

## Case Report

# Brucellosis complicating chronic non-infectious disorders: diagnostic and therapeutic dilemmas

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There is little information in the literature on the clinical progress of brucellosis in patients affected by other non-infectious diseases; however, the infection can often trigger an exacerbation of existing underlying conditions in certain target organs. In this report we present four cases of brucellosis complicating previous diseases, and the difficulties in relation to their diagnosis and treatment. The study involved four patients with the following disorders: polycythaemia vera, pulmonary fibrosis, cirrhosis of the liver and arthritis of the knee. Brucellosis was diagnosed by classical serological and bacteriological methods. The strains involved could be isolated only in three of the four patients: two strains were *Brucella abortus* biovar 1 and one was *Brucella suis* biovar 1. Two patients relapsed 10 and 7 months after admission, another presented chronic brucellosis and received various therapy schemes, and one died. Since the best selection of antibiotics and the optimal duration of therapy remain unknown for patients having brucellosis complicated by previous pathologies, these remain at the discretion of the attending physician. Management of our patients was controversial in terms of the selection of antibiotics, duration of treatment and decision regarding surgery.

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## Introduction

The clinical features of human brucellosis make diagnosis difficult because of their protean manifestations; in addition to non-specific symptoms, another characteristic of the disease is the paucity of physical signs. However, fever may spike and be accompanied by rigors if bacteraemia is present, and may be relapsing, mild or protracted. This disease may lead to changes in haematological parameters, although it has been stated that routine laboratory tests are generally not helpful for diagnosis, except that the white blood cell count is often normal or low (Young, 1995). Physical signs are generally non-specific, although lymphadenopathy, hepatomegaly or splenomegaly is often found (Pappas *et al.*, 2005). Common presentations such as osteoarticular (Ariza *et al.*, 1993; Solera *et al.*, 1999) and hepatosplenic involvement have been described (Cervantes *et al.*, 1982; Akritidis & Pappas, 2001). Some authors have considered abscess

production to be exceptional (Ariza *et al.*, 2001), while respiratory system complications are acknowledged but considered to be rare (Pappas *et al.*, 2003). Bone and joint involvement includes arthritis, spondylitis, osteomyelitis, tenosynovitis and bursitis (Weil *et al.*, 2003).

There is little information in the literature regarding the clinical progress of brucellosis in patients affected with other diseases; however, this infection can often trigger an exacerbation of existing underlying conditions in certain target organs (Akritidis & Pappas, 2001). The objective of this report is to present four cases where brucellosis complicated previous diseases that were followed up over an extended period of time, and to discuss the difficulties in relation to treatment, as well as diagnostic problems.

## Case reports

### Case 1

A 52-year-old man, a livestock breeder living in a rural area, had a previous history of polycythaemia vera (PV) of 12 years

Abbreviations: CELISA, competitive ELISA; FPA, fluorescence polarization assay; Hb, haemoglobin; OA, osteoarthritis; PF, pulmonary fibrosis; PV, polycythaemia vera.

duration. He had been treated with 1 g hydroxyurea once a day for 1 year and had a haemoglobin (Hb) level of 15 g dl<sup>-1</sup> before assisting a pregnant cow with brucellosis and coming into contact with fetal membranes without protection. One month after exposure he sought medical treatment for asthenia, generalized arthralgia, myalgia, fever, and paleness of skin and mucous membranes. There were no changes in spleen size (splenomegaly was detected when he was diagnosed with PV). At that time, his laboratory results were 11.2 g Hb dl<sup>-1</sup> and 113 fl mean corpuscular volume, with normal leukocyte and platelet counts. The reticulocyte percentage was 14 %, 49.59 µmol total serum bilirubin l<sup>-1</sup>, 11.97 µmol direct bilirubin l<sup>-1</sup>, 19.98 µkat lactic acid dehydrogenase l<sup>-1</sup>, <5 mg haptoglobin ml<sup>-1</sup>. Direct and indirect Coombs' tests were negative. Alpha- and beta-thalassaemias were discounted by DNA sequencing. The expression of glycosylphosphatidylinositol-linked proteins (CD59, CD58 and CD14) performed on granulocytes by flow cytometry required for the diagnosis of paroxysmal nocturnal haemoglobinuria was normal. Our patient was not medicated with any drug other than hydroxyurea. Glucose-6-phosphate dehydrogenase deficiency, and related deficiencies involving the pentose phosphate pathway and glutathione metabolism, were not checked because the patient had no history of these pathologies and no previous evidence of anaemia or haemolytic events and Heinz bodies were not observed in blood smears. No schistocytes were found in blood smears.

