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Molecular detection of *Bartonella henselae* DNA from fleas obtained from dogs, Korea

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Purpose : The purpose of this study was to investigate the presence of *Bartonella henselae* DNA, which is known as an etiologic agent of lymphadenitis, in fleas from dogs.
Methods : The *Bartonella henselae* infection was investigated in 42 fleas from 22 dogs in Korea. By using seminested PCR targeting *pap31* gene, *B. henselae* DNA was amplified from fleas.
Results : *B. henselae* DNA was detected in seven fleas (7 of 42 fleas, 16.7 percent) from four dogs (4 of 22 dogs, 18.2 percent). To confirm these findings, we performed sequencing and identified the seven PCR products. Sequence analysis revealed that six sequences belonged to Huston-1 genogroup and one sequence to Marseille genogroup.
Conclusion : These results may suggest that dogs could be an important source of *B. henselae* infection in children in Korea. This is the first report about the detection of *B. henselae* in fleas from dogs in Korea. **(Korean J Pediatr 2006;49:983-986)**

Key Words : *Bartonella henselae*, Fleas, Dogs, Polymerase chain reaction

Inroduction

Cat scratch disease (CSD), caused by *Bartonella henselae* or possibly *Bartonella clarrridgeiae*, is a worldwide zoonosis associated with a variety of clinical manifestations[1]. Cats are known to be the most important reservoir of the bacteria and can transmit Bartonella to humans by a scratch or a bite[2]. Cats are most contagious through their blood, which may mix with a cat's saliva in cases of gum bleeding. It is also possible that infected cats with *B. henseae* may transmit CSD after a flea bite[2, 3]. There are worldwide reports about *Bartonella* infections in cats and *B. henselae* infections in cats and *Bartonella* transmission by cat fleas (*Ctenocephalides felis*)[4-6].

Although dogs can also transmit *Bartonella*[7], there have only been a few reports regarding *Bartonella* infection in dogs and the route of *B. henselae* transmission to dogs by fleas[8, 9]. In the USA, there is an estimated 140 million pets, of which the majority (about 77 million cats) of are cats. In fact, the pet dog population in Korea is estimated at more than 4 million, which is 19 times more pet dogs than pet cats[10, 11]. Recently, *B. henselae* seropositivity was reported to not be closely associated with cat contact in Korea[12] and CSD cases involving "dog-only" contact have been reported[12-14]. Although these reports might suggest dogs as a more important reservoir of *B. henselae* than cats in Korea, there has not been any report of *B. henselae* infection in dogs or from fleas collected from dogs so far in Korea. Therefore the aim of this study was to confirm the presence of *B. henselae* in fleas recovered from dogs in Korea and to characterize the *B. henselae* strains.

Materials and methods

One hundred five dogs were enlisted from three animal sanctuaries in Gyunggi-do, Korea. From August 2004 and August 2005, fleas were obtained by combing the dogs for

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10 minutes. The obtained fleas were transferred into tubes containing 1.0 mL 70% ethanol and kept at room temperature before identification and DNA extraction. The fleas were identified under microscopy by morphologic characteristics as described previously by Menier and Beraucorunu[15]. The fleas were washed for 5 minutes in phosphate buffered saline then crushed in sterile tubes with the tip of a sterile pipette. DNA was extracted by using a QIAamp Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instruction for tissue DNA extraction. The tissue was lysed overnight at 55℃ and the DNA was eluted in 100 μL of elution buffer. To detect *B. henselae* DNA, seminested PCR targeting of the *pap31* gene was performed according to the protocols described by Zeaiter et al.[16]. PAPn1 and PAPn2 were used for the 1st round, and PAPn1 and PAPns2 were used for 2nd round. The PCRs were carried out with a DNA thermal cycler (iCycler Thermal Cycler, Bio-Rad Laboratories, Hercules, CA, USA) using a *Taq* DNA polymerase kit (AccuPower PCR PreMix, Bioneer, Daejeon, Korea). The 20 μL reaction mixture containing the following: 0.2 μM of each primer; 200 μM (each) of dATP, dCTP, dTTP, and dGTP; 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 40 mM KCl; 1 unit of *Taq* polymerase; and 1 μL of DNA sample (or product of 1st PCR reaction). The first and second rounds of the amplification were done under the same conditions, utilizing thirty-five cycles of amplification. Each cycle consisted of the following: pre-denaturation at 94℃ for 1 minute, 35 cycles of denaturation at 94℃ for 1 minute, primer annealing at 55℃ for 1 minute, extension at 72℃ for 1 minute. The amplified DNA fragments were 277 bp and 211 bp for 1st and 2nd rounds respectively. PCR products were sequenced in both directions with BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster, CA, USA). Sequencing products were resolved with ABI 3730 KL autoanalyzer (Applied Biosystems, Foster, CA, USA). Sequence analysis was performed using BioEdit (North Carolina State University, Raleigh, NC, USA) and MEGA3 (Biodesign Institute, Tempe, AZ, USA) and aligned with the *pap31* sequences available from GenBank for the *B. henselae* isolates.

