A Multidisciplinary Approach to the First Autochthonous Case of Tularemia Reported in Portugal

Abordagem Multidisciplinar do Primeiro Caso Autóctone de Tularémia Notificado em Portugal

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ABSTRACT

Francisella tularensis, a Gram-negative coccobacillus, is a highly virulent pathogen responsible for several zoonotic outbreaks in Europe in the last few decades. The authors report the case of a 46-year-old male who developed fever, myalgias and headache a week after having contact with animal feed contaminated by rodents. Serological tests were positive for *Francisella tularensis*. This first case of autochthonous tularemia in Portugal led to an intensive investigation involving several healthcare services and national governmental authorities. The authors address the possible underdiagnosis of this infection in the country.

Keywords: Francisella tularensis; Portugal; Public Health; Tularemia

RESUMO

Francisella tularensis, um cocobacilo Gram-negativo, é um microrganismo infecioso altamente virulento, responsável por vários surtos de doença na Europa nas últimas décadas. Os autores apresentam o caso de um homem de 46 anos com febre, mialgias e cefaleias cerca de uma semana após contacto com ração de animais contaminada por roedores. O estudo serológico foi positivo para *Francisella tularensis*. Este caso, o primeiro caso de tularémia autóctone notificado em Portugal, originou uma intensa investigação envolvendo diversas autoridades de saúde e governamentais portuguesas. Os autores alertam para a possibilidade de subdiagnóstico desta doença no país.

Palavras-chave: Francisella tularensis; Portugal; Saúde Pública; Tularémia

INTRODUCTION

Francisella tularensis is the etiologic agent of tularaemia. There are four subspecies that have been described: *F. tularensis tularensis* (type A strain), *F. tularensis holarctica* (type B strain), *F. tularensis novicida* and *F. tularensis mediasiatica*.[1–3] The subspecies *tularensis* and *holarctica* are responsible for the majority of tularaemia infections worldwide.[1] Small rodents and lagomorphs are the main hosts of *F. tularensis*, but a wide range of both wild and domestic animals may be infected. A number of different arthropods can also act as vectors.[1–3] Transmission to humans occurs directly from the animal reservoir (handling tissues or fluids, ingestion of undercooked meat or animal bite), through arthropod bites, following exposure to contaminated environmental sources (water, soil, dust) or during sample manipulation in the laboratory.[1,2,4] Hunters, trappers, veterinarians, animal trimmers, landscapers, farmers and laboratory workers are deemed as high-risk groups.[1,2]

Tularaemia has a short incubation period, ranging from 3-5 days up to two weeks.[1,5] Six major clinical syndromes have been described: ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic and typhoidal, usually associated with the route of inoculation.[1]

Due to its high virulence, possibility of aerosolization and ability to cause severe disease, *F. tularensis* is classified as Category A potential bioterrorism agent, according to the Centers for Disease Control and Prevention (CDC). Tularaemia is under epidemiological surveillance in Europe since 2003. Despite being a disease of compulsory notification in Portugal since 2014, there were no human cases reported until 2018.[6]

The authors report the first notified autochthonous case of tularaemia in Portugal and intend to raise clinical awareness to this diagnosis.

CASE REPORT

A previously healthy 46-year-old male was admitted with a 4-day history of fever (40ºC maximum), malaise and myalgia. He was a small game hunter and had regular contact with rabbits in a domestic warehouse, apart from a history of contact with livestock feed contaminated by rodents the week before, in the store he owned. He denied recent travel, consumption of suspicious food or water or tick bites.

On admission, he was febrile and hypotensive with no further findings in the physical examination. Laboratory results (Table 1) revealed leucocytosis, thrombocytopenia, elevated C-reactive protein and mild cytocholestasis. Renal function was preserved. Abdominal ultrasound revealed no abnormalities.

Considering the epidemiology, leptospirosis was suspected. Fluids and empiric antibiotic treatment with doxycycline 100 mg twice a day (bid) were administered. Serological tests for *Brucella* spp., *Rickettsia* spp., *Coxiella burnetti*

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Table 1 – Laboratory tests performed during hospital admission

Laboratory analysis (units)	**Results** (reference values)
Hemoglobin (g/dL)	15.6 (11.8 - 15.8)
Leucocytes (x10^9/L)	21.8 (3.6 - 10.5)
Neutrophils (x10^9/L)	18.3 (1.5 - 7.7)
Platelets (x10^9/L)	148 (150 - 400)
CRP (mg/dL)	22.4 (< 0.5)
ALT (U/L)	69 (< 34)
AST (U/L)	58 (< 31)
Total bilirubin (mg/dL)	1.8 (0.2 - 1.2)
Direct bilirubin (mg/dL)	0.5 (< 0.5)
LDH (U/L)	338 (< 247)
HIV antibodies	Negative
HAV IgM/IgG	Negative
HBsAg	Negative
HBsAb	Negative
HBcAb IgM	Negative
HCV IgM/IgG	Negative
EBV IgM	Negative
CMV IgM	Negative
RPR	Negative
Coxiella burnetti IgG	Negative
Rickettsia sp. IgG	Negative
Brucella sp. IgM/IgG	Negative
Leptospira sp. IgM/IgG	Negative
Leptospira 16S (RNA) - urine	Negative
***Francisella tularensis* (TAT)**	**Positive**
Blood cultures	Negative
Urine culture	Negative

