Case Report Diagnosis and follow-up of *Bartonella henselae* infection in the spleen of an immunocompetent patient by real-time quantitative PCR

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Systemic *Bartonella henselae* infections are unusual in immunocompetent adults. However, here we report one such case of bartonellosis in a 34-year-old patient, who presented with fever and multinodular splenomegaly. We also describe a novel method of identifying *Bartonella henselae* by real-time quantitative polymerase chain reaction and sequencing of amplified products. This could prevent splenic bartonellosis being mistaken for lymphoma and thereby avert unnecessary splenectomy.

Introduction

Cat-scratch disease (CSD) is a self-limiting regional lymphadenitis that typically follows a cat scratch or bite; however, in children, as well as immunocompetent adults, disseminated infections by *Bartonella henselae* associated with hepatosplenic manifestations have been described (Scolfaro *et al.*, 2008; Gilad *et al.*, 2003).

We report here an uncommon case of CSD featuring multinodular splenomegaly in an immunocompetent patient. Suspected bartonellosis was investigated by serology and diagnosis was confirmed by real-time PCR in the patient’s blood samples (Liberto *et al.*, 2011).

We also describe the follow-up of *B. henselae* DNA both during and after a course of combined antibiotics.

Case report

On 10 January 2011, a 34-year-old Caucasian woman, affected by mild fever (37.5 °C) followed after 3 days by neck pain with increasing fever throughout the evening, dry cough and epigastralgia, was admitted to her local hospital. Despite the persistence of symptoms, she was discharged with a diagnosis of ‘fever in a patient with multiple splenic nodules’. Subsequently, on 2 February 2011, she was referred to the Operational Unit of Internal Medicine at the University Hospital of Catanzaro, Italy, with suspected splenic lymphoma.

Upon admission, the patient’s blood pressure was 110/60 mmHg, her pulse 62 beats min⁻¹ and no symptoms of fever were present. Nevertheless, during the evening, the patient exhibited a high temperature (peaking at 38.5 °C) on both the first and second days of her hospital stay. The only significant finding in the physical examination was a small papule on the second finger of the left hand (where the patient reported being bitten by a cat 2 months previously), in conjunction with axillary lymphadenopathy featuring large firm, mobile nodes measuring about 5 mm, but causing no pain.

White blood cell count was 4500 cells μl⁻¹ [normal value (n.v.) 4500–9000], platelet count was 487 000 μl⁻¹ (n.v. 150 000–450 000), haemoglobin 12.7 g dl⁻¹ (n.v. 11.5–16), and erythrocyte sedimentation rate was 80 mm h⁻¹ (n.v. 7–30). Ultrasound (US) of the spleen documented the presence of at least four hypoechochogenic nodular formations, of maximum size 15 mm.

Magnetic resonance imaging (MRI) showed four lesions with hyperintense signal on the T2-weighted images. Moreover, such lesions appeared hypointense on T1-weighted images, with postcontrast progressive enhancement from the periphery to the centre of each lesion.

The results of serology testing for *Toxoplasma gondii*, Epstein–Barr virus, cytomegalovirus, human immunodeficiency virus, *Brucella* spp. and *Salmonella* spp. were all negative. In contrast, *B. henselae* IgG and IgM titres, tested...
by indirect fluorescent antibody analysis (Euroimmun, Medizinische Labordiagnostika, using an IgG titre $\geq 1:64$ and IgM titre $\geq 1:20$ as cut-off, according to the manufacturer’s instructions) were both significantly raised (Table 1); serology for Bartonella quintana was negative.

No bacterial growth was observed in any of several blood cultures, which were checked daily for 10 days. During such time, blind cultures were performed every other day.

Due to clinical suspicion of CSD, we also performed real-time PCR assays for B. henselae on the patient’s whole blood samples as indicated in Table 1.

The sampling regimen for both serology and PCR testing schedules for B. henselae was as follows: the first samples were taken the day after admission, the next three samples within 1 month of admission, the fifth at the end of an approximately 6 week course of antibiotics; the sixth 1 month after the end of treatment, and the last roughly 4 months after cessation of antibiotic therapy.

