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*Case Report*

# Imported brucellosis and Q-fever coinfection in Croatia: a case report

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## Abstract

The brucellosis and Q-fever coinfection is very rarely reported. To our knowledge, this is the first case report of concomitant brucellosis and Q-fever, most likely imported in Croatia. A 30-year-old male agricultural worker was hospitalized on 22 April 2017 after a ten days fever up to 40°C with chills, shivering, excessive sweating, general weakness, loss of appetite and headache. A month and a half prior to the hospitalization he lost 18 kg of body weight. Three weeks before hospitalization the patient returned from Kupres (Bosnia and Herzegovina) where he was working for the past year on a sheep farm and consumed unpasteurized dairy products of sheep origin. At admission, his condition was moderately severe due to pronounced dehydration. Routine laboratory tests showed slightly elevated erythrocyte sedimentation rate, anemia, thrombocytopenia and elevated liver transaminases. The chest X-ray showed an inhomogeneous infiltrate of the lower right lung. Three sets of blood culture were cultivated. After 48 hours incubation, bacterial growth was detected in aerobic bottles. Gram-stained smear revealed small, gram-negative coccobacilli. Specimens were subcultured on blood and chocolate agar plates. Using a Vitek GN identification card, the isolated organism was identified as *Brucella melitensis*. 16S rRNA gene sequencing of the isolate confirmed it as a *Brucella* sp. Rose-Bengal test was positive, while Wright agglutination test showed a significant increase in antibody titer from 80 to 640 in paired sera. Using indirect immunofluorescence assay (IFA), *Coxiella burnetii* phase II IgM/IgG titers were 50 and 1024, respectively indicating acute Q-fever. The patient was treated with doxycycline and rifampicin. So far, there has been no relapse or signs of chronic infection.

**Key words:** brucellosis; Q-fever; coinfection; Croatia.

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## Introduction

Brucellosis is a zoonosis attributed to at least four *Brucella* species in terrestrial mammals: *B. melitensis, B. abortus, B. suis* and *B. canis*. *B. melitensis* is the most widespread and the most virulent. Reservoirs of *B. melitensis* are sheep and goats, but other species including dogs and cattle may also be infected [1]. Infection is transmitted to humans by inoculation through skin cuts and abrasions, inhalation of contaminated aerosols, conjunctival inoculation or ingestion of contaminated milk and dairy products [2]. Brucellosis is a systemic infection with a broad clinical spectrum, from asymptomatic to severe and sometimes fatal disease [3,4]. In Croatia, brucellosis was first recorded in Istria in 1947. During 1940-1950s, it was known to be endemic in many parts of Istria, but thereafter only sporadic cases were recorded until 1980. From 1981 to 1994, a total of 45 cases of brucellosis were reported in northern Croatia with no further cases in the period 1995-2003. During 2004, two sporadic cases and one family outbreak occurred in Dalmatia. In the last decade (2006-2016), a total of 39 cases of human brucellosis were notified in north-western and southern coastal counties (data from the Reference Center for Epidemiology Ministry of Health Republic of Croatia, Croatian Institute of Public Health; CIPH).

Q-fever is a worldwide zoonosis caused by *Coxiella burnetii*. The majority of human infections occur by inhalation of contaminated aerosols mainly from infected sheep and goats, however transmission by

