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< Case Report >

Ehrlichia ewingii infection in a dog from South Korea – A case report

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Abstract

The report describes a case of natural *E. ewingii* infection confirmed by microscopic examination and molecular analyses in a domestic dog with acute lameness. After the diagnosis, the dog was successfully treated with oral doxycycline. To authors' knowledge, this is the first case report of natural *E. ewingii* infection in dogs in South Korea.

Key words : Dog, *Ehrlichia ewingii*, Lameness, Korea

INTRODUCTION

Ehrlichiosis is a tick-borne bacterial infection caused by bacteria of genus *Ehrlichia* (Rikihisa, 2006). Currently, several species of *Ehrlichia* is known to infect and cause diseases, and can result in fever, myalgia, depression, leucopenia and thrombocytopenia which may lead to bleeding disorders. In dogs, ehrlichiosis is primarily caused by *E. canis*, which is transmitted by the brown dog tick, *Rhipicephalus sanguineus*. When the infection is established, the disease is composed of 3 distinct phase described as acute, subclinical, and chronic (Schaefer et al, 2007). Symptoms are similar for all stages; however, the chronic stage is more severe than other stages. In chronic stage, bone marrow becomes hypoplastic, resulting in severe pancytopenia. Clinical signs can vary depending on the predominant organs affected, and may include bleeding tendency (epistaxis, hematuria, melena, and petechiae/ecchymoses), splenomegaly, glomerulonephritis, renal failure, interstitial pneumonia, anterior uveitis, meningitis and severe weight loss.

This report describes a history, clinical presentation, and outcome of treatment on a case of *E. ewingii* infection diagnosed by microscopic examination and molecular analyses in a domestic dog. To author's knowledge, this is the first case report of natural *E. ewingii* infection in dogs in South Korea.

CASE DESCRIPTION

Complete blood count (CBC), serum biochemistry profiles, electrolytes, and urine dipstick test were examined by using IDEXX Vet Autoread™ Hematology analyzer, VetTest® Chemistry Analyzer, VetLyte® Electrolyte Analyzer, and VetLab® UA™ Analyzer (IDEXX Laboratories, Westbrook, ME, USA).

After total nucleic acid extraction from EDTA-treated peripheral blood by using MagMAX™ Total Nucleic Acid Isolation Kit (Applied Biosystems, Austin, TX, USA), real-time polymerase chain reactions (PCRs) for *E. canis*, *E. chaffeensis*, and *Anaplasma phagocytophilum* were performed on Illumina Eco Real-Time PCR system

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(Illumina Inc., San Diego, CA, USA). Multiplex PCR kit (QuantiTect Multiplex PCR kit, Qiagen, Valencia, CA, USA) was used by following the manufacturer's recommended protocols in a reaction volume of 20 μL. The primers and probes for the *Ehrlichia* species were adopted from published information (Doyle et al, 2005). A 65-bp oligonucleotide internal control (IC) was designed to monitor for false negative result due to failure of the PCR process causing the presence of inhibitory substances in reactions. The IC contained a non-specific 16S ribosomal RNA (rRNA) gene sequence flanked by the *uvr*C gene sequence of *Mycobacterium bovis* to minimize cross-reactivitiy with *M. bovis*. For every reaction, 0.001 μM of IC was added, which resulted in the positive signal with Ct values of 36 to 38 if PCR inhibitory substances were not present in the reaction. Recombinant vectors for each pathogen were used as positive controls.

For the PCR of *Babesia* and *Thelieria* species, the genomic DNA was extracted by using a Dynabeads® DNA DIRECT™ Universal Kit (Invitrogen Life Technologies, Inc., Carlsbad, CA, USA). The genomic DNA encoding the small subunit rRNA gene (18S rDNA) region was amplified using primers RIB-19 and RIB-20, as described previously (Zahler et al, 2000). Field isolates of *Babesia gipsoni* or *Theileria* spp. were used as positive controls.

CASE PRESENTATION

A 2-year-old intact female Beagle dog was presented with left hindlimb lameness. The symptom was firstly detected 1 week previously, and was slowly progressive. Before the onset of clinical signs, the dog was found after spending 6 days lost in a nearby hill. At the rescue, the dog had ixodid tick infestation, thus had received antiparasitic treatment (Frontline®, Merial, Duluth, GA, USA) in a local hospital. On presentation, the dog appeared to be normal otherwise, except for mild fever (39.8℃) and lameness. The dog tested negative to antibodies for *E. canis*, *B. burgdorferi*, and *A. phagocytophilum* (4Dx® kit, IDEXX Laboratories). CBC showed mild neutrophilia (1.74×10^4 cells/μL, reference interval [RI] $0.6-1.69\times10^4$ cells//μL) and thrombocytosis (5.52×10^{11} cells/μL, RI $1.75-5.00\times10^{11}$ cells/μL). Serum biochemistry profiles, electrolytes, urinalysis and radiographic examination were unremarkable.

