Acute Q fever in Portugal. Epidemiological and clinical features of 32 hospitalized patients

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Abstract

Introduction Q fever is a worldwide zoonosis caused by *Coxiella burnetii*. The main characteristic of acute Q fever is its clinical polymorphism, usually presenting as a febrile illness with varying degrees of hepatitis and/or pneumonia. Q fever is endemic in Portugal, and it is an obligatory notifiable disease since 1999. However, its epidemiological and clinical characteristics are still incompletely described.

Methods We performed a retrospective study of 32 cases admitted in the Infectious Diseases Department, Santa Maria's University Hospital, from January 2001 to December 2010, in whom acute Q fever was diagnosed by the presence of antibodies to phase II *Coxiella burnetii* antigens associated with a compatible clinical syndrome.

Results Out of the 32 cases recorded, 29 (91%) were male, with a male:female ratio of 9.7:1. Individuals at productive age were mainly affected (88%, n=28, with ages between 25 and 64 years). Clinically, the most common manifestation of acute Q fever was hepatic involvement (84%, n=27), which occurred isolated in 53% (n=17) of the cases. Hepatitis was more severe, presenting with higher values of liver function tests, in patients presenting both pulmonary and hepatic involvement. Additionally, we report one case of myocarditis and another one with neurological involvement. Empiric but appropriate antibiotic therapy was given in 66% (n=21) of the cases. There was a complete recovery in 94% (n=30) of the patients, and one death. We confirmed the sub-notification of this disease in Portugal, with only 47% (n=15) of the cases notified.

Conclusion In Portugal further studies are needed to confirm our results. From the 32 cases studied, acute Q fever presented more frequently as a febrile disease with hepatic involvement affecting mainly young male individuals. Furthermore, acute Q fever is clearly underdiagnosed and underreported in Portugal, which suggests that an increased awareness of the disease is needed, together with a broader use of serological testing.

Keywords *Coxiella burnetii*, acute Q fever, epidemiology, hepatitis, cholestasis, pneumonia, Portugal

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Introduction

Q fever is a zoonotic disease caused by *Coxiella burnetii*, a strict intracellular Gram negative coccobacillus found worldwide,^{1,2} with the exception of New Zealand.³ It is endemic in many countries, including Portugal.^{1,4} The main reservoirs responsible for transmission to humans are cattle, sheep and goats and also pets, such as cats and dogs, which are responsible for urban outbreaks.^{1,5,6} Infected animals eliminate *C. burnetii* in secretions such as urine, feces or milk and by way of birth products through the placenta or amniotic fluid (tissues that may contain more than 10⁹ bacteria per gram).^{1,5,6}

C. burnetii is highly infectious and can cause disease with a small inoculum (between one to five microorganisms).^{1,5} Additionally, *C. burnetii* is

able to survive in the environment, even under adverse conditions.^{1,5,7} Human infection occurs mostly by aerosols, through inhalation of spores coming from infected secretions.^{5,6} Less frequently, there are cases of transmission by ingestion of unpasteurized milk or fresh cheese^{8,9} and more rarely through blood transfusion,^{1,10} vertical route^{11,12} or by sexual transmission.¹³

The main characteristic of acute Q fever is its clinical polymorphism, manifesting itself over a wide range of symptoms.¹ About 50% of infected individuals are asymptomatic, and only 2-5% of the symptomatic patients require hospitalization.¹⁴ Acute infection can affect any organ but it frequently produces a self-limited febrile syndrome that can occur either isolated or with varying degrees of hepatitis and/or pneumonia.^{1,9,15} There is considerable geographical variation of the main clinical presentation, with an isolated febrile syndrome being the most common manifestation in Australia,¹⁶ whilst hepatitis is the main clinical presentation in France,⁹ and pneumonia the main outline of disease in Croatia,¹⁷ Switzerland¹⁴ and Greece.¹⁸ It is thought that these regional variations may result from biological characteristics of the host, exposure-related factors, or even different strains of *C burnetii*.^{19,22} The mortality rate of acute Q fever is low, approximately 1-2%,^{9,15} with myocarditis being the main cause of death.²³ As it is a non-specific illness presenting a wide range of symptoms, the diagnosis is essentially serological.^{1,5}

Q fever was identified for the first time in Portugal in 1948 by Fonseca et al.^{24,26} For many years it had been thought that acute Q fever merely expressed itself in Portugal as a pulmonary disease,²⁵ but in 1974, the first case with hepatic involvement was identified in the Infectious Diseases Department at Santa Maria's University Hospital (HSM).²⁷ Three seroepidemiological studies have been conducted to date in Portugal confirming that the disease is endemic in the country.^{4,28,29} Studies of cattle have also shown a high seroprevalence of *C burnetii* infection suggesting that Q fever is far from being a negligible disease in Portugal.^{30,31} Despite several clinical cases of Q fever reported in the Portuguese literature,³²⁻³⁴ only two studies have

investigated the clinical and epidemiological situation of the disease in the country.^{27,35} Q fever is a notifiable disease in Portugal since January 1, 1999 and an average of 0.1 cases per 10⁵ inhabitants per year have been reported.³⁶ Although no epidemics have been reported, it is believed that the disease is underdiagnosed and underreported.² For instance, a study performed by the Centre for Vectors and Infectious Diseases of the Ricardo Jorge National Health Institute (CEVDI/INSA) found serological confirmation of 32 cases between 2004 and 2005, as opposed to the 12 cases reported to the General Directorate of Health (DGS) countrywide for the same period.^{2,36}

Although Q fever is found globally and seroepidemiological research suggests that the incidence is greater than that reported, several problems hamper its diagnosis, including: its wide range of clinical symptoms, a lack of awareness on the part of physicians, as well as the large number of subclinical or self-limited cases.

Therefore, given the lack of national data on this issue, our study investigated the situation in the Infectious Diseases Department of a main hospital in Lisbon, focusing on the clinical and epidemiological characteristics of the hospitalized patients.

Methods

We performed a retrospective study using data from medical records, including laboratory data, of patients hospitalized with acute Q fever in the Infectious Diseases Department, HSM, between January 1, 2001 and December 31, 2010. HSM is part of the North Lisbon Hospital Centre and it provides direct care to around 350 000 inhabitants. The study was approved by the Ethical Committee of the Faculty of Medicine, Santa Maria's Hospital, Lisbon.