ALT: alanine transaminase; AST: aspartate transaminase; CMV: cytomegalovirus; CRP: C-reactive protein; EBV: Epstein-Barr virus; HAV: hepatitis A virus; HbsAg: hepatitis B surface antigen; HbsAb: hepatitis B surface antibody; HbcAb: hepatitis B core antibody; HCV: hepatitis C virus; HIV: human immunodeficiency virus; Ig: immunoglobulin; LDH: lactate dehydrogenase; RNA: ribonucleic acid; RPR: rapid plasma regain; TAT: tube agglutination test

and *Leptospira* spp. turned up negative, as well as for HIV, hepatitis, EBV, CMV and syphilis. *Leptospira* spp. was not detected in urine. Blood cultures were negative.

The patient's condition improved, and he was discharged without a final diagnosis. The case was later reviewed, and less frequent causes of zoonosis were considered, namely tularaemia. Due to this suspicion, he completed 21 days of treatment with doxycycline 100 mg bid in outpatient setting, without further complications. Convalescent serum samples were tested either for the previous zoonotic agents (without seroconversion) and *F. tularensis*. *F. tularensis* antibodies were detected by an agglutination test (TAT) with a titre of 1:40 (cut-off for antibody detection is a titre > 1:20).[7] Typhoidal tularaemia was diagnosed, considering the presence of fever with no specific signs or symptoms and the immunological response for *F. tularensis*.

Public Health investigation and control

After being notified, local and regional Public Health professionals conducted an epidemiological investigation. The patient mentioned a history of direct manipulation of dead rats in the animal feed warehouse where he worked (which led him to relocate) and domestic rabbits. Sherman traps (rectangular-shaped box traps designed for the live capture of small mammals) were placed next to the store, the previous warehouse and its surroundings. The traps were checked three times a day during the investigation, that lasted for several days, and complied with animal comfort and safety; no rats or other vectors were captured. Water samples collected from a stream nearby were within normal microbiological parameters. However, signs of recent environmental cleaning and disinfestation in the nearby area where detected. The environmental assessment also involved the National Institute of Health, Portuguese National Authority for Animal Health, Food and Economic Safety Authority, Institute for Conservation of Forests and Nature and the Environment Protection Police Department services.

DISCUSSION

Typhoidal tularaemia is a potentially severe systemic disease and may be the result of any transmission pathway. It is a less prevalent presentation, accounting for 7.7% - 14.4% of cases in several case series.[8,9] Its diagnosis may represent a challenge for physicians, particularly in non-endemic areas, due to the absence of pathognomonic signs and symptoms. Two large outbreaks were reported in northwestern Spain, with over 1000 human cases during 1997 - 1998 and 2007 - 2008.[3,10] Due to geographic proximity, a seroepidemiological study was conducted in the northern region of Portugal after the first outbreak in Spain and found a seroprevalence rate of 8.9%.[11] The *F. tularensis* subspecies *holarctica* was detected in one human sample.[12] *F. tularensis* subsp. *holarctica* was also found in different tick species and lagomorphs.[13] Given these results, awareness of autochthonous tularaemia should be raised. In this case, the presence of a febrile illness, nonspecific laboratory findings and exposure to rats led to the hypothesis of leptospirosis. Considering the higher prevalence of leptospirosis and other zoonosis in Portugal, tularaemia is not often suspected and may be underdiagnosed. Cross-reactivity between zoonotic agents should be considered despite its weak impact in the diagnosis of *F. tularensis*,[14] and was excluded in this case.

Aminoglycosides remain the first line treatment of all forms of tularaemia, except for meningitis and endocarditis, where combination therapy is recommended. Oral fluoroquinolones and doxycycline can be used for mild disease. Empirical therapy failure and symptom relapse have been described, either due to delay in the correct treatment or treatment regimens shorter than 14 days.[4,7] Considering the uncertainty of the diagnosis at discharge and subsequent suspicion of tularaemia, physicians chose a doxycicline 21-day regimen.

After six months, the laboratory test was repeated and

it was negative for *F. tularensis*, In tularaemia, antibodies appear approximately two to three weeks after infection and may be detected several years after recovery, depending on the patient's immune system.[15] In this case, the serological test used detects primarily IgM-type antibodies,[14] suggesting resolution of the acute infection.

CONCLUSION

In this case, despite the epidemiological investigation that was conducted, the source was not confirmed, possibly due to the time lapse between clinical presentation and its notification. Disease notification in real time, whenever possible, is crucial for an effective intervention by Public Health departments.

We highlight raising awareness to the possible misdiagnosis of tularaemia and the importance of coordination between physicians, laboratory and Public Health departments. A prompt response is crucial in order to determine the source of the infection and contain a potential outbreak.

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AUTHORS CONTRIBUTION

FC, ILC, CT: Case description and discussion.

RG: Critical review of the work.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

INFORMED CONSENT

Obtained.

COMPETING INTERESTS

All authors report no competing interests.

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