Bone marrow aspiration showed erythroid hyperplasia and the bone marrow biopsy revealed an absence of erythrophagocytosis and granulomas. There was no increased marrow reticulin. Serological tests to detect antibodies to *Mycoplasma pneumoniae* and Epstein-Barr virus were negative, but tested positive to brucellosis, and *Brucella abortus* biovar 1 was isolated from the blood cultures. Treatment with hydroxyurea was discontinued.

### Case 2

A 64-year-old-man who had worked for over 40 years in a pig abattoir was hospitalized with a diagnosis of pulmonary fibrosis (PF). The patient presented with pain in the lower back and a sacroiliac abscess on which a biopsy of the involved tissue and surgical drainage were performed, and pieces of adjacent muscle and bone extracted. One part of these samples was spread directly onto solid medium and another part inoculated into liquid medium. Forty-four hours later, the bone sample showed growth of *Brucella suis* biovar 1 on both solid and liquid media. At this time brucellosis serology was tested with positive results.

### Case 3

A 41-year-old man was hospitalized with cirrhosis, ascites and oesophageal varices grade I, and evaluated for liver transplant. The patient presented symptoms of asthenia, generalized myalgia, weight loss, anorexia, night sweat and fever over the previous 6 months. He remembered having

had a brucellosis infection 18 years earlier, and having been treated with tetracycline and streptomycin for 2 months. He also reported a history of tuberculosis in 1978, for which he recalled receiving a proper course of treatment. The patient also affirmed alcohol and tobacco consumption for the previous 27 years. Brucellosis serological tests were positive and a liver biopsy was performed, but *Brucella* sp. could not be isolated.

### Case 4

A 58-year-old man who worked in a cow slaughterhouse had previous chronic osteoarthritis (OA) in the left knee. At the time of the consultation, the patient self-reported limitations in his activities, such as difficulties in walking, standing up from a seated position and climbing stairs. He presented symptoms of malaise, generalized myalgia, fever and sweats. At that time he had pain, tenderness and swelling in the left knee. Joint effusion was diagnosed by puncture and the sample cultured yielded *B. abortus* biovar 1. Additional imaging studies were performed and serological tests for brucellosis were positive.

### Treatment

For all four patients the initial treatment approach was not standardized but most cases received 1 g intra-muscular streptomycin once a day for 15 days and 100 mg oral doxycycline twice a day for 45 days. During the longer term treatment period the patients received different schedules of antibiotics.

### Microbiological methods

Serological tests were carried out on serum samples obtained from the patients. The buffered plate agglutination test, Rose Bengal test, standard tube agglutination test, 2-mercapto-ethanol test and complement fixation test (Lucero & Bolpe, 1998) used antigens prepared at the National Administration of Laboratories and Health Institutes Dr C. G. Malbrán (ANLIS) with *B. abortus* 1119-3 strain. Competitive ELISA (CELISA) was carried out as previously reported (Lucero *et al.*, 1999); the antigen (S-LPS from *B. abortus* 1119-3) and the mAb were standardized and supplied by the Brucellosis Centre of Expertise and OIE Reference Laboratory, Animal Diseases Research Institute (ADRI), Canada. The test was considered positive when %I is >28 (Lucero *et al.*, 1999, 2007).

Fluorescence polarization assays (FPA) were run with antigen supplied by the National Atomic Energy Commission, Argentina, as previously reported (Lucero *et al.*, 2003). The test was considered positive when the millipolarization unit value was >72.

