INTRODUCTION

Cat scratch disease (CSD) is a worldwide zoonosis caused by *Bartonella henselae* or possibly by *Bartonella clarridgeiae* (1–3). It is characterized usually a self-limiting regional lymphadenopathy, associated with a cat scratch or bite. Originally considered rare, it is now recognized as a common cause of lymphadenopathy in children and young adults (2). Classic systemic disease includes a cutaneous inoculation by a scratch or bite followed by a regional lymphadenopathy after a variable period, ranging from 1 to 8 weeks. The number of pet cats is increasing in developed countries including Korea. According to the increase in number of pet cats, zoonosis like CSD has risen as a health problem in human society. In the past most cases of CSD were diagnosed by clinical manifestations and intradermal reaction with specimens taken from patients before isolation of the causative organisms. Due to the difficulty of isolation of *B. henselae* from CSD patient, the diagnosis is usually based on serologic data and clinical history when informative. Recently polymerase chain reaction (PCR) analysis using aspirates of cervical lymph node was performed and the presence of *B. henselae* DNA was demonstrated. This is the first reported case of cat scratch disease in Korea confirmed by PCR for *B. henselae* DNA.

CASE REPORT

A 25-yr-old previously healthy woman visited Sanggye-paik Hospital with high fever over 7 days and painful mass in the left neck. In spite of medication of oral antibiotics at a private clinic, her symptoms were not improved. She had been admitted to our hospital on 7 May 2004. She had been keeping a dog for 4 months before admission but had no history of contact with a cat.

On admission there were multiple palpable mass in the left neck area. The masses were 2 cm and 1.5 cm in diameter, and were tender. On physical examination liver and spleen was not palpable. There was no skin lesion or scratched wound in the face, extremity and trunk.

She had a white cell count of $3,490 \times 10^9/L$ (neutrophil, 87%; lymphocyte, 8.6%; monocyte, 3.2%), with platelets 100 $10^9/L$, a hemoglobin of 12.1 g/dL. Blood chemistry revealed: AST 71 IU/L, ALT 62 IU/L, total bilirubin 0.3 mg/dL, BUN 8 mg/dL, creatinine 0.7 mg/dL. Laboratory findings showed elevated CRP, but ESR was 3 mm/hr. ANA and anti-DNA was negative. The computed tomography of the patient’s neck showed multiple variable-sized lymph nodes (maximum 16 × 10 mm). The serum sample from the patient was tested for *B. henselae* antibodies by using a commercial immunofluorescent assay (Bartonella IFA IgG; Focus technologies, Cypress, CA, U.S.A.). The IgG titer was 1:64 positive. Aspira-
tion cytology of lymph node of left neck revealed reactive hyperplasia.

The patient started receiving clindamycin intravenously for 6 days after lymph node aspiration. The fever and pain in the left neck area persisted during the treatment. Under the impression of reactive lymphadenitis she had been discharged without medication. During the outpatient clinic follow up her symptoms improved gradually without medication and completely recovered one month later. She had remained asymptomatic for 3 months.

The detection of \textit{B. henselae} DNA from lymph node aspirate using PCR

To prepare template DNA from the lymph node aspirate, QIAamp DNA Tissue Mini Kit (QIAGEN GmbH, Hilden, Germany) was used. \textit{B. henselae} Huston-1 (ATCC 49882) DNA was used for positive control. We selected the primer sets (TN-1, TN-2, and IP) for the \textit{gltA} gene used by Margolis et al. (5) and the primer sets (PAPn1, PAPn2, and PAPns2) for the \textit{pap31} gene used by Zeaiter et al. (6). Seminested PCR protocols for amplification of the \textit{B. henselae gltA} and \textit{pap31} genes were applied to the sample (Table 1). The size of the amplified DNA fragments was 139 bp and 211 bp for the \textit{gltA} and \textit{pap31} genes respectively (6, 14). \textit{B. henselae} DNA was detected from patient’s lymph node aspirate (Fig. 1). PCR products were sequenced. For \textit{gltA} gene, IP and TN-1 were used (5) and for \textit{pap31}, PAPns2 and PAPns1 were used (6). They were sequenced at both directions with BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster, CA, U.S.A.). Sequencing products were resolved with ABI 3,730 XL autoanalyzer (Applied Biosystems, Foster, CA, U.S.A.). The sequences were aligned with the \textit{gltA} or \textit{pap31} sequences available in GenBank for \textit{B. henselae} isolates. The patient’s PCR product for \textit{gltA} had a consistent sequence of \textit{B. henselae} and

| Primer | Target gene | Nucleotide sequence          | Position (direction)* |
|--------|-------------|------------------------------|-----------------------|
| PAPn1  | \textit{pap31} | TCTAGGAGTTGAAACCGAT         | 438-457 (↑)           |
| PAPn2  | \textit{pap31} | GAAACACCACCACGCAACATA       | 695-714 (↑)           |
| PAPns2 | \textit{pap31} | GCACCAGACCACATTTTCCCTT      | 629-648 (↑)           |
| TN-2   | \textit{gltA} | TGTTGGAGCTAAGGAAAGCAG       | 796-814 (↑)           |
| TN-1   | \textit{gltA} | CCTCATGGCAGGTTTGTGCG        | 957-976 (↑)           |
| IP     | \textit{gltA} | GTTCTGTTGAAAAGATTCTTGA      | 838-860 (↑)           |

*: Primer positions are numbered to the gene of \textit{B. henselae} (strain Houston-1).

Fig. 1. Results of seminested polymerase chain reaction (PCR) for \textit{gltA} gene and \textit{pap31} gene of \textit{B. henselae}. Lanes 2-4 for PCR of \textit{gltA} gene (139 bp), lane 6-8 for PCR of \textit{pap31} gene (211 bp). Lane 1 and 5, DNA ladder marker (Bioneer, Daejeon, Korea); lane 2 and 6 positive control (Houston-1, ATCC 49882); lane 3 and 7, negative control; lane 4 and 8, lymph node tissue from the patient with cat scratch disease.

Fig. 2. Partial \textit{pap31} sequences of two main genogroups \textit{B. henselae} and case (red color-primer, blue color-different sequence between genogroups).
DISCUSSION

Cat scratch disease is usually a self-limiting disease and does not require therapy. But, some patients with multi-system involvement may benefit from antibiotics treatment, so it is necessary to identify the organism rapidly by clinical laboratory assay. The diagnosis of CSD is made currently on the basis of clinical criteria in addition to a recent history of cat or dog exposure, a scratch or a flea bite plus bacteria culture, histologic examination of tissue biopsies and serologic test. Although serologic analysis by immunofluorescence or enzyme linked immunosorbent assay is a useful tool for the diagnosis of B. henselae infection, the specificity of serological assay has been questioned due to the cross reactivity between B. henselae and other species (13). Also antigenic variability in the species can partly explain inconsistent results in the serological diagnosis of CSD.

PCR assays appear to be very useful in confirming clinically suspected CSD and have advantage of rapid diagnosis with species polymorphism (14). PCR assay using the amplification of 16S rRNA gene which is present in all bacteria offers a rapid and specific means to detect the organism directly from clinical specimens in CSD patients. And it is more sensitive than isolation when performed on suitable clinical samples such as fresh or frozen lymph node tissues.

In conclusion, when presented with lymphadenopathy, physicians should inquire about recent cat, dog, or pet's contact and/or animal scratches considering the possibility of CSD.

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