**Ehrlichia chaffeensis** Infection in Dogs in South Korea

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**ABSTRACT**

*Ehrlichia chaffeensis* is one of the causative agents of canine ehrlichiosis and human monocytic ehrlichiosis (HME). Canine ehrlichiosis caused by *E. chaffeensis* was diagnosed in two dogs in South Korea based on clinical findings, and the diagnosis was confirmed by polymerase chain reaction (PCR) and DNA sequencing. A 5-year-old intact male American Pit bull terrier allowed outdoors was found to be concurrently infected with *Babesia gibsoni* and *E. chaffeensis*. The major clinical findings were lethargy and reddish urine, and laboratory analysis revealed severe hematuria and thrombocytopenia. In addition, a 3-year-old neutered male Shih-tzu was also found to be infected with *E. chaffeensis*. Although this dog was an indoor companion animal, he was frequently allowed outside for exercise. The clinical signs observed in this dog included generalized purpura with petechiae and ecchymoses due to thrombocytopenia. A 390-bp partial portion of *E. chaffeensis* 16S rRNA gene was amplified in both cases, and nucleotide sequence analysis revealed 99% homology of this fragment with other *E. chaffeensis* isolates. These findings demonstrate the presence of *E. chaffeensis* infection in dogs in South Korea, and this is the first report to confirm clinical cases of *E. chaffeensis* infection in dogs. Key Words: *Ehrlichia chaffeensis*—Dog—PCR—Korea.

**INTRODUCTION**

*Ehrlichiae* are gram-negative obligate intracellular bacteria that cause disease in people as well as in several species of domestic and wild animals. They require a vector for transmission and are maintained in nature in a cycle involving at least one vertebrate reservoir host (Paddock et al. 2003). *Ehrlichia chaffeensis* was the first documented ehrlichial pathogen of people and can cause human monocytic ehrlichiosis (HME), an emerging tick-borne infectious disease in humans (Dumler et al. 1995, Paddock et al. 2003). Most interest in *E. chaffeensis* is related to its effects in humans; however, dogs are also susceptible to infection by *E. chaffeensis*. Although dogs experimentally infected with *E. chaffeensis* developed thrombocytopenia without other systemic manifestations of the disease (Zhang et al. 2003), more serious symptoms, including vomiting, epistaxis, lymphadenopathy, and anterior uveitis, have also been documented in three dogs that were naturally infected with *E. chaffeensis* (Breitschwerdt et al. 1998).

We recently reported the presence of *E. chaffeensis* and other ehrlichial/rickettsial organisms in ticks (*Haemaphysalis longicornis*) and small mammals (*Apodemus agrarius*) in South Korea (Kim et al. 2003, 2006). In addition, evi-

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The presence of ehrlichial agents in dogs in South Korea has not yet been investigated. In this report, the cases of two dogs in South Korea that had clinical ehrlichiosis are described. Nested polymerase chain reaction (PCR) and sequencing of the amplified products were used to confirm the natural infection of *E. chaffeensis*.

**METHODS**

Dog 1 was a 5-year-old intact male American Pit bull terrier that was moved to South Korea from Yugoslavia in his adult age and was admitted to the animal hospital in May 2006. The dog had been allowed outdoors and was brought to the clinic after exhibiting signs of lethargy and reddish urine. Dog 2 was a 3-year-old neutered male Shih-tzu dog that was examined for generalized purpura with petechiae and ecchymoses in March 2006. He was an indoor companion animal; however, he was taken for frequent walks.

Complete blood counts (CBC) and serum biochemistry analysis and urinalysis were performed following clinical examinations at each hospital. In addition, a molecular diagnosis was conducted to characterize the infectious agents that caused these clinical signs, as well as to exclude the possibility of co-infections with other agents. A nested PCR assay was conducted using primers designed to amplify 16S rRNA gene fragments of each of the following organisms: *A. phagocytophilum*, *A. platys*, *E. canis*, and *E. ewingii* (Kim et al. 2006). Amplified 390-bp partial sequences of *E. chaffeensis* were aligned for maximum homology with the 16S rRNA gene sequences at the National Center for Biotechnology Information (National Institutes of Health) BLAST network service.