Sampling Procedures

We included all cases reported to the DGS as Q fever (ICD10: A78) as hospitalized patients in the Infectious Diseases Department during the period under study. Additionally, all the discharge notes from the department were analyzed, seeking any clinical syndrome compatible with acute Q fever (such as

undetermined febrile syndrome, atypical pneumonia, hepatitis or meningoencephalitis of unknown origin). For the identified cases, serologies were analyzed in search of serological data compatible with acute Q fever. The inclusion criteria were serology and clinical condition compatible with acute Q fever.

Serology

The Clinical Pathology Department of HSM uses the indirect immunofluorescence antibody test (IFA) which is the technique recommended by the World Health Organization (WHO) for diagnosis of Q fever.⁵ Cut-off titers varied throughout the study period (table I) due to two changes of the company providing the antigen reagent.

Period	Cut-off titer				Antigen reagent company
	IgM II	IgG II	IgM I	IgG I	
A: Jan/2001 - Jul/2007	1:16	1:16	1:16	1:16	Focus technologies
B: Aug/2007 - May/2010	1:12	1:64	1:12	1:64	Vircell
C: Jun - Dec/2010	1:16	1:16	1:16	1:16	Focus technologies

Table I – Antibody anti-*Coxiella burnetii* cut-off titers used at the Clinical Pathology Department, HSM, during 2001-2010.

The following criteria were used for the serological diagnosis of acute Q fever:

- seroconversion (absence of antibodies during the acute phase sample with their presence in the convalescence sample); or
- a fourfold increase in antibody titers (between acute and convalescence samples); or
- presence of IgM anti-phase II with titers at least two times the cut-off level ($\geq 1:64$ in period A and C and $\geq 1:48$ in period B); or
- if only IgG anti-phase II were reactive, the disease was considered acute if titers $\geq 1:256$ were found in any of the periods under study.^{37,38}

Clinical Syndromes

Patients were classified by clinical syndrome.^{9,15}

1. Isolated febrile syndrome: fever (axillary temperature $\geq 38.2^{\circ}\text{C}$ – the cut-off value used at the Infectious Diseases Department, HSM) without symptoms or signs of pneumonia, hepatitis or other specific clinical syndrome.

2. Hepatic involvement: fever associated with hepatic abnormalities:

a) Hepatitis: transaminase levels at least two times the upper normal limit (aspartate aminotransferase (AST) > 68 U/L and/or alanine aminotransferase (ALT) > 98 U/L).

b) Cholestasis: elevation of at least one of the cholestasis markers (gamma-glutamyltransferase (GGT) > 73 U/L, alkaline phosphatase (ALP) > 129 U/L or direct bilirubin > 5.1 $\mu\text{mol/L}$).

3. Pulmonary involvement: fever associated with chest X-ray abnormalities, with or without pulmonary clinical syndrome (including shortness of breath, coughing, chest pain or hemoptysis).

4. Nervous system involvement: cerebrospinal fluid (CSF) analysis conducted by lumbar puncture is thought to be the best indicator of neurological abnormalities, however any neurological symptom or sign of undetermined origin may be taken into consideration. Three types of clinical patterns are normally found: meningitis (increased level of leukocytes in the CSF), meningoencephalitis or peripheral neuropathy.³⁹

5. Cardiac involvement: pericarditis defined by chest pain, electrocardiographic abnormalities (ST segment elevation in the absence of Q waves) or pericardial effusion; or myocarditis defined by chest pain, palpitations, electro- or echocardiographic abnormalities, or elevated cardiac biomarkers, when a possible acute coronary syndrome had been ruled out.

With the exception of isolated febrile syndrome, the above-mentioned clinical syndromes were not mutually exclusive and patients could belong to more than one group, depending on their specific organ involvement. In order to categorize the patients, epidemiological, clinical, laboratory and imaging data were collected for each case (appendix). Since a list of symptoms denied during

examination did not always exist, those not listed were defined as being absent.

Statistics

Results were analysed statistically using SPSS version 19. Pearson's χ^2 test and Fisher's exact test were used to compare qualitative data. Quantitative data were compared using the Student's t test when a normal distribution was present, and the Mann-Whitney U test when this condition was not verified. Any difference with a P-value <0.05 was considered indicative of statistical significance.

Results

The process described above yielded 32 cases with symptoms and serological tests indicative of acute Q fever. Eleven patients showed evidence of seroconversion; four had a fourfold increase in antibody titers; eight patients had IgM anti-phase II titers at least two times the cut-off level; and nine patients had IgG anti-phase II titers $\geq 1:256$.

Epidemiological data

The annual distribution of the 32 cases is shown in figure 1. The number of cases varied between one in 2004 and 2005 and five (15.6%) in 2002.

Figure 1 – Annual distribution, over a ten-year period (2001-2010), of 32 cases of acute Q fever admitted in the Infectious Diseases Department, HSM, with reference to the number of notified cases.

Only half of the cases (46.9%, n=15) were reported to the DGS as Q fever. In fact, there was a statistically significant decrease in notification during the 2006-2010 period (3 out of 18 cases) when compared with 2001-2005 (12 out of 14 cases) ($\chi^2=15.1$; $p=0.000$), without any official reports in the years 2007, 2009 and 2010 (figure 1).

The seasonal distribution (figure 2) shows higher levels of infection in the spring (34.4%, n=11) with the highest frequency in May (25%, n=8). We also analyzed data regarding patients' county of residence (namely in rural areas).

Figure 2 – Seasonal distribution, per month of onset of symptoms, over a ten-year period (2001-2010), of 32 cases of acute Q fever admitted in the Infectious Diseases Department, HSM.

Figure 3 illustrates the geographic distribution of the cases, with the highest number of patients in Loures (25%, n=8), Lisbon (21.9%, n=7) and Odivelas (18.8%, n=6). Three additional cases came from outside the Lisbon District (one from Tomar, Santarém, one from Grândola, Setúbal and another case was that of a patient residing in Canada). Of all patients, only nine (28.1%) reported living in rural areas (four in Loures, two in Odivelas, one in each of the following: Mafra, Grândola and Canada).

