

Case Report

Unusual clinical presentation of brucellosis caused by *Brucella canis*

Nidia E. Lucero,¹ Nestor O. Jacob,² Sandra M. Ayala,¹ Gabriela I. Escobar,¹ Patricia Tuccillo³ and Isabelle Jacques⁴

Correspondence
Nidia E. Lucero
nidia@elsitio.net

¹Brucellosis Laboratory, Administración Nacional de Laboratorios e Institutos de Salud Dr C. G. Malbrán (ANLIS), Avda. Velez Sarsfield 563, 1281 Buenos Aires, Argentina

²Unidad de Transplante Renal, Hospital Cosme Argerich, Margall 750, 1155 Buenos Aires, Argentina

³Hospital Naval 'Pedro Mallo', Patricias Argentinas 351, 1405 Buenos Aires, Argentina

⁴Laboratoire de Pathologie Infectieuse et Immunologie, Institut National de la Recherche Agronomique, 37380 Nouzilly, France

Brucella canis is considered a rare cause of human brucellosis. The clinical importance of this infection may have been underestimated so far because of difficulties with presumptive diagnosis. The case described here presented symptoms compatible with brucellosis but the routine tests using *Brucella abortus* antigen were negative. The infection would have remained undiagnosed if culture had not been positive. This case illustrates the potential for a favourable outcome in *Brucella canis* diagnosis and supports recommendations for the use of *B. canis* serology. The infection should be suspected in patients with compatible symptoms and negative serology for *B. abortus* antigen.

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Introduction

Although brucellosis is a worldwide zoonosis, it predominates in Mediterranean countries, the Middle East and Latin America. The *Brucella* species that are frequently associated with human brucellosis are *Brucella melitensis*, *Brucella suis* and *Brucella abortus*. *Brucella canis* is considered a rare cause of human brucellosis (Young, 1983); the most common type of contagion is through contact with infected dogs or their secretions. Dogs infected with *B. canis* appear to be relatively healthy but persistent bacteraemia without fever or symptoms is common and the strain may remain in the tissues for a long time (Carmichael & Shin, 1996).

Human *B. canis* infection is infrequently recognized, probably due to the lack of serious consideration to the disease as a diagnostic possibility. Another limiting factor is the general unavailability of the specific serological tests needed in the absence of cross-reactivity between antibodies to *B. canis* and smooth *Brucella* species pathogenic to humans. *Brucella* species with smooth surface antigens react in agglutination tests with antibodies against smooth *Brucella* cultures. Rough *Brucella* species such as *B. canis* are not agglutinated by anti-smooth sera but by anti-rough *Brucella* sera.

We present a case of infection with *B. canis* and describe serological tests that appear to be promising for presumptive diagnosis.

Case

Initially, a 15-year-old boy with oral lesions and fever up to 40 °C was empirically treated for 2 days with oral penicillin but this treatment was suspended because of an increase in levels of transaminase. After a week the patient worsened, with weakness, persistent fever, liver and spleen enlargement and submaxillary adenopathy, and was admitted to the hospital with a suspected diagnosis of cytomegalovirus (CMV) infection.

Routine laboratory tests, urinalysis, C-reactive protein, rheumatic factor, C3 and C4, were normal. Chest X-ray and echocardiography were also normal, but subsequent thoracic, abdominal and pelvic computer tomography showed spleen enlargement. Skin test with PPD 2 UT (purified protein derivate, 2 unit tuberculin) was negative, as were human immunodeficiency virus (HIV), hepatitis A virus, hepatitis B virus, Epstein–Barr virus and routine brucellosis serology tests, while IgM and IgG antibodies to CMV were positive. Pharyngeal swab, urine and blood cultures were performed on admission. Ten days later, a Gram-negative coccobacillus was obtained from blood cultures (Soloaga *et al.*, 2004), and treatment with ceftriaxone 2 g q.i.d. intra-venous was started and continued for 21

Abbreviations: CELISA, competitive ELISA; CMV, cytomegalovirus; IELISA, indirect ELISA; RSAT, rapid screening agglutination test.

days. The boy's fever subsided and he showed remarkable clinical improvement in 48 h. The strain isolated, presumptively identified, was sent to our laboratory, where it was confirmed as *B. canis*. The treatment was modified to doxycycline 200 mg b.i.d. per os and rifampicin 600 mg q.i.d. per os for 6 weeks.

The patient was discharged symptom-free from the hospital. Neither signs nor symptoms of relapse were detected during the follow-up period (8 months) in the outpatient service.

Methods

Bacteriological studies. The strain isolated from the patient was identified and typed by CO₂ requirement and its agglutination pattern with monospecific anti-A, anti-M and anti-R sera. *Brucella* cultures are smooth or rough and are agglutinated by their respective antisera. Cultures of the smooth form can be examined for their predominant agglutininogen A (*B. abortus* and *B. suis*) or M (*B. melitensis*) but cultures of the rough form are agglutinated by unabsorbed antisera prepared with *B. canis* or *Brucella ovis* cultures.