**RESULTS AND DISCUSSION**

Laboratory analysis showed severe thrombocytopenia in both dogs (platelet count 27 × 10⁹/L of dog 1 and 50 × 10⁹/L of dog 2) and hematuria (4+, 250 erythrocytes/µL) in dog 1. In a Diff-Quick blood smear, basophilic inclusion bodies suspected ehrlichial morulae within monocytes and *Babesia gibsoni* merozoites within erythrocytes were observed in dog 1, supporting a diagnosis of concurrent infection of *E. chaffeensis* and *B. gibsoni*. However, no remarkable abnormalities were observed in both cases in other hematological parameters and routine serum chemistry profiles.

The results of nested PCR showed that the samples were negative for *A. phagocytophilum*, *A. platys*, *E. canis*, and *E. ewingii*. However, *E. chaffeensis* DNA was amplified from both blood samples (Fig. 1), and sequence analysis of these amplified products confirmed that the products were from the *E. chaffeensis* 16S rRNA gene. The amplified PCR products were 99.7% identical to each other, and were also >99% identical to sequences identified in Arkansas (accession no. AF416764; 99.7% of identity), Connecticut (accession no. AF305074; 99.7% of identity), Sapulpa (accession no. U60476; 99.7% of identity), Southern China (accession no. DQ324547; 98.2% of identity), and Korea (from *H. longicornis* ticks; accession no. AY350424; 99.7% of identity). In addition, the PCR products were 97.9% identical to the products amplified from *E. chaffeensis* found in small mammals from Korea (*Apodemus agrarius*; accession no. DQ402484). The sequences from the *E. chaf-
feensis 16S rRNA PCR amplicons from dogs 1 and 2 were deposited into GenBank and assigned accession numbers EF621763 and EU099990, respectively.

Finally, we diagnosed that dog 1 had concurrent infection with *B. gibboni* and *E. chaffeensis* and dog 2 had *E. chaffeensis* infection based on the laboratory results and molecular methods, and they were treated with antibiotics such as doxycycline.

A number of *Ehrlichia* species can infect dogs, and their affinity for hematopoietic cells may lead to leucopenia and thrombocytopenia. Although *E. chaffeensis* can cause HME in humans, only a mild febrile response with no hematological disorders has been observed in experimental infections in dogs (Zhang et al. 2003). However, the two dogs evaluated in this study showed clinical and hematological signs consistent with clinical ehrlichiosis. To the best of our knowledge, this study is the first to provide molecular evidence of *E. chaffeensis* infections in dogs in South Korea.

In 2000, the first suspected case of *E. chaffeensis* was reported in an active-duty American soldier stationed in South Korea (Sachar et al. 2000). Heo et al. (2002) subsequently identified antibodies against *E. chaffeensis* from patients with febrile illnesses in South Korea. In addition, *E. chaffeensis* was identified in *H. longicornis* ticks from South Korea using molecular methods (Kim et al. 2003, Lee et al. 2005), and tick-borne rickettsial pathogens, including *E. chaffeensis*, were investigated in ticks and small mammals in a prevalence study (Kim et al. 2006). Because dogs (*Canis familiaris* or *C. lupus*) could potentially be the most important reservoir affecting humans (Paddock et al. 2003), the molecular detection of *E. chaffeensis* in this study could have very important epidemiological implications in South Korea. Additionally, domestic animals could also be infected with and transmit this organism in South Korea. Therefore, additional studies should be conducted to isolate and identify this organism from humans and animals in South Korea to confirm their presence and evaluate their possible impact on human and animal health.

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**REFERENCES**


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