□ Case Report □

A Case of Cat Scratch Disease Confirmed by Polymerase Chain Reaction for *Bartonella henselae* DNA

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We report a case of cat scratch disease (CSD) caused by *Bartonella henselae* in a 14-year-old boy who developed lymphadenopathy in the right cervical area, after a raising canine pet for 10 months. The cervical lymphadenopathy persisted for 14 days. Immunofluorescent antibody testing for *B. henselae* with the patient's serum was 1:64 positive. Polymerase chain reaction (PCR) analysis using the patient's lymph node aspirates for *B. henselae* DNA was also positive. This is the first case of cat scratch disease confirmed by PCR for *B. henselae* DNA in children. **(Korean J Pediatr 2005;48: 789-792)**

Key Words : *Bartonella henselae*, Cat scratch disease, PCR, Children

Introduction

Cat scratch disease (CSD) is usually characterized as a self-limiting regional lymphadenopathy associated with a cat scratch or bite, caused by *B. henselae* or possibly *B. clarridgeiae*. *B. henselae*, a fastidious gram-negative bacterium has been known as the major causative agents of CSD only since 1992[1]. CSD has emerged as a relatively common zoonotic disease among children and adults, and is recognized as an important cause of lymphadenopathy or fever of unknown origin (FUO) in children and young adults[2]. Typical clinical manifestation of CSD is a regional lymphadenopathy after cutaneous inoculation by a cat or dog scratch. Atypical manifestations of CSD occur in 5-25% of all cases, and many different organs can be affected[3]. Before the causative organisms could be identified, CSD was diagnosed by clinical manifestations, and intradermal reaction tests with specimens taken from CSD patients. After the identification of *B. henselae* as an etiologic agent, the diagnosis of CSD was based on serologic tests and the clinical history of contact with pet animals due to the difficulty of isolating the organism from patients. Recently, the detection of *B. henselae* DNA by using PCR with specimen of lymph nodes from patients and blood is available for genetic diagnosis of CSD[4-6]. In Korea, few cases of lymphadenitis showing positive results by immunofluorescent assay for *B. henselae* have been reported in adults. However, no case has ever been diagnosed by PCR with lymph node aspirate specimens in children and adults. We report a case of CSD confirmed by PCR of *B. henselae* DNA in a 14-year-old boy with a history of canine pet contact.

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Case Report

1. Clinical history and physical examination

A 14-year-old boy visited our hospital for painful enlargement of the right cervical lymph node and a five-day fever. He had been raising a pet for 10 months and experienced frequent enlargement of the cervical lymph nodes.

On physical examination, his body temperature was 38℃ and blood pressure 120/80 mmHg. Multiple tender masses, sized about 1 cm, were palpable in the right neck area. Liver and spleen were not palpable in the abdomen. There was no skin lesions or scratched lesion on the body or both extremities.

2. Laboratory examinations

Laboratory examinations showed a white cell count of $5,270\times10^9$/L (neutrophil : 61.7%, lymphocyte : 31.9%, monocyte 5.3%), with platelets 236×10^9/L, a hemoglobin of 14.4 g/dL. The results of the blood chemistry were AST 19 IU/L, ALT 12 IU/L, total bilirubin 0.3 mg/dL, BUN 9.9 mg/dL, creatinine 0.78 mg/dL and ESR 2 mm/hr. The neck sonography showed multiple variable-sized lymph nodes. Immunofluorescent assay for IgG antibody of *B. henselae* (Focus technologies, Cypress, CA) with the serum sample from the patient during acute stage showed 1 : 64 positive. The pathologic findings of lymph node aspirates showed chronic inflammation with granulaoma (Fig. 1). The culture of aerobic and anaerobic bacteria, and PCR for *M. tuberculosis* were negative.

