Natural co-infection of *Ehrlichia chaffeensis* and *Anaplasma bovis* in a deer in South Korea

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**ABSTRACT.** Both ehrlichioses and anaplasmoses are zoonotic, fatal infectious diseases that caused by ticks. White-tailed deer (*Odocoileus virginianus*) are known to be important hosts for *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum* and *Anaplasma*-like organisms. In the present study, an evaluation of infection with tick-borne pathogens was conducted using a PCR assay on the blood of a deer that expressed anorexia and decreased activity. The results of the PCR assay revealed natural co-infection of *E. chaffeensis* and *A. bovis* in the deer. This indicates that deer may be a natural reservoir of both *E. chaffeensis* and *A. bovis* in South Korea.

**KEY WORDS:** *Anaplasma bovis*, deer, *Ehrlichia chaffeensis*.

Both ehrlichioses and anaplasmoses are potentially fatal infectious diseases caused by obligatory intracellular gram-negative bacteria [3]. These tick-borne zoonoses are causative agents that are maintained through enzootic cycles between ticks and animals [12]. These diseases adversely affect humans and animals and cause economically devastating diseases in livestock [15].

White-tailed deer (*Odocoileus virginianus*) are known to be important hosts for all three life stages of the lone star tick (*Amblyomma americanum*) [1] and to be a reservoir for *Ehrlichia chaffeensis* (*E. chaffeensis*), *Anaplasma phagocytophilum* (*A. phagocytophilum*) and *Anaplasma*-like organisms [8, 9]. In addition, the presence of *Anaplasma* sp., *Babesia* sp. and *A. ovis*, as well as the presence of antibodies reactive to *E. chaffeensis* and *A. phagocytophilum*, has been detected in mule deer (*O. hemionus*) from Arizona and California [16]. Furthermore, *Anaplasma* and *Ehrlichia* spp. have been identified in deer (*Cervus nippon*) in Japan [5]. However, few studies evaluating infection with tick-borne pathogens have been conducted in deer in South Korea. Therefore, we conducted the present study to evaluate the presence of tick-borne pathogens in a deer in South Korea.

