International Journal of Infectious Diseases 15 (2011) e710–e715

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Strikes, flooding, rats, and leptospirosis in Marseille, France

Cristina Socolovschi [a], Emmanouil Angelakis [a], Aurélie Renvoisé [a], Pierre-Edouard Fournier [a], Jean Lou Marié [b], Bernard Davoust [b], Andreas Stein [c], Didier Raoult [a,*]

[a] Université de la Méditerranée, URMITE UMR 6236, CNRS-IRD, Faculté de Médecine et de Pharmacie, 27 Bd Jean Moulin, 13385 Marseille cedex 05, France
[b] Direction Régionale du Service de Santé des Armées de Toulon, Toulon, France
[c] Service des Maladies Infectieuses, Hôpital de la Conception, Marseille, France

ARTICLE INFO

Article history:
Received 2 February 2011
Received in revised form 10 May 2011
Accepted 24 May 2011

Corresponding Editor: William Cameron, Ottawa, Canada.

Keywords:
Leptospirosis
Emergence
Urban area
Rainfall
Rat
Garbage collection strike

SUMMARY

Objectives: The aim of this study was to examine the mechanisms by which weather conditions, the incidence of Leptospira in reservoir populations, and various socio-ecological factors are driving the emergence of leptospirosis in Marseille, France.

Methods: Over the last 30 years our laboratory has surveyed all human cases of leptospirosis in Marseille using clinical, epidemiological, serological, and molecular tools. Data for the weather conditions in Marseille were collected from the official meteorological station, and garbage management strikes were monitored through the local press. Rats were trapped in alleys near to where the patients may have acquired leptospirosis.

Results: Three new cases of autochthonous leptospirosis are reported. The global tendency for rainfall showed a slight increase over the period under analysis. The index case of leptospirosis occurred after a heavy rainfall with flooding. Over the last 10 years Marseille has undergone 82 days of garbage management strikes. Leptospira DNA was detected in two of 11 rats.

Conclusions: The emergence of leptospirosis has become a public health problem in Marseille, and is associated with a combination of heavy rainfall and garbage collection strikes in which garbage is left on the street and thereby contributes to the expansion of the rat population on the surface.

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1. Introduction

Leptospirosis is a zoonotic disease caused by spirochetes of the genus *Leptospira*, which are distributed worldwide.[1] The annual number of estimated severe leptospirosis cases is around 500 000 worldwide, with an average case fatality rate approaching 10%.[2] This estimate probably underestimates the incidence of leptospirosis because of the lack of reporting systems in most endemic countries and its frequent misdiagnosis as another infectious disease. Transmission to humans results from exposure to the urine of infected carriers, either directly or indirectly via contaminated soil or water, as pathogenic Leptospira may survive in many environments for an extended period of time.[1,2] The incidence of this disease is higher in tropical regions with low socioeconomic conditions, and where there is environmental proximity to infected animals.[1,3,4] Recently, leptospirosis has become an urban health problem, not only in developing countries, but also in industrialized countries, such as Germany, Japan, and the USA.[4–7] Urban epidemics of leptospirosis in developing countries occur in unsanitary environments, especially during periods of seasonal rainfall or flooding in cities with inadequate sewage and garbage management systems.[8–10] In this setting, outbreaks are often caused by the transmission of Leptospira that is associated with the reservoir host *Rattus norvegicus*.[6,11] However, household pets, particularly dogs, or wild boars may also serve as reservoir hosts in urban areas.[12–14]

Marseille is the second biggest city in France and is located on the Mediterranean coast. Marseille has a Mediterranean climate with mild, humid winters and hot, dry summers. In the Köppen climate classification system, which is based on average annual and monthly temperatures, precipitation, and their cyclic variation, known as the seasonality of precipitation zone, Marseille is classified as 'Csa': 'C' represents temperate/mesothermal climates, 's' indicates dry summers, and 'a' indicates the warmest month with an average temperature above 22 °C. The rainfall intensity can vary between heavy rain, when the precipitation rate is between 10 mm and 50 mm per hour, and violent rain, when the precipitation rate is >50 mm per hour. The residents of Marseille prefer seawater to fresh water activities.[15] This factor limits the transmission of leptospirosis via recreational activities involving immersion in fresh water.[16,17] Leptospirosis is a reportable disease in France, and the disease has been surveyed in Marseille over the

* Corresponding author. Tel.: +33 491 38 55 17; fax: +33 491 83 03 90.
E-mail address: didier.raoult@gmail.fr (D. Raoult).