Tests for urease, production of H₂S, growth on dyes, erythritol and penicillin sensitivity, and lysis by Tb, Wb, Iz and R/C phages were performed (Table 2) following procedures described previously and including typed *Brucella* strains of each species in all tests (Corbel & Brinley-Morgan, 1984; Alton *et al.*, 1988). First the colonial morphology was studied by direct observation, acriflavine test and staining of colonies with crystal violet. Since biochemical tests were consistent with *B. canis*, molecular typing was performed in order to confirm these results (Vizcaino *et al.*, 1997).

Epidemiological data. Given these findings, the patient was questioned about exposure to dogs. He had three dogs, one of which was a stray. Clinical study, serum and blood samples were obtained through a veterinarian about 4 months after the initial diagnosis. At this time the stray was unavailable.

Serological tests. Serum samples from the patient and his dogs were obtained and serological tests for brucellosis were performed. The

buffered plate agglutination test, rose bengal test, plate agglutination test, tube agglutination test and complement fixation were performed (Lucero & Bolpe, 1998) using antigens prepared at ANLIS Dr C. G. Malbrán with *B. abortus* strain 1119-3. Competitive ELISA (CELISA) was done 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, Canada.

Rapid screening agglutination test (RSAT) was used as a screening test for the detection of anti-*B. canis* antibodies (Carmichael & Joubert, 1987), with serial dilutions in order to determine the final titre and

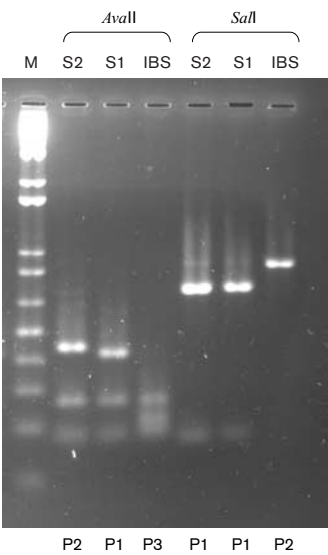


Fig. 1. Molecular typing of the isolated *Brucella* strain by PCR-RFLP of the *omp31* gene. Lanes: M, molecular size marker; S1, *B. suis* biovar 1; S2, *B. suis* biovar 2; IBS, isolated *Brucella* strain; P1, P2, P3, restriction patterns obtained with *Avall* and *Sall*.

Table 1. Serological results of tests on human and dog sera

BPA, Buffered plate agglutination; CELISA, competitive ELISA; CF, complement fixation test; IELISA, indirect ELISA; PAT, plate agglutination test; RB, rosa bengal; TAT, tube agglutination test.

| Sera | Time since first symptoms (months) | <i>B. abortus</i> antigen | | | | | | <i>B. canis</i> antigen | |
|-------|------------------------------------|---------------------------|-----|-----|-----|-----|--------------|-------------------------|--------------|
| | | PAT | BPA | RB | TAT | CF | CELISA* (%I) | RSAT† | IELISA‡ (%P) |
| Human | 2 | Neg | Neg | Neg | Neg | Neg | 11 | 32 | 87 |
| | 3 | Neg | Neg | Neg | Neg | Neg | 10 | 16 | 73 |
| | 8 | Neg | Neg | Neg | Neg | Neg | 17 | 2 | 36 |
| Dog 1 | 3 | | Neg | Neg | | | | 4 | 0·532 |
| | 8 | | Neg | Neg | | | | Pos+/- | 0·230 |
| Dog 2 | 3 | | Neg | Neg | | | | Pos+/- | 0·223 |
| | 8 | | Neg | Neg | | | | Neg | 0·153 |

*CELISA cut off %I > 28. %I = 100-[OD₄₁₄ of test sample/OD₄₁₄ of conjugate control]×100.
†Presented as reciprocal of titres. Pos+/-, weakly positive.
‡Dog IELISA cut off OD₄₁₄ > 0·281; Human IELISA cut off %P > 27 (Lucero *et al.*, 2005). %P = [OD₄₁₄ of the test sample/OD₄₁₄ of the control serum]×100.

including a control standard serum with a known titre. The antigen was prepared at ANLIS Dr C. G. Malbrán using the (M–) variant strain of *B. canis*.

Indirect ELISA (IELISA) with *B. canis* antigen was used as a confirmatory test for the detection of dog anti-*B. canis* antibodies (Lucero *et al.*, 2002), including positive, weak positive and negative sera as control. For the detection of human anti-*B. canis* antibodies a previously established cut-off value was used (Lucero *et al.*, 2005). A recombinant protein combining the immunoglobulin binding sites of proteins A and G conjugated with horseradish peroxidase was used for the assessment of antibodies to rough lipopolysaccharide in dogs and humans. The use of this conjugate has been suggested (Nielsen *et al.*, 2004) as a universal detection reagent for the diagnosis of brucellosis caused by smooth and rough *Brucella* species in sera from cattle, sheep, goats, dogs and pigs.