3. Detection of *B. henselae* DNA

Template DNA was extracted from the lymph node aspirate of patient using QIAmp. DNA Tissue Mini Kit (QIAGEN GmbH, Hilden, Germany). The DNA of *B. henselae* (ATCC 49882) was used as positive control. From the patient's lymph node aspirate, *B. henselae* DNA was confirmed by PCR assays that amplified two different genes (Fig. 2).

The primer sets, TN2 (5′-TGGTGGAGCTAATGAAGCATG-3′), TN-1 (5′-GCAACAAACCTGCCATGAGG-3′), and IP (5′-GTTCTGTTGAAAGAATTCCTGA-3′) were used to amplify 211-bp fragment of the *Bartonella gltA* gene by seminested PCR, as described by Margolis et al[5].

Other primer sets, PAPn1 (5′-TTCTAGGAGTTGAAACCGAT-3′), PAPn2 (5′-GAAACACCACCAGCAACATA-3′), PAPns2 (5′-GCACCAGACCATTTTTCCTT-3′), and PAPns1 (5′-CAGAGAAGACGCAAAAACCT-3′) were used to amplify the 139-bp fragment of the Bartonella PAP31 gene by seminested PCR, as described by Zeaiter et al[6]. Sequencing at both directions of PCR products were conducted with BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster, CA, USA) and sequencing products were resolved with ABI 3730 XL Autoanalyzer (Applied Biosystems, Foster, CA, USA). The sequences were aligned with the *pap31* and *gltA* sequences available in GenBank for *B. henselae* isolates. PCR products of the patient for pap31 gene and *gltA* gene showed corresponding sequences to *B. henselae* (Fig. 3).

4. Clinical courses

Oral antibiotics (Augmentin®) were administrated for three days, but stopped under the impression of reactive hyperplasia. Seven days later, mild fever and pain in the left neck were improved without further treatment.

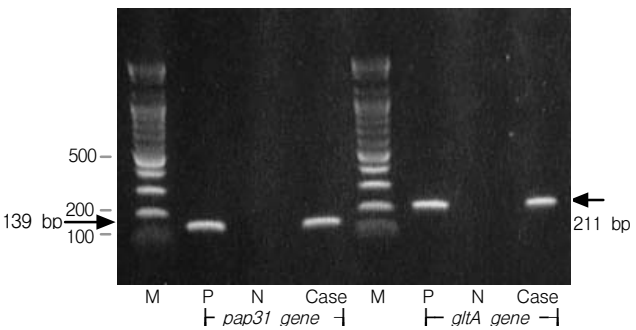

Fig. 2. Detection of *B. henselae* DNA for pap31 gene and *gltA* gene by polymerase chain reaction. M : standard ladder marker, P : positive control from *B. henselae* (ATCC 49882), N : negative control, Case : DNA obtained from the lymph node aspirates of patients.

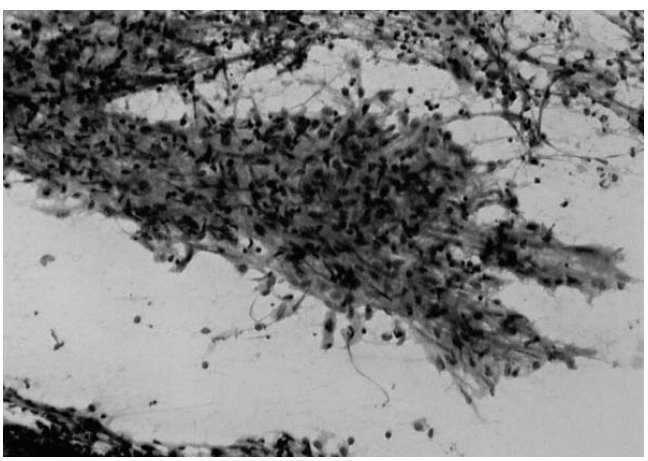

Fig. 1. This figure shows granuloma consisted of epithelioid histiocytes and lymphocytes with some neutrophils in the backgroud (Papanocolaou stain, ×200).