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doi:10.1016/j.ijid.2011.05.017



Figure 1. Garbage pile in the city center of Marseilles during a strike by garbage collectors (October 12–26, 2010) that attracted rats looking for food, in an area where two of the patients lived. Inset: an urban rat in the middle of the garbage pile.

last 30 years.[18–21] In 1984, three cases of leptospirosis occurred near Marseille, and two cases were imported from the Union of the Comoros and Singapore.[15] Later, one additional case of imported leptospirosis from Spain was reported.[22] Since that time, there have been no cases of leptospirosis identified in Marseille.

The history of Marseille reports a considerable role of the rat in the spread of contagious diseases, such as the plague.[23] For a long time, the monitoring of wild rodent reservoirs in the control of permanent foci was undertaken by a special laboratory in Marseille, but this supervision has been stopped. Since 1976, garbage management strikes have been monitored in Marseille by the public authority. In this setting, the accumulation of garbage attracts rats from the city, which are easily visible during their journey and at night on the surface, thus increasing the risk of contracting rodent-borne diseases in Marseille (Figure 1).[24]

Here, we report three human cases of leptospirosis in Marseille residents identified concomitantly with an increase in the urban rat population infected with *Leptospira sp.* The emergence of leptospirosis in Marseille has become a public health problem and highlights the putative roles of increased surveillance, the development of new tools, and socioeconomic and environmental factors in the transmission of leptospirosis.

2. Materials and methods

2.1. Clinical study

Our study was approved by the Ethics Committee of the Université de la Méditerranée, Marseille. Human serum, blood, urine, and cerebrospinal fluid (CSF) samples were obtained from clinical specimens taken for routine clinical purposes from patients with a suspected leptospirosis infection and from patients with unrelated illnesses. The presence of immunoglobulin M (IgM) was determined by an ELISA.[22] DNA samples were extracted from blood, urine, and CSF samples using a QIAamp Tissue Kit (Qiagen, Hilden, Germany). The DNA was used as a template in a real-time PCR (qPCR) assay for detection with specific primers and a probe targeting the 16S rRNA of pathogen Leptospira: Lep16SMGBd: 5′-GCG-GCG-AAC-GGG-TGA-GTA-A-3′ with Lep16SMGBr: 5′-GGA-AAG-TTA-TCC-AGA-CTC-3′ and the probe Lep16SMGB: 6FAM-ACG-TGG-GTA-ATC-TT-Mgb. *Leptospira interrogans* serovar Icterohaemorrhagiae DNA was used as a positive control. For culture, lithium–heparin anticoagulated whole blood and CSF were spread onto 10 ml of Leptospira medium, a polysorbate medium similar to Ellinghausen and McCullough modified Johnson and Harris medium (EMJH) (Bio-Rad Laboratories, Inc., Aulnay/Bois, France) and incubated at 30 °C. For urine samples, the specimens were first filtered through 0.45-μm and then 0.22-μm filters before inoculation. Once a week, 10 μl of each culture was examined with dark-field microscopy, and cultures were surveyed for 8 weeks.

2.2. Reservoir investigation

To identify the presence of Leptospira in rats, a total of 18 Tomahawk trap cages were used on a central street in Marseille. In each trap baits consisted of pieces of apple and bread. Three capture sessions (six cages each night) were carried out in July 2009. All rats were photographed and identified. Rats were euthanized while under injection anesthesia with ketamine. Kidneys were collected, weighed, and immediately frozen. Serum samples were not collected. Animal procedures were conducted according to local regulations on animal welfare. This was a timely study carried out to test the presence of rat-associated bacteria in

Marseille. Total genomic DNA was extracted from the kidneys, which were handled under sterile conditions to avoid the risk of cross-contamination. Samples were screened for the presence of Leptospira DNA using the qPCR assay described above. All kidneys were cultured in a Leptospira medium as described above.