The CBC findings were consistent with inflammation. The blood smear contained basophilic intracellular organisms (Fig. 1). The organisms, ranging in diameter from 2 to 3 μm, had a mulberry-shaped clustered body with serrated margin and were frequently observed in the cytoplasm of neutrophils (1～2 parasites/50 neutrophils). The organisms were compatible with morula stage of *Ehrlichia* or *Anaplasma* spp., and a diagnosis of canine rickettsial disease was made. To identify the organism, real-time quantitative PCR (qPCR) and gel-based PCR analyses for medically important *Ehrlichia* species, *Anaplasma phagocytophilum*, *Babesia* and *Theileria* species were performed as described previously (Zahler et al, 2000; Doyle et al, 2005; Santos et al, 2011). While PCRs for *E. canis*, *E. chaffeensis*, *A. phagocytophilum*, *Babesia* and *Theileria* species were negative, qPCR for *E. ewingii* was positive, indicating *E. ewingii* infection in the dog.

The dog was treated with oral doxycycline twice daily for 2 months. A month after treatment, the dog was fully recovered from the fever, lameness and CBC abnormalities. After 8 months of the treatment, qPCR for *E. ewingii* was negative.

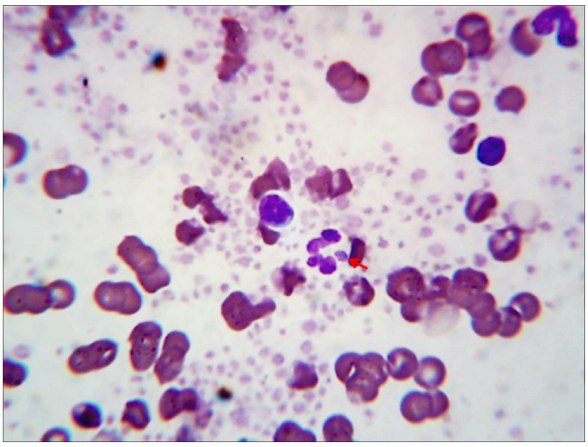

Fig. 1. Perpheral blood smear showing intracytoplasmic morula in the neutrophil. Wright-Giemsa stain, ×100 objective.

DISCUSSION

This report describes a case of *E. ewingii* infection in a dog with acute lameness. Due to the history of tick infestation, the dog was initially confirmed negative to antibodies for *E. canis*, *B. burgdorferi*, and *A. phagocytophilum*, common tick-borne pathogens in South Korea. Based on the mild inflammatory changes and no other abnormality in laboratory and radiographic examinations, the dog was suspected of suffering a musculoskeletal disorder because of highly dynamic personality of the dog. However, blood smear examination revealed unexpected intracytoplasmic morula in neutrophils and molecular analyses confirmed *E. ewingii* infection. The dog was treated with oral doxycycline for 2 months and the lameness was completely resolved. After 8 months of the treatment, qPCR for *E. ewingii* was negative.

In 1992, *E. ewingii* was firstly identified as a pathogen of ehrlichiosis in dogs on the basis of 16S rRNA gene sequence differences between the 2 most closely related species, *E. canis* and *E. chaffeensis* (Anderson et al, 1992). Since then, canine infection of *E. ewingii* has been detected by PCR of 16S rRNA gene in south central and southeastern USA, as well as a recent detection in Cameroon (Stockham et al, 1992; Dawson et al, 1996; Goldman et al, 1998; Murphy et al, 1998; Liddell et al, 2003; Ndip et al, 2005). In contrast to *E. canis* that parasitizes mononuclear cells, *E. ewingii* invades granulocytes causing fever, acute lameness or polyarthritis called as canine granulocytic ehrlichiosis (CGE). The long star tick (*Amblyomma americanum*) has been known as the primary vector. In South Korea, the tick has not been found yet; however, a recent study has demonstrated the presence of another ixodid ticks or wild rodents containing *E. ewingii* in South Korea, indicating that *E. ewingii* may have high prevalence in wild environment in South Korea (Kim et al, 2006). To the authors' knowledge, this is the first report of natural *E. ewingii* infection in dogs in South Korea.

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