Figure 3 – Map of Portugal showing the geographic distribution, by district and county of residence, of 32 cases of acute Q fever admitted in the Infectious Diseases Department, HSM.

Most cases (90.6%, $n=29$) were male, with a male:female ratio of 9.7:1. The average age $\pm$ standard deviation (SD) was 45.2 ± 15 years (range 20-78). The average age for males was 43.2 ± 13.3 (range 20-62) whilst the women's average was 64.7 ± 18.9 (range 43-78). This difference was statistically significant ($p=0.015$). Most patients (87.6%, $n=28$) were between 25 and 64 years of age; in this age range, the male:female ratio was 27:1. Within the 65-84 age group, there were only two cases, both female.

Risk factors, namely occupation, recent time spent in the countryside, contact with animals and consumption of unpasteurized dairy products, were also analysed. Five cases (15.6%) occurred amongst people with at risk professions (one farmer, three rural workers and one horse handler). Only 25 cases (78.1%) had been subjected to any type of epidemiological enquiry. Of those, 23 patients (71.9% of the total sample) had at least one epidemiologic risk factor. Recent time spent in the countryside was noted in 19 out of 24 patients (59.4% of the total sample).

Sixteen out of the 22 people (72.7%) had had recent contact with animals: nine (56.3%) with cattle, sheep or goats; seven people (43.8%) had had contact with dogs; two (12.5%) had had contact with cats; two (12.5%) had been recently bitten by a tick and one (5.3%) had recently been in contact with horses. None of the patients reported contact with parturient or newborn animals. Contact with cats and dogs, very common in Portugal, was not considered a risk factor. Therefore, only 50% of the cases ($n=11$, 34.4% of the total sample) were found to have had contact with animals of risk. Only 11 patients had been asked about the consumption of unpasteurized dairy products, and three of them (9.4% of the total sample) had answered affirmatively.

Four patients presented co-morbidities that put them at risk for the development of chronic Q fever: one had severe aortic stenosis, one was HIV infected and two were splenectomized following traffic accidents.

Clinical data

The average length of illness preceding hospitalization was 10.1 ± 8.4 days (range 2-35). For men, this period was 8.8 ± 7 days (range 2-30) whilst for women it was 22 ± 13.5 days (range 8-35). This difference was statistically significant ($p=0.035$). The average length of hospitalization was 12.3 ± 11.4 days (range 2-52) with no differences encountered between genders or different clinical syndromes. Table II presents the frequency of symptoms, signs and laboratory abnormalities of the 32 patients. The average period with fever (before and during hospitalization) was 12.4 ± 11.1 days (range 5-60). There was no statistically significant difference between the most frequent symptoms and different gender or clinical syndrome. Autoantibodies were investigated in four patients, and were positive only in two: one case with positive rheumatoid factor (RF) test, anti-mitochondrial M2 and lupus anticoagulant antibodies; and another patient with positive antinuclear antibodies (ANA) with a titer of 1:160.

	Total (32 cases) % (no)	Maurin et Raoult, 1999 ¹ (%)
Fever ($\geq 38.2^{\circ}\text{C}$)	100 (32)	88-100
Average time with fever (days)	Not quantified: 15.6 (5) 12.4 (± 11.1) (5-60)	-
Myalgia	68.8 (22)	47-69
Headache	62.5 (20)	68-98
Asthenia	56.3 (18)	97-100
Diaphoresis	53.1 (17)	31-98
Chills	43.8 (14)	68-88
Anorexia	40.6 (13)	-
Nausea	34.4 (11)	22-49
Vomiting	31.3 (10)	13-42
	31.3 (10)	24-90
Cough	Dry: 70 (7)	-
	Productive: 30 (3)	-
Diarrhea	21.9 (7)	5-22
Abdominal pain	21.9 (7)	-
Weight loss	9.4 (3)	-
Arthralgia	6.3 (2)	-
Dyspnea	3.1 (1)	-
Photophobia	0	-
Chest pain	0	10-45
Normal PE	6.3 (2)	-
Abnormal pulmonary auscultation	40.6 (13)	-
Hepatomegaly	40.6 (13)	-
Splenomegaly	25 (8)	-
Jaundice	18.8 (6)	-
Choluria	18.8 (6)	-
Lymphadenopathies	18.8 (6)	-
Exanthema	15.6 (5)	5-21
Purpuric rash	6.3 (2)	-
Neurological disturbances	6.3 (2)	-
Acholia, meningeal signs or psychiatric disturbances	0	-
ESR > 10 mm/h	87.5 (28)	43-87.5
CRP > 5 mg/L	96.9 (31)	-
	Normal count: 71.9 (23)	90
Leukocytes ($\times 10^9/\text{L}$)	Leukocytosis: 9.4 (3)	-
	Leukopenia: 18.8 (6)	-
	Normal count: 68.8 (22)	-
Neutrophils ($\times 10^9/\text{L}$)	Neutrophilia: 15.6 (5)	-
	Neutropenia: 15.6 (5)	-
	Normal count: 28.1 (9)	-
Platelets ($\times 10^9/\text{L}$)	Thrombocytosis: 9.4 (3)	-
	Thrombocytopenia: 62.5 (20)	25
AST > 68 U/L	68.8 (22)	45-85
ALT > 98 U/L	71.9 (23)	-
GGT > 73 U/L	75 (24)	25-75
ALP > 129 U/L	50 (16)	27.7-57
Total bilirubin > 17.1 $\mu\text{mol/L}$	46.9 (15)	9-14.3
Direct bilirubin > 5.1 $\mu\text{mol/L}$	10 cases requested: 70 (7)	-
LDH > 378 U/L	78.1 (25)	33.3-40
CK > 294 U/L	12.5 (4)	29
Urea > 17.9 mmol/L	12.5 (4)	-
Creatinine > 99.1 $\mu\text{mol/L}$	12.5 (4)	29-40
	Normal: 28.1 (9)	-
Sodium (mmol/L)	Hyponatremia: 0	-
	Hyponatremia: 71.9 (23)	-

ALP alkaline phosphatase; ALT alanine aminotransferase; AST aspartate aminotransferase; CK creatine kinase; CRP C reactive protein; ESR erythrocyte sedimentation rate; GGT gamma-glutamyltransferase; LDH lactate dehydrogenase; PE physical exam.