Bacteriological studies were performed following published procedures and typed *Brucella* strains of each species were included in all tests (Corbel & Brinley-Morgan, 1984; Alton *et al.*, 1988).

## Discussion

A high degree of suspicion is needed to make the correct clinical diagnosis of brucellosis because of the vagueness of the systemic features. A presumptive diagnosis may be made by demonstrating specific antibodies in the serum, but isolation of the causal agent from blood, bone marrow or other tissues is the determinant. Diagnosis is more complicated when the patient presents symptoms stemming from other causes preceding the development of the infection. By examining the records of patients studied at ANLIS after the implementation of the National Human Brucellosis Network, we selected four cases of patients with PV, hepatic cirrhosis, arthritis of the knee or PF previous to brucellosis infection (Table 1).

Following serological results, the patient in case 1 received the therapy described in Table 2. After 8 weeks of treatment his Hb level was high and hydroxyurea treatment was successfully reinstated at the usual dose. Ten months later he relapsed with mild haemolytic anaemia (direct and indirect Coombs' tests were negative, indirect bilirubin and lactic acid dehydrogenase levels were high). Treatment with hydroxyurea was suspended, and brucellosis serology and bacteriology studies were repeated. CELISA indicated titre persistence, but the FPA showed error and could not be read, while the blood culture was negative. The patient was retreated for 3 months with rifampicin, ciprofloxacin and doxycycline, and recovered his usual Hb level. Treatment with hydroxyurea was resumed, to which he responded satisfactorily. A review of a large series of cases of brucellosis revealed that anaemia is not uncommon in this disease but that if present it is usually mild. Haemolytic anaemia is infrequent and leucopenia occurs in 20 % of patients as a result of decreased numbers of granulocytes. Absolute lymphocytosis is also found but thrombocytopenia is rare (1–8 %) (Troy *et al.*, 2005; Mousa *et al.*, 1987; Franz *et al.*, 1997; Colmenero *et al.*, 2002; Buchanan *et al.*, 1974). Pancytopenia attributed to excessive peripheral destruction of blood cells, hypersplenism and bone marrow involvement have been reported (Pappas *et al.*, 2005). Rarely, marked pancytopenia, haemolytic uraemic syndrome and severe microangiopathic haemolytic anaemia have also been associated with brucellosis (Young *et al.*, 2000; Söker *et al.*, 2001; Pappas *et al.*, 2004; Altuntas & Eser, 2005).

This case of brucellosis in a patient with PV is the first that we have diagnosed, and we are not aware of any previous

reports of this infection associated with PV and haemolytic anaemia. We consider that the anaemia could be associated with the underlying illness, but autoimmune abnormalities such as auto-antibodies, immune complexes, antinuclear antibodies, or a positive Coomb's test are not frequently described in patients with PV as they are in patients with idiopathic myelofibrosis. Autoimmune haemolytic anaemia (AIHA) and PV are a rare association. The negative direct and indirect Coomb's tests excluded this diagnosis in our patient. AIHA has been reported in infections due to *M. pneumoniae* and Epstein–Barr virus but our patient had negative serological tests for both agents. The hydroxyurea was not thought to be implicated as the cause of the anaemia because haemolytic anaemia is not mentioned among its adverse reactions. The pathogenesis of haemolytic anaemia is poorly understood. *Brucella* sp. would, through a direct effect or through the liberation of cytokines, be able to produce the lysis of red cells. Clearance of antigen by immune-complex formation is a highly effective mechanism of host defence. However, depending on the level of immune complexes and their physico-chemical properties, immune complexes may result in cell damage. This situation and/or the liberation of endotoxins could also explain the haemolysis described in this patient. We consider the anaemia directly related to the infectious process because the patient recovered after antimicrobial therapy.

