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Rapid identification of *Bartonella henselae* by real-time polymerase chain reaction in a patient with cat scratch disease

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Abstract

We report a localized submandibular lymph node infection in a patient with cat scratch disease. Directly performing real-time polymerase chain reaction assay on the biopsy sample, *Bartonella henselae* DNA was simultaneously detected and identified.
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Keywords: CSD; Lymph node *Bartonella henselae*; Real-time PCR; Molecular detection

The cat scratch disease (CSD) usually manifests as a subacute regional lymphadenitis following a cat scratch or bite, and *Bartonella henselae* is the major causative agent. The characteristic disease begins with a primary lesion at the site of inoculation, which develops into a papule or pustule, and, generally, 7 to 14 days after infection, regional lymphadenopathy occurs proximal to the primary lesion. Usually, CSD resolves spontaneously within several months (Carithers, 1985). The diagnosis of CSD is frequently supported on serology. The culture of *B. henselae* from affected lymph nodes can be difficult and has low sensitivity. Recently, polymerase chain reaction (PCR) has been recently proposed as a valuable tool for CSD diagnosis (Margileth, 2000).

We hereby report a case of a CSD patient with a localized submandibular lymph node infection. Suspected CSD was serology investigated, but only the real-time PCR on the biopsy specimen confirmed the presence of *B. henselae* DNA.

A previously healthy 7-year-old girl in a good general condition presented with painful and localized right submandibular edema and erythema and moderate fever. Shortly after the onset of symptoms, she was evaluated by her pediatrician and treated with amoxicillin-clavulanate for a week. Despite oral antibiotics, her symptoms worsened and she was taken to the Department of Infectious and Tropical Diseases of La Sapienza University, Policlinico Umberto I of Rome, for further management and therapy. Her history was significant for multiple scratches to her face from a kitten approximately 1 week before symptoms. At admission, the patient's white blood cell count was 9400/mm^3 (64.6% neutrophils, 25.2% lymphocytes, 5.1% monocytes, 2% eosinophils, and 0.5% basophils), platelet numbers were 295 000/mm^3, hemoglobin was 11.4 g/dL, and erythrocyte sedimentation rate was 50 mm/h. Cardiac, pulmonary, liver, renal, and abdominal function tests were within normal limits. Ultrasonography revealed on the right side of the neck a submandibular, firm, elastic mass 3 cm in diameter with several axillary lymph nodes and abscess. Palpation was painful but no other lymphadenopathy was found.

At the time of admission, the patient was treated intravenously with ceftriaxone and teicoplanin. After 2 days, teicoplanin was stopped because of nausea and vomiting, and clarithromycin was started. Ceftriaxone was stopped on day 5, and rifampicin was commenced.

Serological tests for cytomegalovirus, Epstein-Barr virus, hepatitis A, B, and C virus, parvovirus B19, *Toxoplasma gondii*, and HIV were all negative. Standardized tuberculin

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test, Weil-Felix reaction, and throat swab for microbiological cultures were negative. Blast cells were not seen in peripheral blood smears.

After 10 days, response to antibiotic therapy was still unsatisfactory, and ultrasonography showed an increase in length of some lymph nodes but with the same morphology. The submandibular mass was surgically removed, and histoimmulogic examination showed granulomatous inflammation with abundant necrotic lesions.

Postoperatively, a serum sample collected 15 days after CSD onset and a lymph node were sent to the Istituto Superiore di Sanità Department of Infectious Parasitic and Immune-Mediated Diseases of Rome for serodiagnosis, culture, and PCR assay of *B. henselae*. The patient was treated with clarithromycin and rifampicin for 15 days and was discharged without complications.

B. henselae IgM was negative, and IgG was 1:256. Cultures of the lymph node for anaerobic-aerobic bacteria, fungi, and for *Bartonella* and *Mycobacterium* isolation were negative.

Homogenate of the lymph node (25 mg) was subjected to DNA extraction by using the Nucleospin Tissue kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions, but the final eluate was concentrated by evaporation in 25 μL (DNA sample final volume in H_2O). The quantitative real-time PCR was performed as previously described (Ciervo and Ciceroni, 2004). Two microliters of DNA extract was analyzed, and DNAs from *B. quintana* and *B. clarridgeiae*, in addition to *B. henselae* DNA, were used as positive controls because they have also been proposed as possible agents of CSD (Drancourt et al., 1996; Kordick et al., 1997). The quantitative analysis was done with the LightCycler quantification software using the cycle threshold of the standard *B. henselae* DNA (10^4 to 1 genomic copies).

As shown in Fig. 1, the melting curve profiling revealed specific DNA of *B. henselae* in the clinical sample, and 7 genomic copy numbers per milligram were quantitatively detected (data not shown). Thus, the patient was clinically, serologically, and molecular-bacteriologically diagnosed as having CSD.