The real-time PCR assay was performed as previously described (Liberto et al., 2011), albeit with some modifications. Briefly, in the previous report we detailed a real-time PCR with SYBR Green I dye, able to identify and quantify both B. quintana and B. henselae occurring and in those from a patient harbouring a naturally occurring B. quintana infection. The assay targeted the B. quintana transcriptional regulatory protein gene (bqtR), recently reported as invariable among different B. quintana strains (Arvand et al., 2010). Also, a homology of 97 % was described (Liberto et al., 2011), albeit with some modifications. In the previous report we detailed a real-time PCR with SYBR Green I dye, able to identify and quantify both B. quintana and B. henselae occurring and in those from a patient harbouring a naturally occurring B. quintana infection. The assay targeted the B. quintana transcriptional regulatory protein gene (bqtR), recently reported as invariable among different B. quintana strains (Arvand et al., 2010). Also, a homology of 97 % was described (Liberto et al., 2011), albeit with some modifications. In the previous report we detailed a real-time PCR with SYBR Green I dye, able to identify and quantify both B. quintana and B. henselae occurring and in those from a patient harbouring a naturally occurring B. quintana infection. The assay targeted the B. quintana transcriptional regulatory protein gene (bqtR), recently reported as invariable among different B. quintana strains (Arvand et al., 2010). Also, a homology of 97 % was described (Liberto et al., 2011), albeit with some modifications. In the previous report we detailed a real-time PCR with SYBR Green I dye, able to identify and quantify both B. quintana and B. henselae occurring and in those from a patient harbouring a naturally occurring B. quintana infection. The assay targeted the B. quintana transcriptional regulatory protein gene (bqtR), recently reported as invariable among different B. quintana strains (Arvand et al., 2010). Also, a homology of 97 % was described (Liberto et al., 2011), albeit with some modifications. In the previous report we detailed a real-time PCR with SYBR Green I dye, able to identify and quantify both B. quintana and B. henselae occurring and in those from a patient harbouring a naturally occurring B. quintana infection. The assay targeted the B. quintana transcriptional regulatory protein gene (bqtR), recently reported as invariable among different B. quintana strains (Arvand et al., 2010). Also, a homology of 97 % was described (Liberto et al., 2011), albeit with some modifications. In the previous report we detailed a real-time PCR with SYBR Green I dye, able to identify and quantify both B. quintana and B. henselae occurring and in those from a patient harbouring a naturally occurring B. quintana infection. The assay targeted the B. quintana transcriptional regulatory protein gene (bqtR), recently reported as invariable among different B. quintana strains (Arvand et al., 2010). Also, a homology of 97 % was described (Liberto et al., 2011), although with some modifications.

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To further confirm our diagnosis, we sequenced and analysed amplified PCR products. DNA sequencing was carried out using BigDye Terminator v3.1 Cycle Sequencing kits, and amplified products were purified using CentriSep spin columns and run on the ABI 3100 Genetic Analyzer (all from Applied Biosystems); sequences were aligned using the Seqscape v2.5 software (Applied Biosystems) and Seqencer (Gene Codes Corporation).

A BLAST search of the EMBL/GenBank database revealed 99 % homology with the B. henselae batR-1 allele (accession no. AY289790.1), 98 % homology with B. henselae batR-2 allele (accession no. AM294986.1), B. henselae batR-3 allele (accession no. AY289792.1) and B. henselae batR-4 allele (accession no. AY289793.1). Also, a homology of 97 % was described (Liberto et al., 2011), although with some modifications.