The serology of case 2 indicated persistent titres in CELISA and FPA tests compatible with the chronic form of the disease. The patient was initially treated with doxycycline and rifampicin, but on day 6, due to drug intolerance, the latter was replaced by ciprofloxacin (Table 2). He completed the 45 day treatment, but died 8 months later. The cause of death was abdominal perforation. Large studies of patients with brucellosis have only occasionally focused on the respiratory system, with an estimated rate of involvement of <1–5 % (Lubani *et al.*, 1989). However, the incidence of respiratory involvement has been reported to approach 7 % (Pappas *et al.*, 2003). Moreover, pulmonary complications are rarely serious and respond to standard treatment for brucellosis. Traditional theories have postulated that they might be an autoimmune disorder or after effects of an infection. While the exact cause remains unknown this patient who had been working in a pig abattoir for 40 years could have been infected previously with brucellosis or PF could have been exacerbated by a latent infection. Because the origin and development of PF

**Table 1.** Cases of brucellosis in humans with other pre-existing pathologies

Case	Age (years)/sex	Previous illness	Clinical form	Brucellosis serology	Culture source	<i>Brucella</i> sp. isolated
1	52/male	Polycythaemia vera	Bacteraemic	Positive	Blood culture	<i>B. abortus</i> biovar 1
2	64/male	Pulmonary fibrosis	Localized	Positive	Bone sample	<i>B. suis</i> biovar 1
3	41/male	Cirrhosis and oesophageal varices	Chronic	Positive	Liver biopsy/blood/abscesses	Negative
4	58/male	Arthropathy in the knee	Localized	Positive	Synovial fluid	<i>B. abortus</i> biovar 1

**Table 2.** Treatment administered for the patients

Patient	Time since initial admission	Treatment/dose	Period
1	0	1000 mg hy once a day	12 months
	12 months	100 mg dox twice a day + 300 mg rif three times a day + 750 mg ci twice a day	8 weeks
	14 months	1000 mg hy once a day	10 months
	24 months	100 mg dox twice a day + 300 mg rif three times a day + 750 mg ci twice a day	3 months
	27 months	1000 mg hy once a day	
2	0	100 mg dox twice a day + 300 mg rif three times a day	6 days
	6 days	750 mg ci twice a day + 300 mg rif three times a day	45 days
3	0	100 mg dox twice a day + 300 mg rif three times a day + 750 mg ci twice a day	3 months
	12 months	200 mg dox twice a day + 1 g st once a day + 750 mg ci twice a day	3 months
	24 months	200 mg dox twice a day + 160–800 mg TS three times a day + 750 mg ci twice a day	3 months
	36 months	100 mg dox twice a day + 300 mg rif three times a day + 750 mg ci twice a day	3 months
	48 months	100 mg dox twice a day + 300 mg rif three times a day + 160–800 mg TS three times a day	3 months
4	60 months	200 mg dox twice a day + 1 g st once a day + 750 mg ci twice a day	3 months
	0	100 mg dox twice a day + 300 mg rif three times a day	50 days
	9 months	100 mg dox twice a day + 300 mg rif three times a day + 1 g st once a day for the first 15 days	90 days
	21 months	100 mg dox twice a day + 300 mg rif three times a day + 750 mg ci twice a day	45 days

ci, Ciprofloxacin; dox, doxycycline; hy, hydroxyurea; rif, rifampicin; st, streptomycin; TS, trimethoprim-sulfamethoxazole.

is not completely understood, misdiagnosis is common. Patients with PF sometimes receive systemic corticosteroids and non-steroid anti-inflammatory drugs that could mask signs of infection (Duyur *et al.*, 2001). The potency of glucocorticoids as inhibitors of diverse inflammatory disorders guarantees their continued use as therapeutic agents. However, because some of these mechanisms are also involved in physiological signalling rather than inflammation, the therapeutic effects of glucocorticoids in inflammation are often accompanied by clinically significant side effects (Rhen & Cidlowski, 2005).