2.3. Meteorological study

All daily rainfall measurements over the last 10 years (January 1, 2001–November 7, 2010) were obtained from Météo-France (the French National Institute of Climatology). The Marignane station is located in a suburban area close to Marseille. All data were analyzed using Microsoft Excel version 2007.

2.4. Laboratory investigation

Serological and molecular data used to diagnose cases of leptospirosis between January 2001 and July 2010 were obtained from the archives of the Microbiological Laboratory, Hôpital de la Timone, Marseille. Sera were obtained from all the public hospitals in Marseille. With regard to serological data, a micro-immunofluorescent antibody assay (MIFA) was used during the period January 2001 to July 2004, and since that time an IgM ELISA test has been employed.[22]

2.5. Garbage collector strikes in Marseille

All data on the dates of the garbage collector strikes in Marseille were obtained from the documentation service of the newspaper *La Provence*, Marseille.

2.6. Statistical analysis

The program GraphPad Prism™ v2.0 was used to perform the statistical analyses, employing linear regression tests for laboratory data for the period January 2001 to July 2010. The incidence of leptospirosis was analyzed using Fisher's test. Statistical significance was defined as a p-value of <0.05.

3. Results

3.1. Clinical study

Case 1: On October 29, 2009 a 76-year-old woman presented to the emergency department at the Hôpital de la Conception, Marseille, France with a relapsing fever, headache, disordered consciousness, general malaise, and vomiting. Fifteen days prior to admission she had presented to the emergency room with fever, headache, and abdominal and lumbar pain. The woman was empirically prescribed 800 mg norfloxacin daily for a possible urinary infection. She lived in a building in the urban center of Marseille and had not recently traveled. She had not participated in recreational activities that suggested contact with fresh water. She did not have a pet and had not come into contact with wild rodents.

Upon admission, she presented with a temperature of 38.3 °C and tachycardia, and her blood pressure was 110/70 mmHg. A neurological examination showed signs of meningeal irritation, including cervical rigidity, hyperesthesia, and photophobia. She did not present rash, and the rest of the physical examination was normal. The results from biochemical examinations included increased levels of creatinine (144 μmol/l), urea (8.1 mmol/l), alanine aminotransferase (55 IU/l), and creatine phosphokinase (CPK) (1178 IU/l), associated with hypoproteinemia (58 g/l) and hypoalbuminemia (32 g/l). Her leukocyte count was 12.07×10^9/l, with 61.7% polymorphonuclear leukocytes. CSF examination showed 3×10^6 cells/l, a total protein concentration of 0.25 g/l, and a glucose concentration of 4.2 mmol/l (serum glucose concentration level of 5.7 mmol/l). A chest X-ray showed lung congestion, and computed tomography revealed no dilatation in the biliary system, no enlarged kidneys, and no liver anomalies. The patient was transferred to the infectious disease department on the day of her arrival and given 2 g ceftriaxone administered intravenously (IV) for a suspected pneumonia. Two days later, a diagnosis of leptospirosis was suspected by a consulting infectious disease specialist (DR) on the basis of myalgia with elevated CPK, renal failure, and the presence of neurological manifestations. A qPCR assay specific for leptospirosis was performed on CSF drawn on the first day of admission and was positive for leptospirosis. The patient was treated with ceftriaxone for 7 days, discharged from the hospital, and remains well.

Cases 2 and 3: On January 6, 2010, a 55-year-old man was hospitalized because of a fever of 40 °C, weakness with accompanying tachycardia (130 beats/min), a blood pressure of 90/60 mmHg, jaundice, renal failure, pneumonia, and diarrhea. He was living in the city center of Marseille and had not recently traveled, but was in contact with several pet rats at home. On day 2 of hospitalization, a blood sample was positive in a qPCR assay for *Leptospira sp*. On the third day after admission, his serum was positive by serology and negative by qPCR. On February 3, 2010, a 12 year-old girl, who was living in a camp in a suburb of Marseille, presented with a fever (39 °C), jaundice, renal failure, meningitis, hypotension (70/30 mmHg), and a heart rate of 130 beats/min and was hospitalized in the intensive care unit for 4 days. She did not have a pet and had not recently traveled. On the first day of admission, serology and qPCR assays on serum samples were positive for *Leptospira sp*. All cultures remained negative for the presence of Leptospira. Both patients recovered after antibiotic treatment (Table 1).