Leukocytosis > 11.000 $\times 10^9/\text{L}$, leukopenia < 4.000 $\times 10^9/\text{L}$, neutrophilia > 7.500 $\times 10^9/\text{L}$, neutropenia < 1.900 $\times 10^9/\text{L}$, thrombocytosis > 450.000 $\times 10^9/\text{L}$, thrombocytopenia < 150.000 $\times 10^9/\text{L}$, hypernatremia > 145 mmol/L, hyponatremia < 135 mmol/L.

Table II – Frequency of symptoms, signs and laboratory abnormalities of 32 cases of acute Q fever, admitted in the Infectious Diseases Department, HSM, compared to data from Maurin and Raoult.¹

Chest X-ray results were available for 31 patients, out of which 35.5% (n=11) revealed abnormalities. Of those eleven patients, eight (72.7%) had interstitial infiltrates, two cases (18.2%) presented with condensation and three cases (27.8%) had pleural effusion. The most common pattern was the involvement of the lower lobes (72.7%, n=8), especially on the right side (36.4%, n=4). Only three patients (9.4%) underwent liver biopsies. One of them revealed granulomatous hepatitis compatible with acute Q fever and the other two had nonspecific changes. Two patients (6.3%) underwent lumbar punctures and in both cases the cytochemical and microbiological analysis of the CSF were normal.

Figure 4 displays the classification of patients by clinical syndromes. Overall, 84.4% (n=27) of patients showed liver involvement (which was isolated in 53.1%, n=17), 34.4% (n=11) had pulmonary involvement, 9.4% (n=3) had isolated febrile syndrome, and 3.1% (n=1) each had myocardial and neurological involvement.

Furthermore, we investigated whether there was any relationship between different clinical

syndromes and epidemiological/clinical data, with no statistically significant differences found for age, risk factors or symptoms occurring in the different clinical syndromes. As far as the physical examination data were concerned, patients with isolated liver involvement (n=17) more rarely showed abnormal pulmonary auscultation ($\chi^2=4.39$; $p=0.036$) but more often had splenomegaly ($p=0.041$). It was also found that patients with liver involvement of any type (n=27) were more likely to have increased lactate dehydrogenase (LDH) (OR:32; 95% CI: 2.63-389.25; $p=0.004$). We also investigated whether there was any difference in the average laboratory data values between the two most common clinical syndromes in this study: patients with hepatic and pulmonary involvement only (n=8) and those with isolated hepatic involvement (n=17) (table III). Patients presenting both hepatic and pulmonary involvement had significantly higher levels of AST ($p=0.003$), ALT ($p=0.041$) and ALP ($p=0.048$) and appeared to be more likely to have higher levels of LDH.

Figure 4 – Clinical syndrome distribution of 32 cases of acute Q fever admitted at the Infectious Diseases Department, HSM.

	Hepatic and pulmonary involvement (8 cases)	Isolated hepatic involvement (17 cases)	P value	Total (32 cases)
ESR (mm/h)	37.4 (±36.7)	50.6 (±34.6)	0.31	51.9 (±36.8) (7-130)
CRP (mg/L)	153.2 (±74.8)	156.4 (±79.1)	0.92	157 (±81.3) (5-315)
Leukocytes (x 10 ⁹ /L)	5.945 (±2.441)	8.090 (±3.500)	0.15	7.714 (±3.699) (2.720-17.800)
Neutrophils (x 10 ⁹ /L)	3.6 (±2.1)	5.3 (±2.7)	0.12	5.1 (±3) (1.1-13.1)
Platelets (x 10 ⁹ /L)	100.000 (±37.766)	181.824 (±178.500)	0.45	171.813 (±153.594) (28.000-652.000)
AST (U/L)	251.9 (±130.1)	120.2 (±83.4)	0.003	137.2 (±113.6) (10-538)
ALT (U/L)	307.5 (±214)	178.9 (±135.1)	0.04	185.5 (±165.5) (12-823)
GGT (U/L)	381.3 (±319.1)	198.8 (±124)	0.15	237.9 (±242.7) (20-1000)
ALP (U/L)	253.6 (±95.9)	167.9 (±108)	0.048	182.4 (±123.6) (47-509)
Total bilirubin (µmol/L)	37.1 (±44.8)	25.4 (±18.6)	0.66	25.1 (±26.8) (5.1-145.4)
Direct bilirubin (µmol/L)	4 cases 30.8 (±41.1)	5 cases 20.6 (±23)	0.46	10 cases 23 (±29.5) (3.4-92.3)
LDH (U/L)	1023.4 (±606.8)	652.5 (±287.2)	0.055	710.8 (±421.3) (192-2381)
CK (U/L)	132.5 (±175.9)	167.3 (±229.9)	0.79	163.9 (±222.8) (16-810)
Urea (mmol/L)	18.9 (±25.7)	12.4 (±4.6)	0.52	14.6 (±13) (5.7-82.1)
Creatinine (µmol/L)	139.2 (±196.8)	84.8 (±22.5)	0.21	98.4 (±98.2) (45.8-625.3)
Sodium (mmol/L)	131.3 (±3.4)	132.1 (±4.5)	0.38	131.8 (±4.3) (119-139)

ALP alkaline phosphatase; ALT alanine aminotransferase; AST aspartate aminotransferase; CK creatine kinase; CRP C reactive protein; ESR erythrocyte sedimentation rate; GGT gamma-glutamyltransferase; LDH lactate dehydrogenase.

Table III – Comparison (p-value) of laboratory data (mean value ± SD) between 8 cases presenting both pulmonary and hepatic involvement and 17 cases presenting isolated hepatic involvement. For reference it includes the values of the total 32 cases of acute Q fever admitted in the Infectious Diseases Department, HSM.

