



Contents lists available at ScienceDirect

## Journal of Infection and Public Health

journal homepage: <http://www.elsevier.com/locate/jiph>



# Human leptospirosis cases in Palermo Italy. The role of rodents and climate

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### ARTICLE INFO

#### Article history:

Received 25 March 2017  
Received in revised form 1 July 2017  
Accepted 9 July 2017

#### Keywords:

Human leptospirosis  
Rodents  
Climate change  
Socio-economic welfare  
Public health concern

### ABSTRACT

Many regions of the world are increasingly exposed to leptospirosis due to poverty, global warming and high urban density. Here, we report a molecular survey for pathogenic *Leptospira* spp. in rodents and two symptomatic human cases of leptospirosis in the city of Palermo, Italy.

Four rodent species were captured in six areas of the city, and a molecular analysis for pathogenic *Leptospira* spp. on DNA from the kidney samples showed a different prevalence of leptospirosis in all the species of rodents. In addition, two human cases that occurred in May and October of 2009 in the city were also reported. A 67-year-old woman recovered after antibiotic treatment, whereas a 71-year-old woman did not survive. The weather during both of those times was notable for a violent cloudburst that caused street flooding.

For the past several years, the incidence of leptospirosis has remained steady at 9 human cases every 10 years across the entire island of Sicily, with a population of almost 5 million inhabitants.

The high prevalence of leptospirosis in rodents and the simultaneous presence of known risk factors, such as a mild/wet climate, street flooding and garbage accumulation, could explain the two cases of leptospirosis within the same city in the same year. This occurrence should raise awareness of this underestimated zoonosis among public health authorities, especially given the potential fatality among elderly and immune-compromised individuals in urban settings in developed countries.

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### Introduction

Leptospirosis is one of the most widespread bacterial zoonoses in the world. It is caused by over 200 different serovars belonging to several serogroups of the genus *Leptospira* [1]. Clinical presentation ranges from mild, flu-like symptoms to severe symptomatology, including Weil's syndrome, with multi-organ failure and, often, fatal pulmonary hemorrhagic syndrome [2]. Contact with stray animals, rodents, poor sanitation, heavy rainfall and flooding are the main risk factors in developing countries, whereas recreational activities, such as freshwater swimming, fishing, or sporting events are associated with clinical leptospirosis in developed countries [3,4]. Many regions of the world are increasingly exposed to leptospirosis infection and disease due to climate change, global

warming, a high urban density and poverty. Developing countries carry the major burden of the disease, with half a million cases reported yearly and a mortality rate ranging from 5 to 10% [5]. However, global warming also impacts leptospirosis cases in developed countries. In the Netherlands, an overall decreasing trend of leptospirosis over the past decades has recently reverted to a marked increase in cases among humans and dogs [6,7]. Rodents are the main animal reservoir in urban settings, with *Rattus norvegicus* primarily involved in pathogenic *Leptospira interrogans* serovar Copenhageni transmission [8]. Leptospirosis is reported at a high prevalence in the rodent population of major cities in developed countries, such as Baltimore in the USA [9], Tokyo in Japan [10] and Copenhagen in Denmark [11]. In Italy, the majority of cases are usually recorded in the northern regions of Lombardy, Piedmont and Veneto, where a decrease in the annual incidence of human leptospirosis has been recorded since 1996 [12]. Sporadic cases of symptomatic leptospirosis have been reported in these northern Italian regions, and they are often related to river flooding [13].

In this work, two human cases of leptospirosis that occurred in spring and fall in 2009 in the city of Palermo are discussed together

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<http://dx.doi.org/10.1016/j.jiph.2017.07.024>

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with the results of a molecular survey of rodents captured in green and residential areas of the same city in 2008–2009 for pathogenic *Leptospira* spp. Usually, only nine cases of clinical leptospirosis in ten years are reported across Sicily (Regional Reference Centre, Vitale G.), while in 2009, a serious case and a fatal case in Palermo were recorded in the same year.

## Materials and methods

### Human leptospirosis cases

In 2009, two clinical leptospirosis cases were reported in the city of Palermo during the spring and fall seasons. Leptospirosis was confirmed using a microscopic agglutination test (MAT) on the patient's serum according to the WHO guidelines [14] and with Elisa Pambio IgM as previously described [15].

### Rodent captures

Based on the number of rodent control interventions, 22 locations were monitored in residential and green areas. The locations were grouped in 6 larger areas (1–6 in Fig. 1), and Havahart traps (for rats) and Longworth traps (for *Apodemus sylvaticus* and *Mus domesticus* species) were used, as previously described [16], to capture the rodents. The rodents were euthanized by a 5-min inhalation of CO<sub>2</sub> followed by a bilateral thoracotomy. Samples from both kidneys (2 g) were used for DNA extraction. A total of