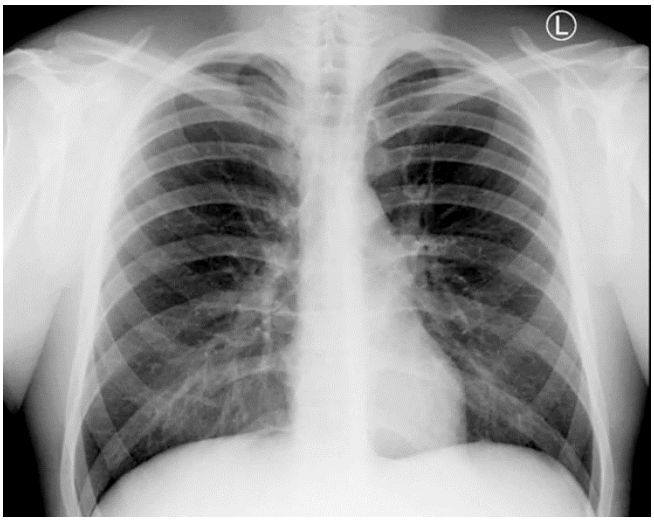
ingestion (mainly drinking raw milk from infected animals) is possible as well [5]. In humans, Q-fever is usually an asymptomatic or nonspecific, flu-like disease with spontaneous recovery, however, atypical pneumonia may occur. A small proportion of patients, especially immunocompromised persons may develop chronic form of disease. Hepatitis, endocarditis or meningitis are among complications observed in the rare chronic course of the disease, with a mortality rate of up to 65%, if untreated, in confront of 1-2% in the acute form [6]. Q-fever still represents an emerging or re-emerging disease in many parts of the world [7,8]. After the first description in 1950, sporadic cases as well as minor or major outbreaks were continuously reported in Croatia. Several large outbreaks were recorded in 1980s (1983-1985), 1991 and 2000s. In 2003 and 2004, two outbreaks with 206 and 104 cases were notified in the Zadar region (middle Dalmatia) [9]. In the last few years, sporadic cases were reported in the coastal Croatian counties with one small cluster (11 cases) in 2017 (data from the Reference Center for Epidemiology Ministry of Health Republic of Croatia, CIPH). In addition, high seropositivity rates to *C. burnetii* from 21.2% to 41.2% were detected in many regions indicating that Q-fever is widespread in Croatia [10].

Data on concomitant brucellosis and Q-fever are very scarce [11]. Herein we report the first case of imported *B. melitensis* and *C. burnetii* co-infection most likely imported in Croatia.

## Case presentation

A 30-year-old previously healthy male agricultural worker was hospitalized at the Clinic for Infectious Diseases at the University Hospital Centre Osijek on 22 April 2017 on the tenth day of the illnesses. The disease was manifested by daily fever up to 40°C with chills and shivering, excessive sweating mostly in the morning, general weakness, loss of appetite, headache which was relieved with analgesics and the occasional arthralgia in the knees and ankles. A month and a half prior to the hospitalization he lost 18 kg of body weight. The epidemiological history revealed that the patient returned from Kupres (southern Bosnia and Herzegovina) three weeks before hospitalization, where he was working for the past year on a sheep farm. During that period he had direct contact with sheep and consumed unpasteurized dairy products of sheep origin.

**Figure 1.** Chest radiograph at admission: inhomogeneous right lower lobe infiltrate.



At the admission, he was febrile (38.8°C), with normal vital functions, but his general condition was moderately severe due to pronounced dehydration. Physical examination was normal. Routine laboratory

**Table 1.** Laboratory results in patient with brucellosis and Q-fever coinfection.

| Parameter | Value | Reference range |
|---|---|---|
| Erythrocyte sedimentation rate (mm/h) | 30 | 2-13 |
| CRP (mg/L) | 53.4 | < 5.0 |
| WBC ($\times 10^9$/L) | 5.6 | 3.4 - 9.7 |
| Platelets ($\times 10^9$/L) | 129 | 158 - 424 |
| RBC ($\times 10^{12}$/L) | 4.28 | 4.34 - 5.72 |
| Hemoglobin (g/L) | 134 | 138 - 175 |
| Hematocrit (L/L) | 0.387 | 0.415 - 0.530 |
| Urea (mmol/L) | 3.4 | 2.8 - 8.3 |
| Creatinine (μmol/L) | 98 | 49 - 97 |
| Bilirubin (μmol/L) | 10 | 3 - 20 |
| AST (U/L) | 99 | 11 - 38 |
| ALT (U/L) | 127 | 12 - 48 |
| GGT (U/L) | 86 | 11 - 55 |
| LDH (U/L) | 550 | 0 - 241 |

CRP = C-reactive protein, WBC = white blood cells, RBC = red blood cells, AST = aspartate-aminotransferase, ALT = alanine-aminotransferase, GGT = gamma-glutamyltransferase, LDH = lactate dehydrogenase.

tests are presented in Table 1. The chest X-ray showed an inhomogeneous infiltrate in the lower right lobe (Figure 1). The abdominal ultrasound showed a marginally enlarged liver. The electrocardiogram was normal.