We therefore set out to see whether the same assay could detect B. henselae DNA in patient whole blood samples. In addition, for quantitative real-time PCR, whole blood samples from healthy volunteers with no evidence of a history of bartonellosis were mixed with serial dilutions of B. henselae, JK9R strain (from $10^6$ to $2.5 \times 10^8$ c.f.u. ml$^{-1}$), a kind gift from Professor Jane Koehler (University of California, San Francisco, USA). DNA was extracted, a standard curve was constructed, and the amount of DNA was extrapolated from the curve and expressed as c.f.u. ml$^{-1}$ values (Al-Ajlan et al., 2011). In the patient whole blood samples tested, PCR products exhibited a $T_m$ 87.39 ± 0.20 °C for the bqtR gene (Fig. 1).

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Table 1. Serological and B. henselae DNA follow-up

<table>
<thead>
<tr>
<th>Date</th>
<th>B. henselae specific antibodies (titre)</th>
<th>Extrapolated c.f.u. ml$^{-1}$ values from a quantitative real-time PCR*</th>
<th>Antibiotics administered to the patient</th>
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<tr>
<td></td>
<td>IgG</td>
<td>IgM</td>
<td></td>
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<tr>
<td>03/02/2011</td>
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<td>1:400</td>
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<td>1:200</td>
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<tr>
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<td>ND</td>
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<td>$3.13 \times 10^3$</td>
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<tr>
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<td>ND</td>
<td>$1.38 \times 10^2$</td>
</tr>
<tr>
<td>04/07/2011</td>
<td>1:640</td>
<td>Negative</td>
<td>$&lt;10^2$</td>
</tr>
</tbody>
</table>

*Al-Ajlan et al. (2011).
found in the comparison between our amplified product and the *B. quintana* batR gene (accession no. AY302696.1), while the homologies vs *Bartonella taylorii* (accession no. AJ302695.1), vs *Bartonella vinsonii* subsp. berkhoefii (accession no. FN652240.1), vs *Bartonella grahamii* (accession no. FN65256.1) and vs *Bartonella clarridgeiae* (accession no. FN645454.1) respectively were 92, 89, 89 and 84 % (data not shown). Thus, in line with CLSI guideline MM18-A for sequence-based identification (CLSI, 2008), justification for species-level identification was found among several *Bartonella* species, using our assay.

Serology test results and PCR data led us to suspect bacteraemia with negative blood cultures, quite often reported during *Bartonella* spp. infections (Foucault *et al.*, 2006). Most importantly, however, our results as a whole (Table 1) permitted us to avoid unnecessary invasive diagnostic procedures such as axillary lymph node biopsy and splenectomy.

Antibiotic therapy was therefore administered, after which the fever disappeared and the patient’s temperature remained stable throughout the day. Namely after the second day of her hospital stay, the patient was started on a course of 500 mg per day azithromycin administered intravenously, in conjunction with doxycycline,100 mg per os (p.o.) (by mouth) twice daily (b.i.d.), for 5 days. Treatment was then switched to 500 mg p.o. twice daily clarithromycin in combination with rifampicin (600 mg per day) for a further 5 weeks. Antibiotic treatment was deemed necessary due to the complicated form of the infection, with visceral involvement (multiple foci in the spleen, which could lead to spleen rupture with minor trauma) and the prolonged fever.

After 4 weeks of treatment, US and MRI showed a reduction in the size of the splenic nodules and an attenuation of lymphadenopathy. Further US and MRI scans taken at 10 weeks showed that both the splenic nodules and lymphadenopathy had disappeared.

The genus *Bartonella* includes facultative intracellular bacteria belonging to the alpha-2 subgroup of proteobacteria, and more than 22 *Bartonella* species have been described, of which at least half have been implicated or confirmed as human pathogens (Chomel *et al.*, 2006). Cats are well known reservoirs of *B. henselae*, and its transmission is reported to occur through scratches or bites, as supported by PCR evidence showing the presence of *B. henselae* in cat tooth pulp (Aboudharam *et al.*, 2005). Unfortunately, the cat that had bitten our patient was a stray animal, and it was not possible to obtain veterinary data or perform microbiological testing to assess for *B. henselae* carriage. *B. henselae* itself is a Gram-negative bacillus that can produce two entirely different pathological reactions, depending on the immune status of the host. In immunocompetent patients, CSD may be observed that is characterized by granulomatous nonangiogenic inflammation. On the other hand in immunocompromised subjects, *B. henselae* infection is associated with angiogenesis, in which a neutrophilic

