

Detection of *Bartonella henselae* DNA Using Polymerase Chain Reaction Assay in Patient with Cat Scratch Disease

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Sir,

Cat scratch disease (CSD) is seen frequently in patients under 20 years of age who usually have a history of being scratched, bitten, or licked by a young cat. Although the course of CSD is benign and often self-resolving, severe complications such as encephalitis, myelitis, pneumonitis or thrombocytopenic purpura have also been described (1). At present, *Bartonella henselae*, a fastidious Gram-negative rod, is recognized as an aetiological agent of CSD. However, isolation of *B. henselae* in culture is difficult and the histopathological findings are not specific. An indirect immunofluorescence antibody (IFA) test for the detection of the IgG and IgM antibodies to *B. henselae* has been used for the diagnosis of CSD (2). The polymerase chain reaction (PCR) assay has been currently developed for the direct detection of *B. henselae* DNA in clinical samples (3–5).

We report a case of CSD diagnosed by direct detecting *B. henselae* DNA extracted from the pus using the PCR method.

CASE REPORT

A healthy 21-year-old Japanese man was referred to the Dermatology Clinic because of a 14-day history of a painful mass in his right axilla, general malaise and recurrent fever up to 39°C. Physical examination disclosed a reddish, febrile, marked tender, fluctuant lymph node measuring 2 cm in the right axilla (Fig. 1), and some scratches on his forearm. Twenty days previously he had been scratched on the right forearm by a kitten. No papules or pustules were observed at the scratch site. Initial laboratory investigations revealed a WBC count of 8.300/μl with a differential of 72% segmented polymorphonuclear neutrophils, 19% lymphocytes, 2% eosinophils and 7% monocytes. Laboratory studies revealed an erythrocyte sedimentation rate of 51 mm for the first hour and C-reactive protein of 0.84 mg/dl. Needle aspiration of the fluctuating lymph node yielded 10 ml of purulent material. Bacterial cultures and a specific culture for the *Bartonella* species performed from the pus were negative. Erythromycin estolate, 900 mg daily, was administered orally, but the patient discontinued taking it after 4 days. The axillary lymph node proceeded to develop a draining sinus tract. The lymph node swelling gradually resolved over the following 3 months. There were no complications and it eventually healed.

The peripheral blood sample and pus obtained from the fluctuant lymph node swelling were evaluated using

PCR and agarose gel electrophoresis of the amplified products. The 16S rRNA gene of *B. henselae* in the blood sample was amplified by PCR as reported by Bergmans et al. (6) with minor modification. The primer pair named 16SF [5'AGAGTTTGATCCTGGCTCAG3'] and 16SR [5'CCGATAAATCTTTCTCCCTAA3'] was used to amplify a 185-bp fragment of the *Bartonella* 16S rRNA gene by PCR. The DNA extraction and the PCR conditions for amplification were as described by Maruyama et al. (7). The extracted DNA obtained either from the patient's blood sample or pus sample was subsequently confirmed as belonging to *B. henselae* using specific primers named 16SR and 16SF generating a 185-bp fragment (Fig. 2).

The titres of the IgG and IgM antibodies against *B. henselae* were determined by indirect immunofluorescence antibody (IFA) analysis with strain ATCC 49882. The intensity of the fluorescence was considered to be positive at a dilution of $\geq 1:64$ for the IgG and $\geq 1:16$ for the IgM antibody. Three weeks after onset of the symptoms, an IFA test for the IgG antibody to