3.2. Epidemiological investigation

Over a 10-year period, a total of 5606 patients were tested for leptospirosis by serology (the number of patients was 523 in 2001, 684 in 2002, 669 in 2003, 735 in 2004, 576 in 2005, 611 in 2006, 622 in 2007, 526 in 2008, 508 in 2009, and 152 in 2010). Before July 2004, a single patient was IgM-positive (1:200) by MIFA.[25] After July 2004, there were eight IgM-positive serum samples using an

Table 1
Three cases of leptospirosis in Marseille

Patient	Case 1, female	Case 2, male	Case 3, female
Age, years	76	55	12
Month	October 2009	January 2010	February 2010
Risk factor	No	No	Living in a camp
Fever	38.5 °C	40 °C	39 °C
Vomiting	Yes	Yes	Yes
Hepatomegaly	Yes	Yes	No
Thrombocytopenia	No, 300×10^9/l	Yes, 89×10^9/l	Yes, 59×10^9/l
CRP	4 mg/l	245 mg/l	215 mg/l
CPK	1178 IU/l	3812 IU/l	2000 IU/l
Creatinine	144 μmol/l	182 μmol/l	322 μmol/l
Urea	8.1 mmol/l	10.31 mmol/l	17.3 mmol/l
Hematuria	No	No	No
PCR			
Blood	ND	Positive	ND
CSF	Positive	ND	Negative
Serum	ND	Negative	Positive
Serology	Negative	Positive	Positive
Culture			
Blood/urine	Negative	Negative	Negative
CSF	Negative	ND	Negative
Treatment	Ceftriaxone	Amoxicillin	Amoxicillin + cefotaxime

CRP, C-reactive protein; CPK, creatine phosphokinase; PCR, polymerase chain reaction; ND, not determined; CSF, cerebrospinal fluid.

ELISA. However, the increase in patients with a positive serology appeared much later, despite more than 500 patients being tested each year by serology. Using molecular diagnostic tools, 142 patients were tested using a qPCR assay specific for leptospirosis and seven positive results were identified (7 patients in 2001, 3 patients in 2002, 8 patients in 2003, 4 patients in 2004, 4 patients in 2005, 11 patients in 2006, 16 patients in 2007, 10 patients in 2008, 25 patients in 2009, 54 patients in 2010). The same molecular detection system was used during this time period. The first case of autochthonous leptospirosis (October 2009) was followed by a significant increase in the number of PCR tests for leptospirosis ($p = 0.0130$). Moreover, between October 2009 and July 2010, 70 patients were tested and four positive samples were detected. In contrast, the number of patients tested by serology significantly decreased after the identification of the first case of leptospirosis in Marseille ($p = 0.0494$). In fact, the incidence of leptospirosis increased significantly from January 2001 through July 2011, with three autochthonous cases of leptospirosis identified in our laboratory precisely between October, 2010 and February, 2011 ($p < 0.0001$, Fisher's test, considering the number of patients tested by serology: 2/259 vs. 0/5359; $p = 0.0187$, considering the number of patients tested by PCR 3/35 vs. 0/68; and $p < 0.0001$ considering the number of autochthonous cases per month: 0/117 vs. 3/5).

3.3. Reservoir study

We captured six male and five female rats (*Rattus norvegicus*) on central streets of Marseille where the first two patients lived. The rate of successful capture was 61% (11/18). We identified the presence of Leptospira DNA in the kidneys of two rats using qPCR assays (18%). Positive and negative controls showed the expected results in all tests. However, all the cultures of kidney samples were negative.

3.4. Meteorological study

The Mediterranean climate is characterized by heavy rainfall at the end of autumn, followed by a long dry period. The global tendency for rainfall in Marseille showed a slight increase over the period analyzed. We observed maximum levels of rainfall between October and November 2002 and also in the last 2 years. The dry period in the summer of 2003 in France was particularly severe in Marseille, and was followed by abundant stormy precipitation (68.2 mm/day) in December. The first case of leptospirosis described in our study occurred after a period of heavy rainfall with flooding over several days in September and October 2009 (34.6 mm/day; 79.2 mm/day; 137 mm/day with a violent episode of 63 mm/3 h). This period was the most severe rainy period over the last 10 years. The other two cases of leptospirosis occurred during a period of high rainfall in this same period (13.6–23.8 mm).