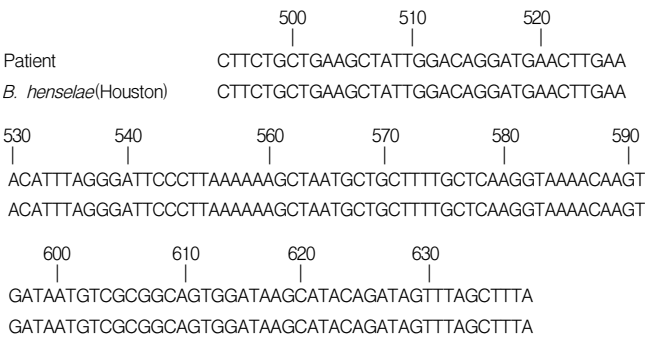

Fig. 3. This figure shows the corresponding sequences of pap 31 gene of *B. henselae* (Houston) and PCR product of the patient.

Discussion

In 1950, CSD was first described by Debre et al.[7] in a boy presented with suppurative lymphadenitis and severe cat scratches. Originally considered rare, CSD is an emerging zoonosis, chiefly by *B. henselae*, with variable clinical manifestations. *B. henselae* is a small gram negative rod of the genus Bartonella, family Bartonellaceae mainly involved in the etiopathogenesis for CSD[1].

CSD has emerged as an important cause of chronic lymphadenopathy in children and young adults, and it occurs more frequently in certain geographical areas. In the USA, it is estimated that over 24,000 cases occur annually, with 2,000 patients requiring hospitalization[8]. A higher rate of CSD cases has been reported in the autumn and winter in temperate climates[1]. Typical manifestations of CSD are nontender papules along the scratched line, a few days after exposure, heals without scarring in two or three weeks. Regional lymphadenopathy follows in more than 80 % of cases, and heals within two months[8]. Atypical presentations of CSD are considered as variable manifestations of Bartonella infection; including fever of unknown origin, nueroretinitis, encephalopathy, and hepatosplenic granuloma[9]. Some reported that *B. henselae* infection is a common etiologic agent of fever of unknown origin[10]. Although CSD is a worldwide zoonosis in several countries[2, 8, 9], clinical studies about epidemiology and clinical characteristics of CSD are rare in Korea. Chae et al.[11] reported that the positive rates of the IgG antibody for *B. henselae* by immunofluorescent assay was about 38.7% (11/31) in adults with lymphadenitis, but they observed a history of close contract with cat in only three patients.

Cats or dogs are the main reservoir of *B. henselae*. Long term bacteremia in cats or dogs and flea-transmission from cat to cat, as confirmed by experimental infection, support a vector borne transmission[7, 12-14]. Some patients with CSD were not associated with any known exposure to cats, suggesting that other animal species may be reservoirs of Bartonella[9]. Recently, new Bartonella species have been isolated from a wide range of mammals, including rodents, carnivores, and cervids[15, 16]. Ticks may play an important role in the transmission of Bartonella species from wild ruminants.

The diagnosis of CSD can be made by clinical criteria including a recent history of cat or dog exposure, culture, histologic examination of tissue biopsies and serologic test. Imunofluorescence or enzyme linked immnuosorbent assay is a useful tool for the diagnosis of *B. henselae* infection, but the specificity of serological assay is questioning due to the cross reactivity between *B. henselae* and other species[17]. In the early stages of disease, antibody titers of both IgG and IgM still might be low, and diagnosis can be only confirmed after increasing titer have been observed in a second serum sample. Histologic findings in CSD are similar to those in other granulomatous disease such as brucellosis and tularemia, but they are found in only 12.5% of the patients with CSD in a study[18]. PCR is regarded as a method to confirm in clinically suspected CSD because it is more sensitive and not influenced by patient's humoral response[4-6, 19]. PCR assay for the amplification of the 16S rRNA gene or citrate synthase gene (*gltA*) of *B. henselae* is a sensitive tool for the detection of *B. henselae* DNA, but it requires large amounts of samples. Recently, it has been reported that the diagnosis of CSD by PCR, with the fine needle aspirates from the lymph nodes, is possible with high sensitivity and minimal invasiveness[19]. In this case, the antibody titer of *B. henselae* by IF was 1:64 positive and *B. henselae* DNA for pap31 genes and for gltA gene were detected by PCR assays with aspirates of lymph node.