CSD is a common cause of subacute regional lymphadenopathy in children and adults, with typical clinical manifestations. It is generally a benign, self-limited disease. Usually, no antibiotic therapy is required, and surgical resection of the lymph nodes is rarely necessary. If CSD is clinically suspected because of the typical lymphadenopathy and a history of cat exposure, the diagnosis is usually confirmed by serology (Regnery et al., 1992; Sander et al., 1998). Suspected CSD should be proved by serological follow-up, by molecular or histopathologic investigations of the affected tissue, or both.

Macrolide antibiotics such as clarithromycin, quinolones, tetracycline, and rifampicin are the usual treatment of CSD, but may have a limited clinical benefit (Margileth, 2000).

The location of the lymphadenopathy in CSD depends on the site of inoculation. Carithers (1985), in an overview of a study of 1200 patients with CSD, reported that 48.8% were affected with axillary lymph nodes, and the cervical or submandibular regions were involved in 28.3% of all patients. In a study of 246 patients with CSD, Hamilton et al. (1995) reported that the most commonly infected sites were the neck, followed by the axilla and groin, and that 37% of the patients had enlarged lymph nodes. Clinically suspected CSD usually is confirmed serologically by antibody titers. IgM antibodies against *B. henselae* are infrequent, even in the early stages of CSD, and negative results do not exclude the presence of acute disease (Regnery et al., 1992; Sander et al., 2001). Ridder et al. (2002) demonstrated that in the early stages of the disease, antibody titers of both IgG and IgM might still be low, and diagnosis can only be confirmed after increasing titers in a second serum sample. In addition, patients without CSD had low antibody titers, up to 1:256, which indicates earlier contact with *B. henselae* or even a nonspecific reaction. In instances in which serological results fail to confirm the

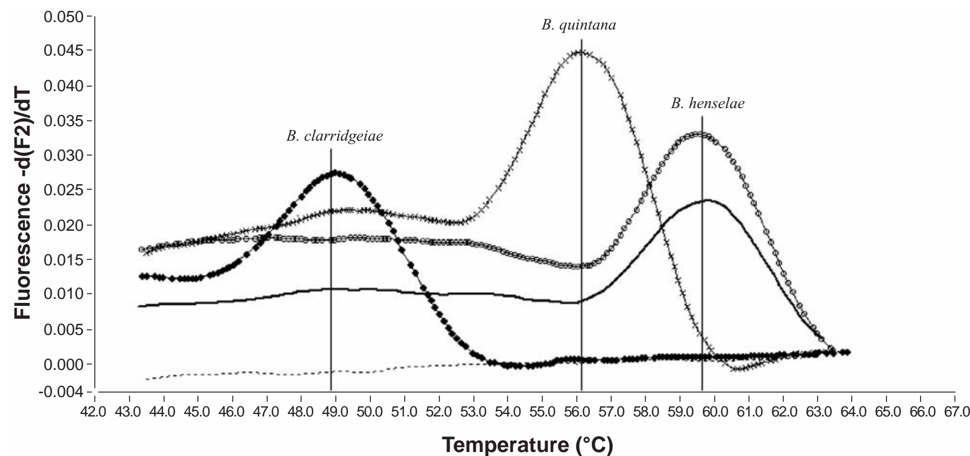

Fig. 1. Detection of *B. henselae* DNA using the LightCycler real-time PCR. Patient's biopsy (-). *B. henselae* Houston-1 (○), *B. quintana* Oklahoma (×), *B. clarridgeiae* (●), and negative (–) controls.

diagnosis of CSD, lymph node excision and histopathologic and molecular diagnostic procedures are recommended.

Amplification of *Bartonella* spp. DNA from lymph nodes, skin, granulomatous lesions, and biopsies from other organs has been reported in patients suffering from CSD (Anderson et al., 1994; Goral et al., 1994; Zeaiter et al., 2002) or bacillary angiomatosis (Gasquet et al., 1998). PCR-based detection of *Bartonella* species from human specimens remains the best method for the diagnosis of CSD. Several PCR protocols were described, and different gene targets were used for the molecular detection of *Bartonella* DNA (Anderson et al., 1994; Johnson et al., 2003; Matar et al., 1993; Norman et al., 1995; Renesto et al., 2001). Nevertheless, these conventional PCR techniques are not suitable to clinical use because contaminations and false positive results may occur. On the other hand, the real-time PCR assay is a good candidate for a clinical diagnosis. Actually, it is an automated technique that presents many advantages, such as high sensitivity and specificity, less possibilities of contamination, and it allows the quantification of genome copy numbers. Another real-time PCR assay was described for patients with suspected *Bartonella* endocarditis, and the method was easily and directly applied to the serum sample (Zeaiter et al., 2003).

In a recent study, we developed a real-time PCR assay for the rapid detection of *Bartonella* spp. involved in endocarditis and CSD in humans (Ciervo and Ciceroni, 2004). Moreover, this molecular assay can detect more than one *Bartonella* species in a sample. The present study proves that this new PCR tool is successful for the amplification of *B. henselae* from fresh tissue biopsy.