3.5. Garbage collector strikes in Marseille

Garbage collector strikes are very common in Marseille. Since 1976, more than 15 garbage collector strikes lasting 1–2 weeks have been recorded in Marseille. French soldiers were requisitioned to remove masses of garbage from the streets of Marseille following a 1-week strike by garbage collectors in June 1976 and after 2 weeks in August 1999. Over the last 10 years, we recorded 10 strikes, corresponding to a total of 82 days of garbage collector strikes; the last one was a 1-week strike in March 2010 (March 6–13). Two strikes were concomitant with rainfall. The first strike was in 2003, which lasted 1 week from November 28 to December 3, after a heat wave that occurred in the summer in Marseille and in all European countries. Heavy rainfall (68.2 mm) was recorded on December 1, which generated flooding in Marseille. Interestingly, preceding the second strike from October 29 to November 5, 2009, several heavy rainy periods with flooding were recorded in September, with a maximum of 137 mm on the 18th, and two days in October, with 34.6 mm on the 21st and 34.4 mm on the 22nd. During the strike, we recorded 9.6 mm of rainfall on November 1. Case patient 1 was admitted to hospital on the first day of the last strike and the two other patients at 2 and 3 months later, respectively.

4. Discussion

Our study describes the first three human cases of leptospirosis identified in the city of Marseille, France. The apparent emergence or re-emergence of leptospirosis is dependent on at least three factors: (1) the interest of physicians and quality of surveillance for the disease, (2) the diagnostic methods implemented to assess the prevalence, and (3) a significant increase in the incidence.[26] In France, leptospirosis is traditionally considered an occupational disease in rural areas, associated with recreational activities, or imported from tropical or endemic areas.[27] Leisure activities, the presence of skin lesions, canoeing, contact with wild rodents, and a home in the countryside are considered risk factors.[28] Leptospirosis became a reportable disease in 1917.[16] There were no significant variations either in the number of leptospirosis cases or in the incidence of disease during the period 1920 -2003.[27] Between 1991 and 2003, the rate of disease incidence in southern France was very low, 0.09 cases/100 000 inhabitants.[27] In 2008 this incidence increased markedly to 0.25 cases/100 000 inhabitants.[16] Over the last three decades, infectious disease physicians from Marseille have reported the epidemiology, clinical course, and treatment of leptospirosis in France, French overseas territories, Indonesia, and in travelers from endemic areas.[15,19,22,29] The constant focus of infectious disease physicians in Marseille on leptospirosis has minimized misdiagnoses, in spite of atypical clinical manifestations.[16,30] Therefore, we believe that the emergence of leptospirosis is not related to a change in disease surveillance or the awareness of physicians.

With regard to the diagnostic tools, we have used the same methods to diagnose leptospirosis: serology, molecular tools, and culture. The diagnosis of leptospirosis is usually done retrospectively by serology because culture is difficult.[1,16] The reference test for leptospirosis is a microscopic agglutination test. This test cannot be standardized as live antigens are often used and several factors, such as the age, density of the culture antigen, and cross-contamination of the culture antigen, can influence the agglutination titer, and the final result requires the testing of acute and convalescent sera.[30] Currently, several assays are commercially available,[31] including IgM ELISAs, hemagglutination assays, and microcapsule agglutination tests, which provide an easier diagnosis of suspected leptospirosis.[32] However, for an early diagnosis of leptospirosis, PCR assays may be a potential tool. In our study, qPCR was positive for different samples drawn during the first and second day of hospitalization, showing promise for the early and rapid diagnosis of leptospirosis. Unfortunately, the Leptospira DNA load that was detected using qPCR was low and we failed to sequence this PCR product. We conclude that new tools have had little impact on the present emergence of urban leptospirosis in Marseille.