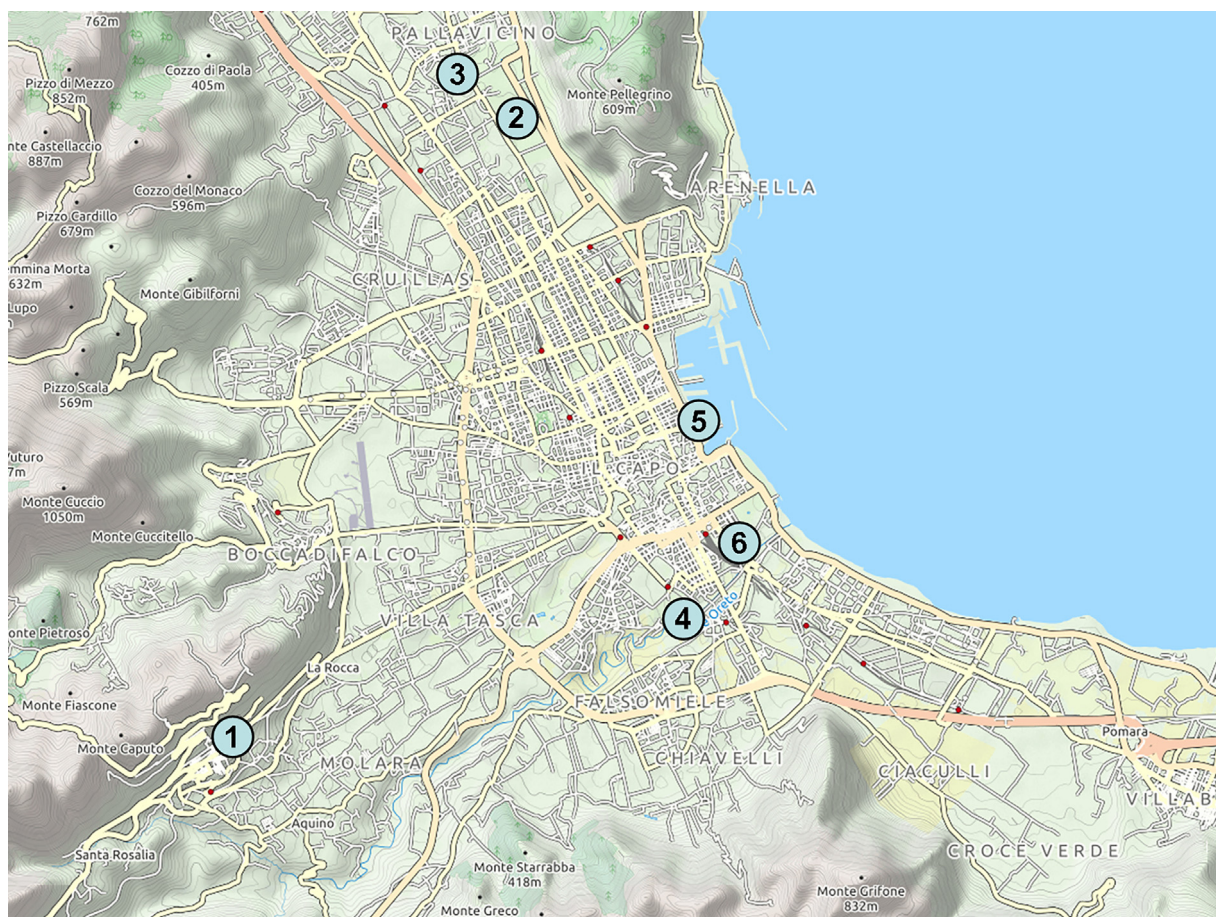
243 rodents, including 27 that had been randomly killed by local residents, were analyzed.

### Molecular analysis of the rodents

Rodent kidney tissues were dissolved in 2 ml of 10 mM TRIS-1 mM EDTA pH 8 and were homogenized using an EDTS VIII homogenizer (Design Village Ltd, U.K.). Total genomic DNA was extracted from the homogenates using a Gene Elute mammalian kit (cat N° G1N350 Sigma-Aldrich St. Louis, MO, USA) following the manufacturer's instructions. A PCR analysis the 16S rRNA gene was conducted as previously described [17]. The primers used to test for pathogenic *Leptospira* spp. were LEPTO E1 GGGAAAAATAAGCAGCGATGTG (forward) and LEPTO E2 (reverse) ATTCCACTCCATGTCAAGCC. The following program, in the Applied Biosystems 9700 thermal cycler, was used: 94 °C for 5 min; 1 cycle followed by 40 cycles at 1 min at 94 °C, 1 min at 60 °C, 1 min at 72 °C; and the final extension step at 72 °C for 5 min.

### Sequencing analysis

Thirty positive amplicons were also confirmed by a sequencing analysis of the amplified fragments. The reactions were carried out with the primers LEPTO E1 and LEPTO E2 using the Big Dye Terminator Cycle sequencing Kit v3.1 (10'' at 96 °C, 5'' at 60 °C, 4' at 60 °C for 25 cycles) and were detected using an ABI PRISM 3130 appara-



**Fig. 1.** Map of the city of Palermo and the main areas of rodents captures.

A schematic map of the city of Palermo in which the 6 main areas where the rodents were captured are shown by numbered circles. (1) Base of Monreale Hill (38.081998, 13.291698); (2) Ippomontato (Favorita Park) (38.161786, 13.337624); (3) Rom Camp (Favorita Park) (38.159367, 13.336788); (4) Oreto river (38.098184, 13.362194); (5) Fish Market (38.121169, 13.366215); (6) Municipal Kennel (38.107053, 13.373096). The captures were planned in collaboration with Ecological service of Palermo.

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