Real-time PCR assay is an attractive alternative to block cycler PCR assays. It could be a useful laboratory support that may potentially be standardized as a 1-step method for the identification and discrimination of *Bartonella* spp. in clinical samples from patients with clinical evidence of CSD.

In conclusion, our findings showed that culture methods have demonstrated poor sensitivity probably because the specimens came from patients who were already treated with antimicrobial agents. The only direct evidence of the etiological CSD agent was confirmed by real-time PCR even in the presence of low number of genomic copies in the biopsy. In any case, further studies and additional testing are necessary to validate our first results and improve the clinical utility of real-time PCR assay of CSD diagnosis.

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References

Anderson B, Sims K, Regnery R, Robinson L, Schmidt J, Goral C, Hager C, Edwards K (1994) Detection of *Rochalimaea henselae* DNA in specimens from cat scratch disease patients by PCR. *J Clin Microbiol* 32:942–948.

Carithers HA (1985) Cat-scratch disease: An overview based on a study of 1,200 patients. *Am J Dis Child* 139:1124–1133.

Ciervo A, Ciceroni L (2004) Rapid detection and differentiation of *Bartonella* spp. by a single-run real-time PCR. *Mol Cell Probes* 18:307–312.

Drancourt M, Moal V, Brunet P, Dussol B, Berland Y, Raoult D (1996) *Bartonella (Rochalimaea) quintana* infection in a seronegative hemodialyzed patient. *J Clin Microbiol* 34:1158–1160.

Gasquet S, Maurin M, Brouqui P, Lepidi H, Raoult D (1998) Bacillary angiomatosis in immunocompromised patients. *AIDS* 12:1793–1803.

Goral S, Anderson B, Hager C, Edwards KM (1994) Detection of *Rochalimaea henselae* DNA by polymerase chain reaction from suppurative nodes of children with cat-scratch disease. *Pediatr Infect Dis J* 13:994–997.

Hamilton DH, Zangwill KM, Hadler HL, Cartter ML (1995) Cat-scratch disease—Connecticut, 1992–1993. *J Infect Dis* 172:570–573.

Johnson G, Ayers M, McClure SCC, Richardson SE, Tellier R (2003) Detection and identification of *Bartonella* species pathogenic for humans by PCR amplification targeting the riboflavin synthase gene (*ribC*). *J Clin Microbiol* 41:1069–1072.

Kordick DL, Hilyard EJ, Hadfield TL, Wilson KH, Steigerwalt AG, Brenner DJ, Breitschwerdt EB (1997) *Bartonella clarridgeiae*, a newly recognized zoonotic pathogen causing inoculation papules, fever, and lymphadenopathy (cat scratch disease). *J Clin Microbiol* 35:1813–1818.

Margileth AM (2000) Recent advances in diagnosis and treatment of cat scratch disease. *Curr Infect Dis Rep* 2:141–146.

Matar GM, Swaminathan B, Hunter SB, Slater LN, Welch DF (1993) Polymerase chain reaction-based restriction fragment length polymorphism analysis of a fragment of the ribosomal operon from *Rochalimaea* species for subtyping. *J Clin Microbiol* 11:1730–1734.

Norman AF, Regnery R, Jameson P, Greene C, Krause DC (1995) Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. *J Clin Microbiol* 33:1797–1803.

Regnery RL, Olson JG, Perkins BA, Bibb W (1992) Serological response to "*Rochalimaea henselae*" antigen in suspected cat-scratch disease. *Lancet* 339:1443–1445.

Renesto P, Gouvernet J, Drancourt M, Roux V, Raoult D (2001) Use of *rpoB* gene analysis for detection and identification of *Bartonella* species. *J Clin Microbiol* 39:430–437.

Ridder GJ, Boedeker CC, Technau-Ihling K, Grunow R, Sander A (2002) Role of cat-scratch disease in lymphadenopathy in the head and neck. *Clin Infect Dis* 35:643–649.

Sander A, Posselt M, Oberle K, Bredt W (1998) Seroprevalence of antibodies to *Bartonella henselae* in patients with cat scratch disease and in healthy controls: Evaluation and comparison of two commercial serological tests. *Clin Diagn Lab Immunol* 5:486–490.

Sander A, Berner R, Ruess M (2001) Serodiagnosis of cat-scratch disease: Response in children and a review of methods. *Eur J Clin Microbiol Infect Dis* 20:392–401.

Zeaiter Z, Fournier PE, Raoult D (2002) Genomic variation of *Bartonella henselae* strains detected in lymph nodes of patients with cat scratch disease. *J Clin Microbiol* 40:1023–1030.

Zeaiter Z, Fournier PE, Greub G, Raoult D (2003) Diagnosis of *Bartonella* endocarditis by real-time nested PCR assay using serum. *J Clin Microbiol* 41:919–925.