In the present study, treatment was found to have been adequate, according to field literature,^{1,2,67,68} in 65.6% of the cases (n=21): 19 received doxycycline 100 mg twice daily (bid) for at least 14 days and two patients were treated with ciprofloxacin, either 500 mg or 750 mg bid for 18 and 21 days, respectively. The other 11 cases received inadequate treatment: five patients (15.6%) received the wrong antibiotic (either beta-lactam, beta-lactam combined with aminoglycoside, macrolide or carbapenem and in one case only symptomatic treatment) and six patients (18.8%) received the correct antibiotic but for insufficient periods (less than 14 days). Thus, of the 27 cases in which the correct antibiotic was prescribed, the average number of

days in the hospital before initiating treatment was 3.3 ± 8.2 days, with 46.9% of the cases (n=15) beginning treatment on the first or second day of hospitalization. The number of patients correctly treated increased significantly between 2001-2005 (5 out of 14 cases) and 2006-2010 (16 out of 18 cases) ($p=0.003$). All cases reported between 2008 and 2010 were correctly treated.

The vast majority of patients (93.8%, n=30) fully recovered. One patient (3.1%), who was not appropriately treated (received doxycycline for only 10 days), recovered only partially, being discharged with mild anemia and kidney dysfunction. One other patient (3.1%), who had a multi-systemic involvement (hepatic, pulmonary, neurological and cutaneous), died.

This patient had been correctly diagnosed, early after hospital admission, and treated with doxycycline and chloroquine for more than 30 days. Despite the appropriate treatment he developed global respiratory failure, attributed to an atypical pneumonia due to *C burnetii*.

Patients with co-morbidities that put them at risk for the development of chronic Q fever showed no statistically significant difference regarding the hospitalization or recovery period.

The period between admission and the first serology request was also analysed. The average period was 1.1 ± 4.5 days, with more than half of the patients (65.6%, n=21) with the first serology requested on the first or second day of hospitalization.

Seventy-five percent of the patients (n=24) had a follow-up including at least one medical appointment. Of these, however, only 28.1% (n=9) had two or more follow-up appointments.

Discussion

This study investigated the epidemiological and clinical characteristics of patients hospitalized in the Infectious Diseases Department of a main hospital in Lisbon (HSM) during the period 2001-2010. Contrary to what was expected, no increase in the incidence rates of acute Q fever was found when the annual distribution was analysed. It was confirmed that Q fever was underreported, since less than half of the cases studied (46.9%) were notified to the DGS. Furthermore, a statistically significant decrease was found in notification over the period evaluated such that in more recent years (2007, 2009, and 2010) none of the diagnosed cases were reported. Q fever is usually a self-limited disease and is normally diagnosed retrospectively, due to the delay in serological confirmation.²² Thus, its notification requires an extra effort by the physician. Moreover, over half of Q fever cases are asymptomatic,¹⁴ a fact which, in conjunction with the underreporting of symptomatic patients, makes it difficult to accurately assess the incidence of the disease. These results must raise physicians' awareness to the importance of notifying Q fever, so that its real incidence in Portugal may be found. If one

were to assume that only 4% of cases need hospitalization,¹⁴ this would suggest the existence of at least 457 cases of acute Q fever in the decade of 2001-2010 in the urban area served by HSM.

Judging by the counties of residence of the 32 patients in this sample, most of them, as expected, lived in the areas served by HSM (Loures, Lisbon and Odivelas).

The illness was shown to be more prevalent during spring, with the highest incidence in May (25%). Higher levels of incidence in April, May and June have been reported in a previous Portuguese study²⁷ as well as in studies performed in other European countries (Spain,²² France,¹⁵ England⁴⁰ and Holland).⁴¹ Most calves are born in the autumn (lambling season) with a smaller number in the spring. The higher prevalence of the illness in spring may result from the births occurring in this season as well as the period of time needed for the desiccation, aerosolization of the spores and incubation in the host following the autumn births.⁴²

The male:female ratio was 9.7:1, which is highly distinct from the Portuguese population ratio: 0.9:1.⁴³ All studies on Q fever report this male predominance, although with lower ratios: 1.7:1 in Holland,⁴¹ 2.5:1 in France,¹⁵ 4.2:1 in Croatia¹⁷ and 5.3:1 in Australia.⁴⁴ A previous Portuguese study²⁷ as well as one performed in the region of Barcelona (Spain)²² also showed much higher rates of incidence for men: 15:1 and 11.6:1, respectively. This male prevalence does not seem to be attributable to any occupational factor, since in seroprevalence studies the male:female ratio is 1:1.⁹ This suggests that, after exposure to *C burnetii*, both genders are equally affected; however, men are frequently more symptomatic.^{20,45} In studies with animal models, estrogen has proven to play a protective role,⁴⁶ a fact that might explain why this predominance in males only occurs after puberty.⁴⁷ Furthermore, the current study observed that the average number of days with symptoms preceding hospitalization was much inferior for men than for women, which leads us to speculate that women, even when symptomatic, appear to have less severe symptoms and as a result are less likely

to present to the Emergency Room, or if they do go, they do so later than men.

Q fever was more frequent amongst the age groups that were economically active (25-64 years, 87.6%). However, it was confirmed that Q fever can affect any age group; this study found cases ranging from 20 to 78 years of age, as had other studies.^{9,15,22,26} No pediatric patients were reported, as the Infectious Diseases Department of HSM only receives patients over the age of 18. Nevertheless, studies with children have shown that Q fever can occur in this group, although it is often more asymptomatic.^{20,45} The tendency of people in their working years to contract the disease may be attributable to an increased risk owing to occupational activities.^{1,15} The average age of the men in this study was significantly lower than that of women, with the patients over 65 years of age being only female. The predominance of working aged men appears to be related to hormonal factors, with post-menopausal women being as likely to become symptomatic as the men. That might explain why in the present study women were diagnosed at higher ages than men. However, any conclusion would need to be confirmed by further studies, given the reduced number of women in this sample.

Acute Q fever risk factors were analysed as well, namely occupation, recent time spent in the countryside, contact with animals or consumption of unpasteurized dairy products. Only 15.6% of the patients had occupational risk factors (one farmer, three rural workers and one horse handler); this coincides with the results of a French study of 323 cases of acute Q fever.⁹ The epidemiological data were not studied in all cases, or at least, they were not recorded in the medical files. Of the entire sample, 71.9% had positive epidemiological history: 59.4% mentioned recent time spent in the countryside, 34.4% had had contact with animals of risk and 9.4% had consumed unpasteurized milk or fresh cheese. The reduced number of patients living in rural areas (28.1%) as compared with those who mentioned recent time spent in the countryside (59.4%) suggests that, in this sample, transmission of acute Q fever occurred more frequently due to recreational activities. The fact

that an epidemiological history was not collected from all patients points to an insufficient collection of clinical data or to the lack of suspicion of Q fever at admission.