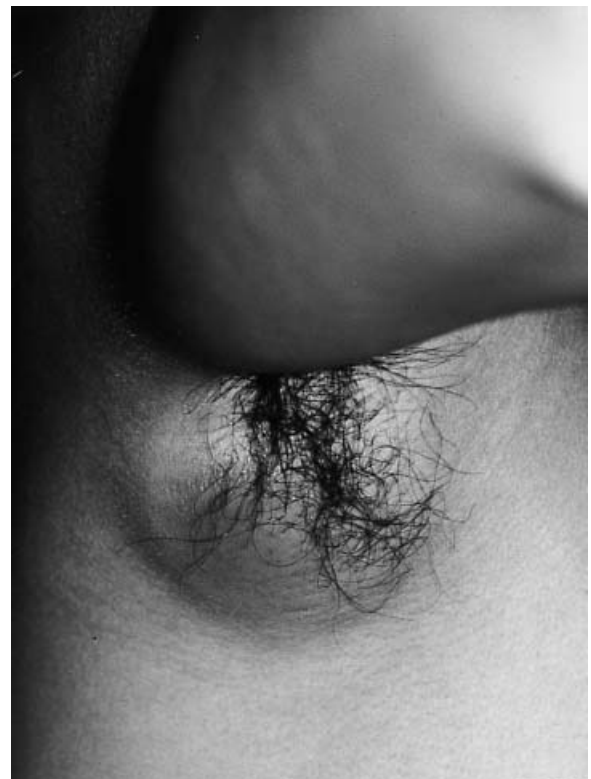


Fig. 1. Fluctuant right axillar lymph node swelling.

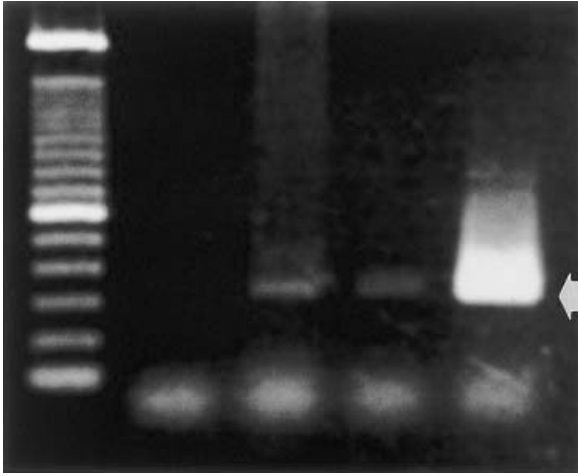


Fig. 2. Detection of *B. henselae* 16S rRNA by PCR with 16 SF and 16SR. Arrow indicates 185 bp fragment of *B. henselae* DNA. Lane-1; DNA ladder, lane-2; negative control, lane-3; pus sample from patient, lane-4; blood sample from patient, lane-5; sample DNA from *B. henselae* ATCC 49882.

B. henselae was positive at a titre of 1:512. However, the IgM antibody to *B. henselae* was not detected in the sera.

DISCUSSION

In the present study, *B. henselae* DNA was detected from pus aspirated by PCR assay and a definite diagnosis of CSD was made. The PCR assay in detecting *B. henselae* may be a more rapid and more sensitive tool than serological tests for the diagnosis of CSD.

The diagnosis of CSD has been based primarily on the clinical criteria (i.e. regional lymphadenitis, fever and skin lesions) in addition to the recent history of cat exposure or a cat scratch. Since atypical cases can cause diagnostic problems, there is a need for methods to allow detection of the bacterial species directly in the clinical samples of CSD suspected patients. Bartonella species can be cultured from blood of bacteraemic humans and cat (8). However, it is difficult to culture from a lymph node of a patient with CSD. Recently, the presence of *B. henselae* DNA has been demonstrated in blood from the patients by PCR (6, 7). Detection of *B. henselae* DNA in blood is not surprising due to the fact that bacteraemia has been reported (9) and that *B. henselae* is capable of surviving within macrophages and erythrocytes (10).

B. henselae DNA was detected in the pus aspirated from the fluctuant regional lymph node in our case. A few reports in which *B. henselae* DNA was detected from the pus specimens have been published (3–5). The involved lymph nodes in patients with CSD either regress over a period of months or proceed to suppuration (1). Although there is wide variation in the reported series of patients, the suppuration rate ranges from 13% to 48% (1). The detection of *B. henselae*

is feasible in the pus specimens from lymph node specimens by PCR (3–6). Bergmans et al. (4) reported that 18 (86%) of 21 CSD cases with titres ≥ 64 were positive in the Bartonella DNA obtained from pus aspirates, whereas 9 (90%) of 10 cases with antibody titre ≤ 64 did not react in the PCR. Their study showed a clear correlation between *B. henselae* PCR positivity and Bartonella antibody titre. Avidor et al. (5) indicated that *B. henselae* DNA was positive in specimens from pus aspirates; however, 4 of the available 19 serum samples were initially negative at the time that PCR assay was positive. The patients seroconverted later. Three of 19 patients tested negative by serological enzyme immunoassay. The authors concluded that PCR assay of pus could detect *B. henselae* before the appearance of IgG antibodies and that some patients with CSD did not develop anti-*B. henselae* IgG detectable serologically (5).

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