The patient in case 3 had sterile blood, liver biopsies and abscess cultures set up (Table 1), and was initially administered doxycycline, rifampicin and ciprofloxacin (Table 2). Splenomegaly without ascites was observed. One year after admission an abscess in the right hypochondrium was drained and the purulent material was studied in respect of bacteriology, fungi and mycobacteria with negative results. The patient received another five therapeutic treatments over the last 5 years due to the recurrence of the infection (Table 2). Because of multiple hepatosplenic lesions no surgical procedure for drainage of collected depth fluid was possible (Fig. 1). Liver enlargement and/or a mild non-specific increase in liver enzyme levels caused by non-specific or granulomatous hepatitis may be detected in about 50 % of patients with brucellosis. Troy *et al.* (2005), observed that more than half of 28 patients had an elevated liver enzyme profile. However, hepatic abscess as a complication of acute brucellosis is exceptional (Cervantes *et al.*, 1982; Williams & Crossley, 1982). Clinical manifestations, clinical progress and radiological studies of this patient were consistent with chronic hepatosplenic suppurative brucellosis. Histological

findings in these patients have been reported to range from cellular infiltration of lymphocytes or polymorphonuclear leukocytes to granulomas and abscesses (Akritidis *et al.*, 2007). Cirrhosis as a result of hepatic lesions caused by brucellosis has been reported but its role in the genesis of hepatic fibrosis has not been demonstrated. Hepatic cirrhosis caused by alcohol, viral hepatitis or autoimmune disorders has been associated with cases of brucellosis complicated by spontaneous bacterial peritonitis, vasculitis and sepsis. But when cirrhosis is prior to brucellosis infection the latter may be the cause of hepatic disorders



**Fig. 1.** Abdominal computed tomography of patient 3 shows the liver with irregular edges, and the left lobe with hypertrophy and hepatosplenomegaly with multiple calcium intraparenchymatous lesions.



(Dizbay *et al.*, 2007; Colmenero *et al.*, 1996; Khateeb *et al.*, 1990).

The patient in case 4 was treated with rifampicin and doxycycline for 50 days after *B. abortus* biovar 1 was isolated from the synovial fluid of the left knee (Table 1). Seven months later (at 9 months after admission) he relapsed and a second series of rifampicin and doxycycline was prescribed for 90 days; however, for the first 15 days streptomycin was also used (Table 2). After the second therapy he felt much better. Twelve months later because of the persistence of the CELISA titre he was treated again with rifampicin/doxycycline and ciprofloxacin for 45 days, and also 150 mg ranitidine every 12 h. A scintigraphical scan with <sup>67</sup>Ga gallium citrate showed a slight increase in radiolabel uptake of the left knee unrelated to the infection. Osteoarticular presentations are sometimes linked to a genetic predisposition with recent data suggesting an association with HLA-B39 (Bravo *et al.*, 2003). Arthritis involving the large joints is the most prevalent clinical form of osteoarticular complication of brucellosis (Ariza *et al.*, 1993; Solera *et al.*, 1999). Peripheral arthritis is the most common complication of brucellosis; it is non-erosive since it usually involves the knees, hips, ankles and wrists in the context of acute infection (Pappas *et al.*, 2005). Mousa *et al.* (1987) and Khateeb *et al.* (1990) studied 169 and 96 cases of brucellosis, respectively, and found 36 and 25 % with arthritis. Previous joint lesions as factors that could determine this particular brucellosis localization were not mentioned in either report. Our patient's symptoms appeared immediately following his activities in a cow slaughterhouse, but he had OA of the left knee several years before this infectious process. He made a good recovery and remains healthy 1 year after the last treatment. The relapse was probably due to the length of treatment, longer courses of between 3 and 5 months have been suggested (Alp *et al.*, 2006).

We are not aware of any previous reports of brucellosis complicating cases of PV and haemolytic anaemia, PF, cirrhosis of the liver or OA of the knee. Classical serological tests were not useful for the diagnosis of the patients involved and have added little information regarding clinical progress of the disease and its response to treatment.

There is little information concerning management of patients having brucellosis complicating previous pathologies. The most appropriate selection of antibiotics and the optimal duration of therapy remain unknown and the literature demonstrates that choices in this regard remain at the discretion of the attending physician. Our cases 1 and 4 could be considered acute infections, while cases 2 and 3 would be chronic. Management of our patients was controversial with regard to the selection of antibiotics, duration of treatment and the decision with respect to surgery in the case of patient 3. The total duration of antibiotic therapy necessary for eradication of infection in such special situations is unknown.

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