The classification by clinical syndromes described above found hepatic involvement in 84.4% of cases and pulmonary involvement in 34.4%. The two previous Portuguese studies, one in Lisbon²⁷ and another in Coimbra,³⁵ had also found this predominance of liver damage. Both the present study and the previous one made in Lisbon²⁷ were undertaken in an Infectious Diseases Department. This may imply a bias leading to underestimation of the number of patients with pulmonary involvement (perhaps more frequently admitted in a Respiratory Diseases or Internal Medicine Department). Thus, further studies, especially including different departments, are needed to confirm the main clinical presentation of acute Q fever in Portugal. The hepatic involvement may take the form of hepatitis or cholestasis, nonetheless, in this study an association of the two was the most common situation. In other regions such as France,⁹ Ontario (California)⁴⁸ and in southern and north-eastern Spain,^{22,49} hepatic involvement is the most frequent clinical expression of the disease. The most common presentation of acute Q fever may vary between two regions of the same country, as is the case of Spain, in which pneumonia is more frequent in the Basque country⁵⁰ and hepatitis is more common in Andalusia.⁴⁹ Further studies are needed in different regions of Portugal to confirm if there is a main clinical presentation of acute Q fever countrywide. Several possible hypotheses exist to explain this geographical variance: different strains of *C. burnetii*,⁵¹ variance in the biological characteristics of the hosts,⁵² the size of the inoculum or the transmission route.^{1,9,15} Studies of animal models have shown that infection by the respiratory route (intranasal inoculation) is more frequently associated with pneumonia whilst intraperitoneal inoculation is more often associated with liver involvement.^{53,54} The consumption of unpasteurized dairy products is associated with a high frequency of liver involvement in some countries,⁸ like in France, a country with extensive rural areas with

considerable cattle production and with the habit of consuming unpasteurized dairy products.⁹ In the present study, all the patients who mentioned consumption of unpasteurized dairy products had hepatic involvement. Nonetheless, due to the reduced number of cases with this history, it was not possible to arrive to any proper conclusions, nor was it possible to find any significant differences between the other risk factors and the various clinical syndromes, which might suggest a more important role of the host factors in the main presentation of the disease.¹⁵ Raoult et al. argue that liver involvement occurs primarily in young people whilst pneumonia is more common in older and/or immunodeficient patients.¹⁵ This study found no statistically significant difference between the presence or absence of pulmonary involvement and immunosuppression or patient age.

Three cases of isolated febrile syndrome, one case of myocardial involvement and one case of neurological involvement were also recorded. Myocarditis is a rare manifestation of acute Q fever, however, it does account for the largest number of acute Q fever deaths.^{15,23} La Scola et al. argue that myocarditis is associated with a larger inoculum.⁵³ The only case of myocarditis in this study was a 62-year-old man with associated hepatitis and cholestasis, without previous cardiac co-morbidities. He was admitted with high fever and a widespread macular rash without any cardiac symptoms. The laboratory results showed an erythrocyte sedimentation rate (ESR) of 102 mm/h and high cardiac biomarkers (troponin I 17 000 µg/L, creatine kinase (CK) 810 U/L and CK-MB 59 U/L), with normal electro- and echo-cardiogram. The patient made good progress during hospitalization with a progressive decrease of the cardiac biomarkers, which returned to normal values within three days. By discharge, he was free of any clinical symptom or laboratory abnormality.

Neurological involvement in acute Q fever usually presents as meningitis, encephalitis or peripheral neuropathy or, more rarely, as a pyramidal, extra-pyramidal or cerebellar syndrome.¹⁵ The only case with neurological involvement in this study was that of a 40-year-old man, admitted with pulmonary symptoms

and a nodular rash. During hospitalization he began to suffer atypical neurological symptoms, such as right eyelid ptosis, dysmetria, ataxia and disequilibrium, suggesting a nervous system disorder, namely of the right oculomotor nerve and the cerebellum. However, no abnormalities appeared in the cytochemical or microbiological analysis of the CSF nor in the computed tomography (CT) scan; the MRI showed moderate cortical and cerebellar atrophy, suggesting a parainfectious cerebellitis. Cerebellar encephalitis is mentioned in literature but very seldomly.⁵⁵ Some studies have mentioned similar cases: Bernit et al. reported a case of paresis of the oculomotor nerve,³⁹ Ropper et al. and Sawaishi et al. each mentioned a case of acute cerebellitis^{55,56} and an English study also reported a case of cerebellar ataxia.⁵⁷

Cutaneous lesions occur in 5-21% of acute Q fever cases,^{9,58} usually in the form of a transient punctuate rash, a maculopapular rash, or, more rarely, erythema nodosum.^{15,20} Five (15.6%) of the patients studied herein had exanthema, macular in two and maculopapular in another two, occurring mostly on the trunk and limbs. In just one case, the rash failed to involve the palms of the hands and the soles of the feet. The patient with neurological involvement also had an atypical rash in the form of painless, bilateral, erythematous skin nodules on the lower limbs. Three separate skin biopsies were conducted to rule out vasculitis, analyse the presence of granulomas suggestive of Q fever and detect DNA of *C burnetii*. However, the results were all negative. This patient had an ESR of 130 mm/h (the highest in the study) and apparently developed a vasculitis associated with the *C burnetii* infection as has been previously reported.⁵⁹

Table II illustrates the frequency of symptoms, signs and laboratory abnormalities in the patients in this sample and compares them with the results compiled by Maurin and Raoult,¹ to our knowledge, the largest review paper to date, which analyses data from nine previous studies. The main clinical picture consisted in fever associated with myalgia, headaches, asthenia and diaphoresis, as reported in most studies

published to date.^{1,26,27,41,60} The most frequent abnormalities found in the physical examination were abnormal pulmonary auscultation, hepatomegaly and splenomegaly. The prevalence of hepatosplenomegaly in the current study was higher than that reported in most studies,^{1,17} probably due to the greater prevalence of liver involvement in this sample. It is worth pointing out that there were no cases of chest pain, meningeal signs or psychiatric disturbances. In a study of 176 Portuguese patients, Mendes et al. found fever in 100%, headaches in 79%, diaphoresis in 77%, chills in 61%, myalgia in 57% and adynamia in 56% of the cases.²⁷ As in the current study, they also found high levels of liver involvement (78%, n=138) including hepatomegaly in 78% of the cases and jaundice in 20%. In 1973, Derrick described the course of infection of 173 acute Q fever patients, concluding that the fever lasted between five to 57 days, with an average of ten days.⁵⁸ The current study found an average duration of fever of 12.4 days with a variation between five and 60 days.