The majority of CSD cases resolves spontaneously within a few weeks and does not require antibiotics therapy. Antibiotics treatment is recommended only in CSD patients with lymphadenopathy associated with significant morbidity, severe systemic disease, and in immunocompromised condition. To date, no standardized antibiotic treatment regimen exists for patient with CSD. Macrolides such as erythromycin, clarithromycin, and azithromycin are often used for treatment of CSD. Rifampicin, quinolones, and tetracycline are reported to have a limited clinical effect in the treatment of CSD[20, 21]. Cats and dogs are the most common household pets, associated with a potential transmission of about thirty infectious agents including *B. henselae* to humans[22]. It seems to be desirable to educate the pet owners for possible infections by *B. henselae*.

We report a case of CSD in a 14-year-old boy, confirmed by PCR with lymph node aspirates of the patient. In children who have lymphadenopathy with feline or canine pets, the possibilities of CSD must be considered.

한글 요약

Bartonella henselae DNA에 대한 PCR 검사로 확진된 Cat Scratch Disease 1례

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CSD는 고양이에 긁히거나 물린 다음에 발생하는 자가제한적 국소 림프선염을 증상으로 하는 질환이다. CSD는 소아와 성인에서 비교적 흔한 인수 공통질환이며, 특히 소아 연령의 림프선염과 원인 불명열의 중요한 원인으로 알려져 있다. CSD는 고양이 벼룩에 존재하는 배설물에 있는 *B. henselae*가 고양이의 발톱이나 이빨에 물리면서 인체에 접종되는 것으로 여겨지지만 감염 경로는 아직 확실하지 않다. *B. henselae*는 고양이 벼룩 뿐 아니라 개벼룩, 쥐이 및 진드기 등의 다양한 매개 곤충에 존재하여 다양한 가축과 야생동물을 감염시킨다. 최근에는 고양이 벼룩에 교차 감염되는 애완용 개가 중요한 중간 숙주로 대두되었다. CSD는 애완용 개나 고양이와 접촉하거나 긁힌 기왕력과 전형적인 임상증상, 조직 소견, 혈청학적 검사에 의해 주로 진단되어 왔지만 최근에는 PCR을 이용하여 최종 진단을 하게된다. 저자들은 10개월간 애완용 개를 키웠던 14세 남아에서 진단된 CSD 1례를 보고하는 바이다. 본 연구는 국내 소아에서 PCR로 진단된 최초의 CSD 증례인 점에 의의가 있다. 환아의 발열과 경부 림프선염은 10일 정도 지속되었으나 특별한 치료를 하지 않고 회복되었다. 환자의 혈청에 대해 시행한 *B. henselae* 간접 면역 형광법 검사는 1 : 64 양성이었으며 경부 림프선 흡인물을 대상으로 시행한 PCR 검사에서 *B. henselae* DNA의 존재가 확인되었다.