Results

A total of 42 fleas (one to three fleas per dog) were collected from 22 dogs. Two species of fleas were found in the dogs, *Ctenocephalides felis* (*C. felis*) and *Ctenocephalides canis* (*C. canis*) (Fig. 1). Twenty-three fleas (23/42; 54.8%) were *C. felis* and the others (19/42, 45.2%) were *C. canis*. *B. henselae* DNA was detected in seven fleas (7 in 42 fleas, 16.7%) from 4 dogs (4 in 22 dogs, 18.2%) (Fig. 2). Out of the seven fleas positive for *B. henselae* DNA, two were *C. canis* and five were *C. felis*. The sequences from the seven positive PCR products were identical to the *B. henselae pap31* gene. Six PCR products from three dogs belonged to the Houston-1 genogroup and one from a dog belonged to the Marseille genogroup (Fig. 3).

Discussion

Although CSD is a worldwide zoonosis[17-19], Korean studies about the clinical characteristics and the epidemiology of CSD are rare. One recent report by a Korean researcher, who used the immunofluorescent assay (IFA) to

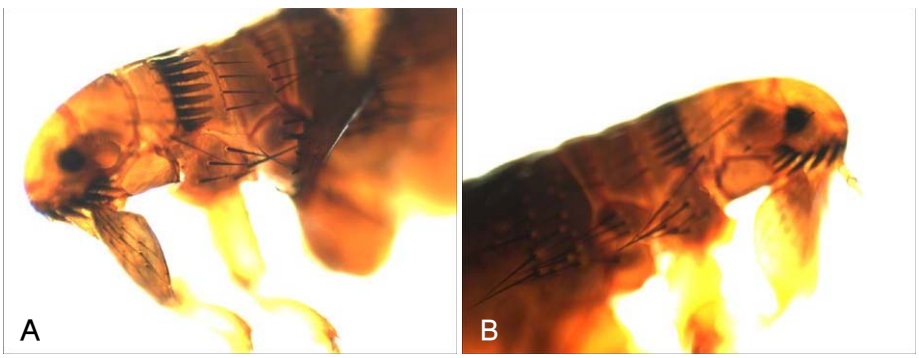

Fig. 1. *Ctenocephalides* species (magnification 40). A) *C. felis* : notice that the first tooth genal ctenidium is as large as the second one. B) *C. canis* : notice that the first tooth genal ctenidium is shorter than the second one. The shape of the head is more round than that of *C. felis*.

Fig. 2. The results of seminested polymerase chain reactions targeting *B. henselae* pap31 gene (211 bp). Lanes 1, DNA ladder; lane 2, positive control (Houston-1, ATCC 49882); lane 3, negative control; Lane 4-9, DNA extracted from fleas. *B. henselae* DNA was detected in lane 8.

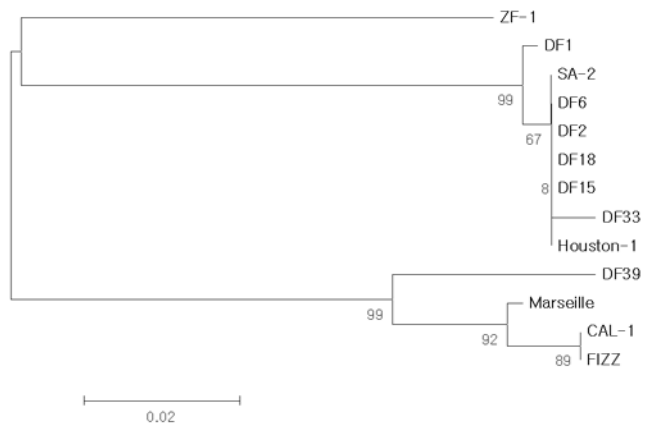

Fig. 3. Neighbor-joining unrooted tree (obtained by using the distance matrix with the Kimura two-parameter method and neighboring-joining method using MEGA3 software package) generated with *pap31*-based sequencing data for six *B. henselae* isolates (Houston-1, ZF-1, SA-2, Marseille, CAL-1, FIZZ). Six isolates (DF1, DF2, DF6, DF15, DF18, DF33) belong to genogroup Houston-1 and one isolate (DF39) belongs to genogroup Marseille.

detect the positive rates for the IgG antibody to *B. henselae*, found 38.7% (11/31) of patients with lymphadenitis positive for *B. henselae*[12]. The study also found that subjective positive for *B. henselae* were more likely to have a history of close contact with dogs only, as opposed to those subjects who only had contact with cats. Also, case reports in Korea of CSD confirmed by detection of *B. henselae* DNA indicate that patients had a history of close contact only with dogs.