The most common laboratory abnormalities were an increase in the C reactive protein (CRP) and the ESR levels with normal leukocytes, thrombocytopenia, elevated LDH, GGT and transaminases as well as hyponatremia. The rates of elevated LDH and total bilirubin, as well as thrombocytopenia were more frequent than those reported in field literature,^{1,5,15,60,61} probably due to the greater prevalence of liver involvement in this sample.

Some studies have suggested a possible autoimmune abnormality associated with liver involvement in Q fever, but this is yet to be clarified.^{5,62} Autoantibodies frequently found include antinuclear (ANA), antimitochondrial, anti-smooth muscle and antiphospholipid (anticardiolipin and lupus anticoagulant) antibodies.^{5,62} Only four cases of the sample had autoantibody analysis performed. Two patients were positive, both with isolated liver involvement. One patient had hepatitis and cholestasis with granulomatous hepatitis found on the liver biopsy, together with a positive RF test, antimitochondrial M2 and lupus anticoagulant antibodies. The other patient

presented with isolated cholestasis and had nonspecific changes on the liver biopsy and positive ANA with a titer of 1:160.

No statistically significant differences were found between the most frequent symptoms in the different clinical syndromes, in contrast to data reported in other studies, that showed a greater frequency of headaches and myalgia in patients with liver involvement.^{9,15} In this study, we found that patients with isolated hepatic involvement were less likely to present an abnormal pulmonary auscultation and more likely to have splenomegaly. Patients with hepatic involvement of any type were more likely to have elevated LDH levels. Also, patients with both hepatic and pulmonary involvement had worse laboratory results with significantly higher levels of AST, ALT and ALP and a tendency towards higher LDH as well, when compared with patients presenting isolated hepatic involvement. The most evident abnormality was the AST level ($p=0.003$), a nonspecific marker of liver cytolysis, which together with the likelihood of elevated LDH, suggests a more virulent infection with greater cellular destruction in patients with hepatic and pulmonary involvement, in other words, with multi-systemic involvement. In these patients we also found higher levels of hepatocellular destruction (indicated by higher ALT values) and more cholestasis (suggested by the ALP values). This greater cellular destruction, especially hepatocellular, may also be related to a reduction of blood oxygenation due to pulmonary involvement. In fact, Marrie et al. observed that 26.6% of their patients with pneumonia had hypoxemia.⁶³ To our knowledge, this is the first time that this fact has been observed and further confirmation is needed with greater sample size studies. It is worth pointing out, however, that Domingo et al., in a report of 38 cases in 1988, conveyed opposite findings with higher levels of transaminases and direct bilirubin in patients with liver involvement without accompanying pneumonia.⁶⁴

The most common pattern found on chest X-ray was interstitial infiltrate with a tendency towards the lower lobes, as was the case in other studies.⁶⁵ Pleural effusion was found in 9.4% of

the cases, which is in line with the 5-10% reported by other authors.⁶⁵ Liver biopsies on acute Q fever patients characteristically reveal doughnut type granulomatous hepatitis consisting of dense, concentric layers of fibrin around a central lipid vacuole.⁶⁶ However, these findings merely appear in 3-10% of biopsies.² In the present study, liver biopsies were performed in only three cases (9.4%) and in one case, granulomatous hepatitis consistent with Q fever was found, while the other two cases had only nonspecific changes. In acute Q fever, lumbar punctures usually reveal normal CSF findings, as was the case in the two patients analysed in this study.

As the results of serological tests are time-consuming and can be negative in the initial period of the disease, upon suspicion of Q fever, empirical treatment should be initiated immediately. The ideal treatment for acute Q fever is doxycycline 100 mg bid for 14 days.^{1,67} In case of intolerance to doxycycline (especially gastric intolerance) or in the case of meningoencephalitis, fluoroquinolones are preferred.^{2,68} Treatment of acute Q fever with doxycycline is only efficient during the symptomatic phase, leading to a reduced period of fever.²² Treating asymptomatic Q fever patients or treating them for periods of less than 14 days are practices commonly found in this and other studies;⁶⁹ however, such treatments are neither considered clinically necessary nor are they suggested in review articles about this disease.⁶⁹ In this study, 65.5% of the patients were appropriately treated, and about half of them began doxycycline on the first or second day of hospitalization, which suggests that the diagnosis of zoonosis was considered early on. We also found a statistically significant rise in the number of cases properly treated in recent years, suggesting that at the Infectious Diseases Department, HSM, physicians are becoming more and more aware of this type of pathology. The vast majority of patients who received inappropriate treatment were probably misdiagnosed. When the pulmonary involvement was predominant, if Q fever was not considered as a probable etiology, the patient was expected to be treated with a beta-lactam or a macrolide. If

instead a zoonosis was thought of, especially Mediterranean spotted fever, which is very frequent in Portugal, the patient was expected to be treated with doxycycline, but in this instance for time spans inferior to 14 days (usually five to ten days).

The date of the request of the first serology for each patient also indicates the point at which a potential Q fever diagnosis was considered. In more than half of the cases (65.5%) the serology was requested on the first or second day of hospitalization, which in fact confirms that the diagnosis was thought of early in most cases. The fact that adequate treatment and serological tests were requested early in most patients suggests that the lack of epidemiological data in the medical files of some patients is due to the insufficient collection of their clinical history since the diagnosis was in fact taken into consideration. Since serological tests are necessary for diagnosing Q fever, it requires considerable physician interest in this illness. We must interpret these results taking into account one of the biases of this study, since it was undertaken at an Infectious Diseases Department and it is therefore expected that early serologies were run in most patients, together with correct empirical treatment.