A blood sample was collected from the horn of a Korean spotted deer (*C. nippon*) that was being kept at private farm in Jeonbuk, South Korea, and observed to have anorexia and decreased activity. Complete blood count (CBC) analysis was then conducted on the sample. In addition, the sample was subjected to a molecular survey for tick-borne pathogens to clarify the possibility of co-infections with other organisms because the deer was located in an area in which it would be exposed to ticks. Total DNA was extracted from the blood, which was collected into an EDTA anticoagulant tube, using a GENE ALL™ Blood SV mini kit (General Bio System, South Korea) according to the manufacturer’s instructions. Extracted DNAs were stored at –20°C and used as the template for PCR amplification. Specifically, PCR and a nested PCR assay were conducted to determine whether *Theileria* spp., *E. chaffeensis*, *E. canis*, *E. ewingii*, *E. muris*, *A. platys*, *A. marginale*, *A. phagocytophilum* and *A. bovis* were present. The nested PCR assay was conducted using primers designed to amplify the 16S rRNA gene of *E. chaffeensis*, *E. canis*, *E. ewingii* [11], *A. platys* [10] and *A. muris*, *A. phagocytophilum* and *A. bovis* [5], and the PCR assay was conducted using primers designed to amplify the 18S rRNA gene *Theileria* spp. [2] and the major surface protein 1 beta gene of *A. marginale* [14]. For all PCR assays, the volume of the total PCR mixture was with 20 µL. Each total PCR mixture included 2 µL template DNA, 2 µL of 10 × PCR buffer including 20 mM MgCl2, 1 µL of a 10 mM deoxynucleotide triphosphate (dNTPs) mixture, 0.3 µL of each primer (10 pmol/µL) and 0.3 µL of 5 U/µL Taq DNA polymerase (Intron Biotechnology, Daejeon, South Korea). The PCR products were then separated by 1.5% agarose gel electrophoresis, stained with ethidium bromide and photographed using a Gel-Doc 2000 system (Bio-Rad, Hercules, CA, U.S.A.). Amplified PCR products were cloned to confirm the nucleotides sequence. Sequence homology searches were conducted using the National Center for Biotechnology Information (National Institute of Health, Bethesda, MD, U.S.A.) BLAST network service. The nucleotide sequences were then aligned and compared using the MultAlin software (Multiple sequence alignment by Florence Corpet) and a genestream program (Genestream network service, IGH, Montpellier, France). Phylogenetic analyses based on the obtained sequences were conducted using Multiple Sequence Alignment and the ClustalW program. The results of the CBC analysis revealed that the hematocrit levels and RBC counts were slightly increased (59.4% and 12.88 × 106/µL, respectively). In addition, although the
WBC counts were within the reference levels, they were slightly decreased (2.8 \times 10^3/\mu L). However, no inclusions of hemoparasites were found in the blood smears. The PCR assay was positive for *E. chaffeensis* and *A. bovis* (Fig 1) and was negative for the remainder of the species. Sequence analysis of the 390-bp product generated for *E. chaffeensis* revealed that it was 99.7% homologous with sequences obtained in the U.S.A. (GenBank accession number AF416764), South Korea (GenBank accession number AY350424) and China (GenBank accession number AF147752). Furthermore, it was found to be 99.2% homologous with *E. chaffeensis* (GenBank accession number EF621763) obtained from a dog in South Korea (Fig. 2A). Additionally, sequencing of the 551-bp product amplified for *A. bovis* revealed that it was 99.8% and 99.3% homologous with the sequences of *Anaplasma* sp. (GenBank accession number EU368731) and *A. bovis* (GenBank accession number AB196475), respectively, both of which were isolated in Japan. It was also 99.6% and 99.1% homologous to sequences of *E. bovis* (GenBank accession number U037755) isolated in South Africa and *A. bovis* (GenBank accession number AF470698) isolated in South Korea, respectively (Fig. 2B). The sequences for *E. chaffeensis* and *A. bovis* obtained in the present study were deposited into GenBank in the National Center for Biotechnology Information (National Institute of Health, Bethesda, MD, U.S.A.) BLAST network service as GenBank accession numbers EU682762 and EU682764, respectively.

In 2000, the first suspected case of *E. chaffeensis* was reported in an active-duty American soldier stationed in South Korea [13]. Recently, antibodies against *E. chaffeensis* and *A. phagocytophilum* have been identified in South Korean patients with febrile illness [4], and the presence of *E. chaffeensis* has been reported in ticks, *Apodemus agrarius* and dogs in South Korea [7, 17]. In addition, the presence of *A. bovis* was detected in a *Haemaphysalis longicornis* tick collected from a hedgehog in South Korea [6]. The nucleotide sequences of *E. chaffeensis* obtained in the present study are very similar to those obtained from ticks, small mammals and dogs in South Korea. Furthermore, the sequences of *A. bovis* in the present study also reveal high identity with the sequences of *A. bovis* obtained...
from the *H. longicornis* tick in South Korea. To our knowledge, the present study is the first mention of infection of *E. chaffeensis* and *A. bovis* in domestic animals in South Korea. Thus, the detection of *E. chaffeensis* and *A. bovis* in the present study indicates important epidemiological implications in South Korea.

In this study, we describe for the first time natural coinfection of *E. chaffeensis* and *A. bovis* in a deer in South Korea. This indicates that deer may be a natural reservoir of agents that cause human monocytic ehrlichiosis (*E. chaffeensis*) and ruminant anaplasmosis (*A. bovis*) in South Korea. However, direct evidence is required to make a definitive conclusion, but detection of *E. chaffeensis* and *A. bovis* using PCR is suggestive of the possibility that infected deer may be a reservoir of these agents for human infections. Therefore, a larger study evaluating tick-borne diseases that can impact the health of animals including, deer populations in South Korea, should be conducted.

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REFERENCES