Taking the clinical picture and the epidemiological history (contact with sheep) into account, brucellosis and Q-fever were suspected.

During the hospitalization, the patient was initially treated with moxifloxacin (400 mg intravenously). After completion of microbiology results, a targeted antimicrobial therapy was given in accordance with the antibiogram: doxycycline (100 mg twice daily) with rifampicin (600 mg once daily) orally. On the third day of the hospitalization, the patient became afebrile with a gradual regression of other symptoms. The control chest X-ray performed on day 7 of hospitalization showed a complete regression of the inflammatory infiltrate of the lung.

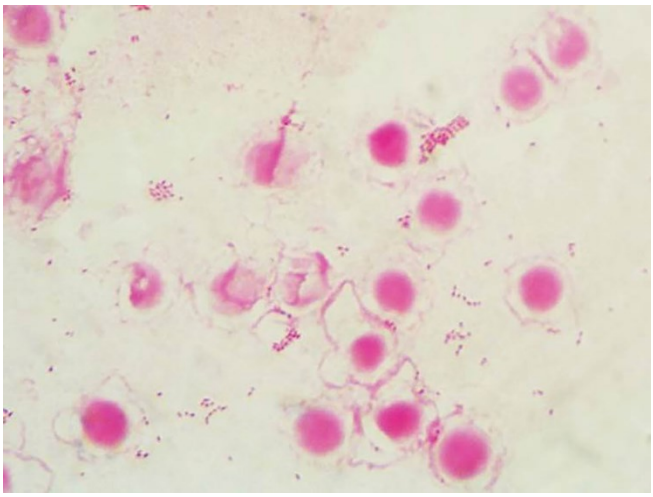
The C-reactive protein normalization followed within a week, and the normalization of the liver enzymes, gamma-glutamyltransferase, lactate dehydrogenase, erythrocytes and hemoglobin within eight weeks.

After 14 days of hospitalization, the patient was released home with prescribed therapy of doxycycline 100 mg twice daily and rifampicin 600 mg once daily mg for a total duration of six weeks. The patient was monitored during the six months. Laboratory parameters and electrocardiogram were examined monthly. The chest X-ray and the abdominal ultrasound were performed once. There was no relapse.

*Microbiology results*

Three sets of blood culture were cultivated using an automated blood culture system (Becton Dickinson, Sparks, USA). After 48 hours incubation, bacterial growth was detected in aerobic bottles. Gram-stained smear revealed small, Gram-negative coccobacilli (Figure 2). Specimens were subcultured on blood and chocolate agar plates. After 48 hours of aerobic incubation, small, smooth and glistening colonies were visible. Biochemical reactions showed positive urease, catalase and oxidase tests. The isolated strain did not ferment glucose and lactose or produce $H_2S$.

**Figure 2.** Blood culture (Gram stain): small, Gram-negative coccobacilli.



The isolated organism was identified as *B. melitensis* using a GN identification card Vitek Compact 2 (bioMerieux, Durham, USA). Subsequently, 16S rRNA gene sequencing using a BigDye Terminator v1.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Applied Biosystems, Austin, Texas, USA) of the isolate confirmed it as a *Brucella* sp. Obtained sequence (GenBank accession number MH217586) was compared to previously deposited 16S rRNA sequences using BLAST; it showed 100% identity to several