References

1) Regnery RL, Olson JG, Perkins BA, Bibb W. Serologic response to “Rocalimaea henselae” antigen in suspected cat scratch disease. Lancet 1992;339:1443-5.
2) Clarithers HA. Cat-scratch disease : an overview based on a study of 1,200 patients. Am J Dis Child 1985;139:1124-33.
3) Anderson BE, Neuman MA. Bartonella spp. As emerging human pathogens. Clin Microbiol Rev 1997;10:203-19.
4) Sander A, Posselt M, Bohm N, Ruess M, Altwegg M. Detection of Bartonella henselae DNA by two different PCR assays and determination of the genotypes of strains involved in histologically defined cat scratch disease. J Clin Microbiol 1999;37:993-7.
5) Margolis B, Kuzu I, Hermann M, Raible M, His E, Alkan S. Rapid polymerase chain reaction-base confirmation of cat scratch disease and Bartonella henslae infection. Arch Pathol Lab Med 2003;127:706-10.
6) Zeaiter Z, Fournier PE, Raoult D. Genomic variation of Bartonella henselae strains detected in lymph nodes of patients with cat scratch disease. J Clin Microbiol 2002;40:1023-30.
7) Debre R, Lamy M, Jammet ML, Costil L, Mozziconacci P. La maladie des griffes du chat. Sem Hop 1950;26:1895-904.
8) Jackson LA, Perkins BA, Wenger JD. Cat scratch disease in the United States. Am J Public Health 1993;83:1707-11.
9) Murakami K, Tsukahara M, Tsuneoka H, Iino H, Ishida C, Tsujino K, et al. Cat scratch disease : analysis of 130 seropositive cases. J Infect Chemother 2002;8:349-52.
10) Jacobs RF, Schutze GE. Bartonella henselae infection as a cause of prolonged fever and fever of unknown origin in children. Clin Infect Dis 1998;26:80-4.
11) Chae MB, Lee JY, Kwak YG, Park SH, Lim HJ, Chung MH, et al. Prevalence of antibodies to Bartonella henselae and Bartonella Quintana in Korean patients with lymphadenopathy. Korean J Infec Dis 2002;5:305-10.
12) Tsukahara M, Tsuneoka H, Iino H, Ohno K, Murano I. Bartonella henslae infection from a dog. Lancet 1998;352:1682.
13) Keret D, Giladi M, Kletter Y, Wientoub S. Cat scratch disease osteomyelitis from a dog scratch. J Bone Joint Surg 1998;80:766-7.
14) Gundi V, Bourry O, Scola B. Bartonella clarridgeiae and B. henselae in Dogs, Gabon. Emerg Infect Dis 2004;12:2261-2.
15) Ellis BA, Regnery RL, Beati L, Bacellar F, Rood M, Glass GG, et al. Rats of the genus Rattus are reservoir hosts for pathogenic Bartonella species : an old World origin for a New World disease? J Infect Dis 1999;180:220-4.
16) Kordick DL, Swaminathan B, Greene CE, Wilson KH, Whitney AM, O'Connor S, et al. Bartonella vinsonii subsp. Berkhoffii subsp. nov, isolated from dogs; Bartonella vinsonii subsp. Vinsonii; and emended description of Bartonella vinsonii. Int J Syst Bacterio 1996;46:704-9.
17) Sander A, Berner R, Ruess M. Serodiagnosis of cat scratch disease : response to Bartonella henselae in children and a review of diagnostic methods. Eur J Clin Microbiol Infect Dis 2001;20:392-401.
18) Demers DM, Bass JW, Vincent JM, Person DA, Noyes DK, Staege CM, et al. Cat scratch disease in Hawaii : etiology and seroepidemiology. J Pediatr1995:127-6.
19) Avidor BA, Varon M, Marmor S, Lifschitz-Mercer B, Kletter Y, Ephros M, et al. DNA amplification for the diagnosis of cat scratch disease in small quantity clinical specimens. Microbiol Infect Dis 2001;115:900-9.
20) Margelith AM. Antibiotic therapy for cat-scratch disease : clinical study of therapeutic outcome in 268 patients and a review of the literature. Pediatr Infect Dis J 1992;11:474-8.
21) Ives TJ, Manzewitsch P, Regnery RL, Butts JD, Kebede M. In vitro susceptibilities of Bartonella henselae, B. quintana, B. elizabethae, Rickettsia rickettsii, R. conorii, R. akari, and R. prowazekii to macrolide antibiotics as determined by immunofluorescent-antibody analysis of infected vero cell monolayers. Anitimicrob Agents Chemother 1997;41:578-82.
22) Tan JS. Human zoonotic infections transmitted by cats and dogs. Arch Intern Med 1997;157:1933-43.