In this sample, the vast majority (93.8%) of the patients fully recovered. Acute Q fever is normally seen as a mild and self-limited disease.¹⁶ However, some rare cases may have a poorer prognosis, and as a consequence acute Q fever presents a mortality rate of 0.9-2.4%.^{9,15} In our study, this rate was 3.1%: one patient (out of 32) died. This patient had a multi-systemic involvement (hepatic, pulmonary, neurological and cutaneous), and despite appropriate treatment, developed global respiratory failure, attributed to an atypical pneumonia with *C burnetii*.

In the vast majority of patients (75%), further serological tests were requested during at least one medical appointment after discharge. However, this high level is due to another bias of this study, since many potential patients were excluded from the study exactly as a consequence of the lack of serological follow-up. The inexistence of a second serology (convalescence

sample) prevented, in many cases, the serologic diagnosis of acute Q fever. This fact obviously prevents the confirmation of the diagnosis and explains, in part, why this illness is underdiagnosed in Portugal. Fournier et al. found that only 39% of patients tested positive in their first serology.⁷⁰ Despite the well-known importance of parity of the serological samples, this criterion is rarely met. CEVDI/INSA receives more than five hundred blood samples for testing for Q fever every year, however, less than 20% of them presented two paired samples for testing.²

None of the patients in this sample appeared to develop chronic Q fever. However, it should be noted that only nine cases were followed in more than one medical appointment. Beyond the serological follow-up, all acute Q fever patients should have a transthoracic echocardiogram performed to rule out any subclinical heart valve lesions, as these are the patients most likely to develop chronic Q fever.^{71,72} It is, therefore, fundamental that all patients receive follow-up medical appointments, especially those with a higher risk of chronic illness (cardiac or vascular prostheses, valvular heart disease, immunodeficiency or pregnant women).¹ Such medical appointments should include monthly serologies at least in the first six to nine months to rule out chronic infection.^{5,73,74} In this study the patients at risk for chronic infection did not receive adequate follow-up. For this reason, it is crucial to point out the importance of this monitoring in order to identify and treat chronic Q fever as early as possible.

Finally, it is necessary to standardize Q fever diagnosis in order to be able to truly compare the incidence and the seroprevalence of the illness in different regions.⁴² Furthermore, it should be emphasized that even when the same method is used, such as the IFA, the results can vary according to the kind of antigen reagent used. For example Tissot-Dupont et al. found that an IgM anti-phase II titer equal or greater to 1:50 associated with an IgG anti-phase II titer equal or above 1:200 had 100% specificity for acute Q fever.⁷⁵ However, as the Clinical Pathology Department of HSM does not use the same antigen reagent referred in the above-mentioned

article, these cut-off values could not be used in this study. Thus, it is important to raise awareness that the cut-off titers used by different laboratories (using different antigen reagents) vary greatly and should not be compared.^{76,77}

Study limitations

One of the important limitations of this study was the lack of a second serology (convalescence sample) in many cases, which prevented the serological diagnosis of acute Q fever. At least nine patients in this condition had to be excluded from the present study, the vast majority of them with epidemiological and clinical history compatible with acute Q fever. Of the 32 patients included in this study, five of them were diagnosed with only one serological sample.

Furthermore, since this study was undertaken at an Infectious Diseases Department there are several biases that should be taken into consideration. On the one hand, it is expected that early serologies and adequate empirical treatment were run in most patients. On the other hand, there may be an underestimation of pulmonary findings since most of these patients will more probably be admitted in a Respiratory Diseases or Internal Medicine Department. Additionally, there may be an over-representation of the severity of the disease, since only patients who need hospitalization were analysed. Therefore this sample may not be fully representative for the general population.

Conclusions

More studies with larger samples and with patients from different regions of Portugal are needed to confirm these results. Although Q fever has been an obligatory notifiable disease since 1999, it is clearly underdiagnosed and underreported and, as a result, its real incidence and impact on Public Health in Portugal are still unknown. Although it was first described 70 years ago, Q fever remains a poorly understood disease. Physicians should be more aware of this illness and of the need of more extensive use of serological tests to ensure its diagnosis. In Portugal, when a young male patient presents with persistent fever and elevated transaminases,

acute Q fever should be considered as a possible etiology, moreover if accompanied by intense headache, normal leukocyte count, thrombocytopenia and negative blood cultures. In these cases, the epidemiological context must be ascertained and serological testing for acute Q fever should be performed. It is also emphasized that follow-up serologies and transthoracic echocardiogram should be undertaken in all patients.

Conflicts of interest All authors – none to declare.

Author contributions CP collected and analyzed data. CP, EV and RB designed the study and wrote the manuscript.

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Appendix 1. Epidemiological, clinical, laboratory and imaging data collected from the medical records of the patients hospitalized at the Infectious Diseases Department, HSM.

- Epidemiological data: name, admission date, discharge date, presence of Q fever notification, notification date, gender, date of birth, ethnicity, profession, county of residence, residence in rural areas, recent time spent in the countryside, contact with animals (cattle, goats or sheep; dogs and cats; horses; ticks; parturient or not), consumption of unpasteurized milk or fresh cheese, month and year of symptoms onset, the presence of co-morbidities.
- Clinical data:
 - Symptoms - date of symptoms onset, number of days with symptoms preceding the admission, fever ($\geq 38.2^{\circ}\text{C}$), total number of days with fever, chills, diaphoresis, asthenia, anorexia, headache, photophobia, myalgia, arthralgias, nausea, vomiting, diarrhea, abdominal pain, chest pain, cough (dry or productive), dyspnea, weight loss.
 - Signs - normal physical examination, abnormal pulmonary auscultation, hepatomegaly, splenomegaly, jaundice, choluria, acholia, exanthema or purpuric rash, lymphadenopathies, neurological disturbances, meningeal signs, psychiatric disturbances.
 - Laboratory data - ESR, CRP, leukocytes, neutrophils and platelets count, AST, ALT, GGT, ALP, total and direct bilirubin, LDH, CK, urea, creatinine, sodium, abnormal urinalysis, autoantibodies.
 - Imaging data - result of chest X-ray, liver biopsy and lumbar puncture (if performed).
 - Treatment - antibiotic type, dosage, frequency and duration of treatment.
 - Response to treatment: total recovery, partial recovery or death.

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