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**Fig. 1.** Melting curve analysis of *B. quintana* Oklahoma strain, *B. henselae* JK9R and whole blood sample of the case under investigation. Samples and controls were run in triplicate. Representative data from independent runs are shown.
inflammatory response to bacilli located within the skin, bone and other organs (bacillary angiomatosis) or the liver and spleen (peliosis) is seen (Zenone, 2011). Visceral CSD occurs mainly in immunocompromised patients (Liston & Koehler, 1996), although the literature shows that a significant number of immunocompetent patients do suffer from disseminated CSD (Renou et al., 2010; Family-Pigné et al., 2006), which is transmitted by different mechanisms (Bass et al., 1997; McGill et al., 2003). In visceral CSD, preliminary diagnosis by radiology assessment must be confirmed by serology and molecular methods. Indeed, sporadic cases of nodules in the spleen may highlight the need to include B. henselae infection in the differential diagnosis in cases of suspected spleen lymphoma and other haematological malignancies (Zenone, 2011). To this end we recently proposed a species-specific real-time PCR assay targeting the transcriptional regulatory protein gene (bqtR) able to identify and distinguish between B. quintana and B. henselae in whole blood samples (Liberto et al., 2011), and here we confirm the value of the assay in the diagnosis and follow-up of splenic bartonellosis in a patient with suspected splenic lymphoma. Even more importantly, using the described RT-PCR assay it is possible to avoid invasive procedures such as splenectomy.

Indeed, isolation of B. henselae DNA from the peripheral blood of a patient with CSD has been reported (Arvand & Schäd, 2006) several months after the initial inoculation, demonstrating that B. henselae DNA may be chronically shed into peripheral blood from tissues such as bone marrow, liver and spleen during the natural course of CSD. Therefore, a rapid and specific method to screen potential blood donors may be a valuable method for identification of Bartonella-infected individuals.

Moreover, platelets represent an intriguing Trojan horse for B. henselae, particularly in splenitis cases (Zuckerman, 2009). Indeed, the physiological removal of blood components by the spleen means that it is easily invaded by Bartonella-filled platelets. Nevertheless, isolated splenic CSD is very uncommon, and few cases in which the diagnosis was confirmed by serological testing or after splenectomy have been reported in the international literature (Van der Veer-Meerkerk & van Zaanen, 2008). This distinction is important, because there are many concerns regarding the interpretation of the radiological features pertaining to nodules occasionally found in spleen tissue, especially when a differential diagnosis is required due to suspected Hodgkin’s or non-Hodgkin’s lymphoma, or acute leukaemia. Moreover, the combination of spleen nodules and lymphadenopathy is seen in various infectious diseases (Van der Veer-Meerkerk & van Zaanen, 2008). In all such cases, our method could be very useful in preventing unnecessary invasive diagnostic or therapeutic procedures.

Another advantage of our assay is its ease of use; B. henselae is difficult to cultivate, and identification of such organisms using conventional techniques is very labour-intensive. Furthermore, any serology testing of Bartonella spp. can be easily compromised by events such as cross-reactions with bacteria from other genera (da Costa et al., 2005), as well as variability, depending on the expertise of the operator.

However, as we demonstrate here, detection and quantification of genes of Bartonella spp. (bqtR, batR, etc.) in peripheral blood represents a novel and rapid noninvasive diagnostic tool able to identify bartonellosis in CSD cases with splenic involvement, without the need for splenectomy. This technique may also be used to provide follow-up findings in such cases, offering valuable feedback regarding the effectiveness of treatment and the outcome of the disease. In addition, this assay may be used together with a panel of Bartonella genes to screen blood destined for transfusion.

The study protocol was approved by our institution’s Ethics Committee/Institutional Review Board. Informed consent was obtained from the patient.

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References