**Table 2.** Results of *Brucella* spp. and *Coxiella burnetii* serology testing.

| Day tested | Brucella (Rose Bengal test) | Brucella (Wright test)[a] | *C. burnetii* IFA IgM[b] (phase II) | *C. burnetii* IFA IgG[c] (phase II) | *C. burnetii* ELISA IgM[d] (phase II) | *C. burnetii* ELISA IgG[e] (phase II) | *C. burnetii* ELISA IgA[d] (phase I) | *C. burnetii* ELISA IgG[d] (phase I) |
|---|---|---|---|---|---|---|---|---|
| 18 | Positive | 80 | Positive (50) | Positive (1024) | NT | NT | NT | NT |
| 37 | Positive | 640 | NT | NT | NT | NT | NT | NT |
| 39 | NT | NT | NT | NT | Positive (4.2) | Positive (68) | Equivocal (1.0) | Positive (1.5) |
| 46 | NT | NT | Negative | Positive (512) | NT | NT | NT | NT |
| 81 | NT | NT | NT | NT | Positive (1.8) | Positive (31) | Negative | Equivocal (0.9) |
| 110 | Positive | 320 | NT | NT | NT | NT | NT | NT |

Reference values: [a]Titer < 80 negative; [b]Titer < 12 negative; [c]Titer < 64 negative; [d]Index < 0.9 negative; ≥ 1.1 positive; [e]U/mL < 20 negative, > 30 positive; NT = not tested.

species of the genus *Brucella*, among others also *B. melitensis* (http://www.ncbi.nlm.nih.gov/GenBank/).

Results of *Brucella* spp. and *C. burnetii* serology testing are presented in Table 2. Spot agglutination test for brucella (Rose-Bengal, Biorad) was positive, while Wright agglutination test showed a significant increase in antibody titer from 80 to 640 in paired sera samples (tested on days 18 and 37, respectively). Using IFA (Vircell, Granada, Spain), *C. burnetii* phase II IgM/IgG titers (day 18) were 50 and 1024, respectively indicating acute Q-fever. *C. burnetii* phase II antibodies tested by ELISA (Institut Virion/Serion GmbH; Wurzburg, Germany) on days 39 and 81 showed IgM decrease from index 4.2 to 1.8 and IgG decrease from 68 U/mL to 31 U/mL.

Viral markers of hepatitis C, hepatitis B and HIV were negative.

## Discussion

The brucellosis and Q-fever coinfection is very rarely reported. To our knowledge, the only one study conducted in Afghanistan (Bamyan province) during the outbreak of brucellosis from May 2011 to the end of 2012 reported concurrent brucellosis and Q-fever infections [11].

Epidemiological data (working on a sheep farm, direct contact with sheep, consumption of unpasteurized dairy products of sheep origin) as well as clinical symptoms in the patient presented in this report were suggestive of concomitant brucellosis and Q-fever. Constitutional symptoms such as anorexia, asthenia, fatigue, weakness and excessive weight loss were indicative of brucellosis [3]. Although a variety of pulmonary complications have been reported in brucella infections including interstitial pneumonitis and bronchopneumonia, the frequency of such complications is low [12,13]. In contrast, pneumonia occurs in almost half of the patients with acute *C. burnetii* infection [14]. In some Q-fever outbreaks, even higher frequency of pneumonia was reported (86% in The Netherlands) [15]. The finding of a pulmonary infiltrate on chest radiograph in our patient suggested acute Q-fever.

Although a very low level of cross-reactivity between *Brucella* and *Coxiella* has been described (10%) [16], cross-reactive antibodies were excluded. CDC case definitions were used as criteria for diagnosing both diseases. An eight-fold increase in brucella agglutinating antibodies in paired sera samples (80 and 640, respectively) in addition to positive PCR and cultivation confirmed acute brucellosis [17]. *C. burnetii* IgG titer of 1024 and IgM antibody titer of 50 to phase II antigen confirmed acute Q-fever [18] which was further supported by antibody kinetics in ELISA consecutive testing.

Since brucellosis and Q-fever are zoonoses which have the same reservoir and similar routes of infection, the case presented in this report highlights the need for awareness of possible brucellosis and Q-fever coinfection, especially in professionally exposed persons.

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