July of 2016.

A set of inclusion criteria was elaborated, such as: (i) being City Hall workers that exercise functions in sanitation sector, WWTP workers or workers from privatized entities with sanitation duties; (ii) owning this occupation for at least one year; (iii) handling wastewater or solid waste direct or indirectly at the workplace; (iv) being workers from Lisbon and Tagus Valley Region.

It was used a questionnaire to obtain demographic data, workplace characteristics, as well as the use of PPE and hygiene measures. After blood collection, samples were transported to Lep-

tospirosis and Lyme Borreliosis laboratory of Institute of Hygiene and Tropical Medicine from NOVA University (Lisbon).

### Serological tests

MACROLepto technique (macroscopic agglutination on glass slide) was performed to all participants, in order to screen anti-*Leptospira* spp antibodies in their sera samples (N = 347).

*Leptospira* pathogenic serovars were selected from the World Health Organization reference battery, according to their prevalence in Portugal [5]. Thus, antigens belonging to Ballum serovar were cultured in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium. At the end, the culture had an optimum density of  $\pm 1,6 \times 10^8$  leptospires/mL. Participants serum were homogenized with the inactive antigen.

“Negative” results did not proceed to Microscopic Agglutination Test (MAT). However, “Positive” and “Non-conclusive” sera were then tested with MAT, according to the standard procedure [5,6].

### Molecular tests

It was extracted DNA from 10% randomly selected serum samples, using *Citogene*<sup>®</sup> DNA Cell & Tissue Kit. After DNA extraction, a nested-PCR protocol using universal primers (LeptoA and LeptoB), targeting *rrs* (16S) gene, was used in the first approach for *Leptospira* genus detection [7]. Samples with leptospiral DNA amplified by LeptoA and LeptoB primers, were then submitted to a new nested-PCR protocol, using primers for the pathogenic *Leptospira* species, targeting *lipL32* gene [8].

Leptospiral DNA amplification was performed according to routine laboratory protocol, using *MyCycler* (BioRad<sup>®</sup>). The amplified products resulting from both nested-PCR protocols were analyzed by agarose gel electrophoresis and observed under UV light.

### Ethical approval

The Ethics Committee of the Instituto de Higiene e Medicina Tropical, Universidade NOVA de Lisboa (Institute of Hygiene and Tropical Medicine, NOVA University of Lisbon) approved the study proposal (Research Protocol 6-2016).

## Results

### Socio-demographic and labor characteristics

During this study, data were collected from 347 sanitation workers in Lisbon and Tagus Valley region, of which 322 were male (93%) and 25 were female (7%).

Socio-demographic characteristics from participants, their labor entities, work area and presence of rodents in the workplace were described in Table 1. City Halls were the labor entities with the highest percentage of individuals (56%; n = 193). There was an evident predominance of workers in contact with wastewater in their work (78%; n = 272). The presence of rodents in the workplace was detected by 77% of the workers (n = 265).

The PPE's were used by 94% of the workers (n = 325) and the gloves were the most used PPE, with 56% (n = 194) (Table 2). About the hygiene measures, “daily bath” was the individual preventive measure practiced for a higher percentage of workers (79%; n = 277) (Table 2).

### Laboratorial results

The MACROLepto test revealed that 98% of the samples (n = 339) were “Negative” and 2% (n = 8) “Non-conclusive”. The “Non-conclusive” samples in MACROLepto were tested by MAT, with

**Table 1**

Sample distribution according with “Gender”, “Age group”, “School degree”, “Labor entities”, “Work area” and “Rodents sight”.

	n	%	
Gender N = 347	Male	322	93
	Female	25	7
	<25	2	1
Age group N = 347	25–34	40	12
	35–44	88	25
	45–54	124	35
	55–64	90	26
	>64	3	1
	No degree	3	1
School degree n = 343	4 years of schooling	73	21
	6 years of schooling	64	19
	9 years of schooling	111	32
	12 years of schooling	72	21
	Higher education	20	6
	City Hall	193	56
Labor entities N = 347	WWTP <sup>a</sup>	79	23
	Privatized company	75	21
	Wastewater	272	78
Work area N = 347	Solid waste	75	22
	Daily	30	11
	At least once a week	77	29
	At least once a month	80	30
Rodents sight n = 265	At least once a year	53	20
	Less than once a year	25	10

<sup>a</sup> WWTP – Wastewater Treatment Plant.

**Table 2**

Frequency of use of PPE's and hygiene measures by the workers in their work routine (N = 347).

	Never n (%)	Not always n (%)	Always n (%)	Total n (%)
<b>PPE's</b>				
Waterproof boots	46 (14)	209 (60) <sup>a</sup>	91 (26) <sup>a</sup>	347 (100)
Gloves	27 (8)	125 (36) <sup>a</sup>	194 (56) <sup>a</sup>	347 (100)
Mask	83 (24)	241 (69)	23 (7)	347 (100)
Disposable uniform	143 (41)	186 (54)	18 (5)	347 (100)
Daily uniform	234 (67)	47 (14)	66 (19)	347 (100)
Disposable gown	309 (89)	31 (9)	7 (2)	347 (100)
<b>Hygiene measures</b>				
Daily bath	16 (5)	54 (16)	277 (79)	347 (100)
Hand hygiene	0	74 (21)	273 (79)	347 (100)
Wound protection	26 (7)	129 (37)	192 (56)	347 (100)
Correct disposal of PPE	52 (15)	114 (33)	181 (52)	347 (100)
Separation of clothes/uniform	59 (17)	75 (22)	213 (61)	347 (100)
Uniform Washing	12 (3)	98 (28)	237 (69)	347 (100)

<sup>a</sup> One participant answered that used the PPE, but didn't specify in which frequency.

no agglutination. It was observed leptospiral DNA amplification by universal primers (LeptoA and LeptoB) in a single serum sample, corresponding to 3% (n = 1/35), with no amplification for the pathogenic *Leptospira* primers.

## Discussion

Leptospirosis is a zoonotic disease with a worldwide distribution. In developed countries, certain work occupations, as sanitation workers, has an increased risk to acquire the infection. [9,10]

In this study, it was verified the existence of rodents by three-quarters of the workers, raising the risk of contact with leptospires. Despite this evidence, there was an absence of positive results in serological tests and it was also verified the presence of DNA from a non-pathogenic *Leptospira* spp in a single serum sample. Thus, the use of PPE's and hygiene measures seem to be important to avoid contact with the bacteria, ensuring health promotion and disease prevention.

According to these results, it is considered relevant to develop a future study, aiming to determine rodents bacterial rate, in order to reinforce preventive measures in this professional group.

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