C. felis (cat flea) and *C. canis* (dog flea) are the most prevalent flea species that infest pet cats and dogs[20, 21], and are the most likely vectors of *B. henselae* infection in pets[22]. Fleas can also serve as a *B. henselae* vector to the wild animals and humans[6]. Recently, Kim et al.[23] demonstrated the presence of *Bartonella* DNA in ticks from Korea and suggested that ticks might have possible roles in *Bartonella* infection cycles.

In this study, *B. henselae* DNA was detected in 7 fleas out of 42 (16.7%). *C. felis* was the predominant species (54.8%) followed by *C. canis* (45.2%). In other studies, *B. henselae* infection in fleas from dogs ranged from 0% to 27%, which was lower than that from cats (22% to 33%) of the same region[6, 24-26]. Regardless, data about the sero-prevalence for *B. henselae* in dogs of Korea is still lacking.

Although *B. henselae* sero-prevalence (as determined by antibody levels against *B. henselae*) and the presence of *B. henselae* bacteremia in dogs are important epidemiological data, this study did not collect such samples from the studied dogs and therefore, such tests were not performed. It has been reported that the sero-prevalence in dogs is much lower than that of cats from the same region[9] and others have indicated that it is more difficult to isolate *Bartonella* species in dogs due to a lower bacterial concentration[6]. This proposed difference has been the source of controversy about the role of dogs as a *B. henselae* reservoir.

Despite existing controversy, this study has identified *B. henselae* DNA in the fleas obtained from dogs. These results indicated that *B. henselae* is not rare among fleas collected from dogs, and dogs of Korea may be a possible reservoir of *B. henselae* other than cats. This is the first report about the detection of *B. henselae* in the fleas from dogs in Korea.

한 글 요 약

개에서 추출된 벼룩에서 분자생물학적 방법에 의한 *Bartonella henselae* DNA의 검출

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목 적 : 소아 임파선염의 흔한 원인균의 하나로 잘 알려진 *Bartonella henselae*는 주로 고양이 벼룩에 감염된 고양이와의 접촉을 통하여 병을 일으키는 것으로 알려졌지만, 최근에는 개도 보유 숙주임이 확인되었다. 국내의 CSD증례 보고들에서는 고양이보다 개와 접촉한 경우가 더 많은 것으로 보고되었다. 하지만

국내에서는 개나 고양이 또는 매개체인 고양이 벼룩 등에서 *B. henselae*가 확인된 경우가 아직 없었다. 이에 저자들은 개에게서 얻은 벼룩을 대상으로 *B. henselae* DNA가 존재하는지 알아보기 위하여 본 연구를 시행하였다.

방 법 : 전체 22마리의 개에서 얻은 총 42마리의 벼룩을 대상으로 연구를 시행하였다. 각각의 벼룩에서 DNA를 추출한 다음 *B. henselae*의 *pap31* 부위 유전자를 이용한 시발체를 사용하여 seminested PCR을 시행하였다.

결 과 : *B. henselae* DNA는 전체 22마리 중 4마리의 개(18.2%)에서 추출되었던 벼룩 42마리 중 7마리(16.7%)에서 확인되었다. PCR 결과를 확인하기 위하여 7개의 PCR 산물에 대한 염기서열 분석을 시행하였다. 염기서열 분석에 의하면 6개는 Huston-1 유전자형이었으며, 한 개는 Marseille 유전자형이었다.

결 론 : 국내 개에서 추출된 벼룩에서 Houston-1유전자형과 Marseille 유전자형을 포함한 *B. henselae* DNA가 존재하는 것으로 확인할 수 있었다. 국내에서 개가 CSD의 중요한 감염 경로일 가능성을 제시한 점에 의의가 있지만, 충분한 수의 고양이 및 개에서 얻은 혈청과 벼룩을 대상으로 한 추가적인 연구가 필요할 것으로 생각된다.

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