Unusual clinical presentation of brucellosis caused by *Brucella canis*

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*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.

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 cocobacillus 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
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 agglutinogen 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

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

<table>
<thead>
<tr>
<th>Sera</th>
<th>Time since first symptoms (months)</th>
<th><em>B. abortus</em> antigen</th>
<th><em>B. canis</em> antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PAT</td>
<td>BPA</td>
<td>RB</td>
</tr>
<tr>
<td>Human</td>
<td>2</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>Dog 1</td>
<td>3</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>Dog 2</td>
<td>3</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Neg</td>
<td>Neg</td>
</tr>
</tbody>
</table>

*CELISA cut off %I > 28. %I = 100−[OD₄₄₄ of test sample/OD₄₄₄ of conjugate control]×100.

†Presented as reciprocal of titres. Pos+/-, weakly positive.

‡Dog IELISA cut off OD₄₄₄ > 0.281; Human IELISA cut off %P > 27 (Lucero et al., 2005). %P = [OD₄₄₄ of the test sample/OD₄₄₄ 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 *Avai*II, strains of *B. canis* present only one pattern, P3, which is different from *B. suis*, which presents P1 or P2 patterns. Using *SalI*, 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-Iдрissi 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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Colony morphology</th>
<th>CO₂ requirement</th>
<th>H₂S production</th>
<th>Requirement</th>
<th>Lysis by phages §</th>
<th>Agglutination in sera †</th>
<th>Grotth on media with ‡</th>
<th>Growth on media with ‡</th>
<th>Thionin Basic Safranin</th>
<th>Urease</th>
<th>Penicillin</th>
<th>Fixed organisms</th>
<th>Flocculating organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. abortus</td>
<td>Smooth</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>B. melitensis 16M</td>
<td>Smooth</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>B. ovis 1330</td>
<td>Smooth</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>B. suis</td>
<td>Rough</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. suis RM66</td>
<td>Rough</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
</tbody>
</table>

†(a) 100 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. ¶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.

Acknowledgements

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References