Results and Discussion

With PCR-RFLP on the *omp31* gene there is no possible confusion between *B. canis* strains and *B. suis* rough (Fig. 1). Using *Ava*II, strains of *B. canis* present only one pattern, P3, which is different from *B. suis*, which presents P1 or P2 patterns. Using *Sal*I, the isolated strain presents a P2 profile that is characteristic for some *B. canis* strains, whereas *B. suis* strains are all P1. Therefore, the combination of results using these two restriction enzymes confirmed that the strain was *B. canis* (Vizcaino *et al.*, 1997).

All the tests using *B. abortus* 1119-3 antigen were negative in the patient and his dogs, but when tests with *B. canis* antigen were used, the patient and dogs gave positive serology results, with titres declining over time (Table 1). CMV antibody detection became negative for IgM but remained positive for IgG antibody.

The clinical complaints and physical findings in human brucellosis are frequently non-specific, and this patient had the symptoms, i.e. fever and oral lesions at first, followed by enlargement of the spleen, for 1 month before diagnosis.

We have no documented information on the frequency of oral lesions in human brucellosis. Probably in this case this was due to CMV co-infection since at admission there were 4000 white blood cells per mm³ (40 % monocytes, 60 % lymphocytes), while the peripheral smear showed low hypochromia, normal platelets and lymphocytes but CMV cultures were not done. The patient's rapid improvement after ceftriaxone treatment can presumably be attributed to this drug's good *in vitro* activity against *Brucella* strains isolated in blood cultures (Bosch *et al.*, 1986), for which it has been considered a second-line therapy for brucellosis in patients unable to receive conventional therapy (al-Idrissi *et al.*, 1989).

The dogs were clinically healthy and their blood cultures were negative, but these were done 4 months after initial diagnosis and only for two of the three dogs since the stray, suspected of transmitting the infection, was unavailable. Five months later one of the dogs remained positive to RSAT (Table 1).

Standard agglutination tests for antibodies to *Brucella* generally use only *B. abortus* antigen, but since *B. canis* antibodies do not cross-react, it is necessary to do tests with

Table 2. Differential characteristics of species of the genus *Brucella*

| Strain | Colony morphology | CO ₂ requirement* | H ₂ S production* | Growth on media with*† | | | | | Agglutination in sera*‡ | | | Lysis by phages*\$ | | | | |
|---------------------------|-------------------|------------------------------|------------------------------|------------------------|------------------|----------------|------------------|----------------|-------------------------|-------|---|--------------------|----|----|----|-----|
| | | | | Thionin (a) | Basic fuchsin(a) | Safranin O (b) | i-Erythritol (c) | Penicillin (d) | Urease (e) | A M R | | | Tb | Wb | Iz | R/C |
| | | | | | | | | | | | | | | | | |
| IBS | Rough | — | — | + / — | + | + | + | + | ++ | — | — | + | — | — | — | + |
| <i>B. melitensis</i> 16M¶ | Smooth | — | — | + | + | + | + | + | + | — | + | — | — | — | + | — |
| <i>B. suis</i> 1330¶ | Smooth | — | + | + | — | — | — | + | ++ | + | — | — | — | + | + | — |
| <i>B. abortus</i> 544 | Smooth | + | + / — | — | + | + | + | + | — | + | — | — | + | + | + | — |
| <i>B. canis</i> RM6/66¶ | Rough | — | — | + | — | — | — | + | ++ | — | — | + | — | — | — | + |

*Symbols: –, negative; +/-, weakly positive; +, positive; ++, strong positive.

†(a) 20 µg ml⁻¹; (b) 100 µg ml⁻¹; (c) 1 mg ml⁻¹; (d) 5 IU ml⁻¹ in base medium; (e) Bauer's method.

‡A, A monospecific antiserum; M, M monospecific antiserum; R, rough *Brucella* antiserum.

§Performed at routine test dilution.

||Isolated *Brucella* strain.

¶Reference strain.

B. canis antigen. Because brucellosis has been associated with various clinical manifestations, it is important to use appropriate tests to clearly distinguish the species of infection.

In this report *B. canis* antigen clearly identified the infection and as a result, inclusion of *B. canis* serology tests in all patients with fever syndrome who have a previous negative screening test for brucellosis using *B. abortus* antigen is recommended. Infection due to *B. canis* is probably not rare, so these recommendations could help to reduce the possibility of an incorrect diagnosis.

Recently we surveyed dogs from an urban area in the course of a Neuter Program and 14% were RSAT positive and *B. canis* was isolated in 11.7% of cases (N. E. Lucero, G. I. Escobar, S. M. Ayala, & G. Lopez, unpublished data). These findings indicate that the disease may persist and that if the infected dogs continue to contaminate the environment, it could be a threat to public health.

It is likely that the full-spectrum pathogenic potential of *B. canis* will be increasingly recognized and that its epidemiology will be further elucidated as more cases are identified. The prevalence and clinical importance of *B. canis* may have been underestimated so far because of difficulties with primary isolation and differentiation. In this case, the infection would have remained undiagnosed if culture had not been positive. On the basis of this experience, serological tests, if used with proper controls, appear to be promising for diagnosis.

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