The known risk factors for the emergence of leptospirosis include the prevalence of leptospirosis in the rodent population neighboring humans.[5,11] A high prevalence of Leptospira infection in rats has been reported in Tokyo (17%), Copenhagen (48–89%), and in Baltimore (90.4%).[5,6,33] When we investigated the subject, no study was available on the total number of rats in Marseille, but

city residents often see rats, despite the 1500 rat exterminations conducted each year by public and private agencies. In Marseille, requests for rat extermination by private companies increased by 20% in 2007. This would be an objective fact when considering the total rat population and rat nuisance in the city. Within the twentieth century, small outbreaks and many sporadic cases of the plague have been recorded in Marseille.[23] Murine typhus, another rat-borne infection caused by *Rickettsia typhi*, was found recently among the homeless population in Marseille.[24] We identified Leptospira DNA in two of 11 *Rattus norvegicus* rats captured on central streets of Marseille.

The close proximity of residences to accumulated garbage is another known risk factor for the emergence of leptospirosis.[4] During one strike, a large amount of garbage remained on the streets (Figure 1). Garbage management during the 10 strikes recorded over the last 10 years helped to fix and concentrate the rat population in urban centers of Marseille, and in that way increased the number of rats locally. Currently approximately 1.1 kg of garbage/day/resident is generated. Thus, for 852 395 residents (2007), there is 937 634 kg garbage/day, twice the amount generated 40 years ago. This favors a context in which the concentration of rats increases around garbage deposits and eventually leads to the spread Leptospira in the rat population via contact with infected rats.

We evaluated rainfall because, although relatively rare in Marseille, rainfall is recognized to be associated with leptospirosis.[1,31] Outbreaks of leptospirosis in Rio de Janeiro, Brazil and among strawberry harvesters in Germany appear to have been caused by transmission during periods of heavy rainfall.[34,35] Our study showed that the first autochthonous case of leptospirosis in Marseille was identified after a period of heavy rainfall with flooding (137 mm/day). This period had the highest average rainfall over the last 10 years. Large amounts of rainfall over a few hours can cause flash floods and, consequently may disseminate Leptospira in the human environment. In Marseille, rainfall measurements over a 10-year period showed showed a slight progressive increase, and particular attention should be given to the occasional heavy downpours that occur from September to March. Children may be exposed when playing in yards (in rain puddles or mud) contaminated with rat urine, as was the case for the 12-year-old girl in our study. Adults may be exposed to contaminated surface water or directly with rat urine near garbage deposits.

Leptospirosis has been declared one of the neglected infections of poverty in the USA and Japan; it has become a health problem in urban slums in developing countries.[5,9,36,37] However, we could not demonstrate formally that city rats were the source of leptospirosis in Marseille, in conjunction with flooding and garbage collector strikes. These animals that are infected with *Leptospira sp* are the most likely reservoir of infection in this urban area. None of our three patients had had contact with dogs, moreover in Marseille public companies capture and impound unlicensed, stray, and uncontrolled dogs. The pet rat of the second patient was not sampled because the rat owner refused to allow this. The hygiene status of commercial pet rats is usually known, because they are monitored for pathogenic agents. Nevertheless rat owners should be considered as a high-risk group for this potentially life-threatening infection.[38] The combination of climatic parameters, deficiencies in infrastructure, including the accumulation of garbage, and a high prevalence of rats as a risk factor for leptospirosis has previously been described,[4,9,10] but not in highly developed cities in temperate countries. As a result, in urban areas like Marseille, better control of litter in the streets is critical to decrease the rat population. These three cases indicate the need for leptospirosis awareness among physicians working in urban areas of developed countries.

Acknowledgements

We would like to thank Brigitte Delmas-Moulard from Météo-France (the French National Institute of Climatology), Marignane, France for providing all the humidity parameters and rainfall measurements, Michelle Fouque from the documentation services at the newspaper *La Provence*, Marseille, France for information about garbage collector strikes, Oleg Mediannikov for photography, and Gilles Audoly for designing the graphical and statistical analyses.

Funding: None of the funders played any role in the design, collection, analysis, or interpretation of data, in the writing of the report, or in the decision to submit the paper for publication.

Conflict of interest: The authors declare that they have no competing interest.

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