

Ehrlichia canis Infection in a Dog

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Abstract : 3-year-old female Pitbull terrier dog that had been moved to Republic of Korea was diagnosed with *Ehrlichia canis* infection. Abnormal findings on a complete blood count (CBC) and serum chemistry profile were moderate anemia, mild thrombocytopenia, hyperproteinemia and hyperglobulinemia. Serologic screening test by a commercial ELISA kit for *Ehrlichia canis* was positive, and serum antibody titer was markedly high ($> 1 : 10240$). The morula of *Ehrlichia* organisms was not detected in buffy coat blood smears. Polymerase chain reaction (PCR) was done using the peripheral blood and the result was negative. Based on the serologic test results and the clinical signs, the dog was diagnosed as ehrlichiosis. The dog responded well to doxycycline and was uneventfully recovered.

Key words : canine, *Ehrlichia*, Korea.

Introduction

Ehrlichia canis infection is a potentially fatal tick borne disease of dogs (2, 10, 18). The agent can cause acute, subclinical, or chronic disease (6, 9, 17). Ehrlichiosis has been reported worldwide in association with the broad distribution of its main vector, *Rhipicephalus sanguineus* (5, 9, 10). In Republic of Korea, many of dogs came from other countries, including USA, where disease prevalence is especially high (2, 5). *Ehrlichia* infection previously has been reported in Japan in dogs moved from the USA and in local dogs (17). It has been also observed in China (16). This paper describes a case of *Ehrlichia canis* infection in Republic of Korea. The diagnosis was made based on the positive ELISA kit result and markedly increased antibody titer in a non endemic area of Republic of Korea.

Case

A 3-year-old female Pitbull terrier dog was referred to Haemaru Referral Animal Hospital on September 2004 for a 10 day history of diarrhea, fever and weight loss. The dog was imported from Texas, USA in March, 2004. On physical examination the dog was lethargic, and superficial lymph nodes were enlarged. Abnormal findings on a CBC included moderate anemia (21.4%: reference interval 37-55%), mild thrombocytopenia ($144 \times 10^3/\mu\text{L}$: reference interval $160-430 \times 10^3/\mu\text{L}$). Abnormal serum chemistry profile was hyperproteinemia (10.13 g/dl: reference interval 5.2-8.2 g/dl) and hyperglobulinemia (7.59 g/dl: reference interval 2.5-4.5 g/dl).

Abdominal radiography revealed splenomegaly. Because of the increased globulin concentration, infectious inflammatory (such as bacterial, fungal, protozoal etc.), immune-mediated (such as SLE etc.) and neoplastic diseases were considered as differential diagnoses.

Serum protein electrophoresis revealed broad γ -globulin fraction peak suggestive of a polyclonal gammopathy (Fig 1). Cytology of the aspirate smears from the enlarged superficial lymph nodes showed heterogeneous population with a predominance of small lymphocyte. Low number of macrophages, plasma cells, and neutrophils were found and that was consistent with reactive hyperplastic lymph nodes (Fig 2). Any infectious organisms were not detected.

Although tick exposure was not recognized at presentation both by physical examination and from history, additional tests included blood smear examination looking for *Babesia* organisms, commercial kit test (ELISA IDEXX 3Dx kit) screening for heartworm infection, *Ehrlichia canis* infection and Lyme disease, *Giardia* test (strip ELISA), fecal occult blood test and fecal parasite examination. All tests were negative except ELISA kit test for *Ehrlichia canis*. To confirm the positive result of the test kit, serum was submitted to Antech Diagnostics for *Ehrlichia canis* antibody titer assay (IFA, indirect immunofluorescence assay). Blood smears were examined for morula and blood was submitted to VeTek, Kyung Gi do, for PCR test using *Ehrlichia* specific primer. The serum antibody titer was $> 1 : 10240$, but morula was not found in blood smears and PCR test was negative. Because ehrlichiosis is not endemic in Republic of Korea, *Ehrlichia canis* infection was diagnosed based on the markedly increased serum antibody titer and the relevant clinical signs.

Treatment for ehrlichiosis began with 10 mg/kg/day of

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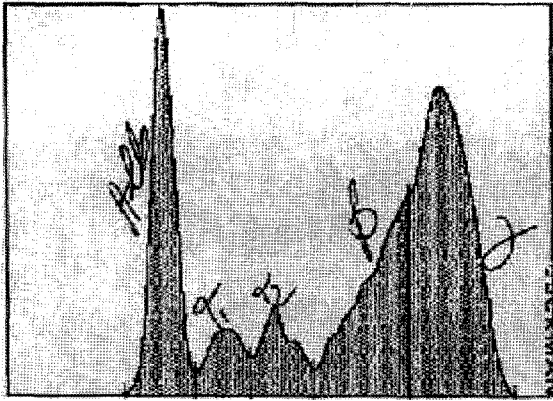


Fig 1. Serum protein electrophoresis revealed broad γ globulin fraction peak suggestive of a polyclonal gammopathy (ANTECH Diagnostics, Southaven, MS).

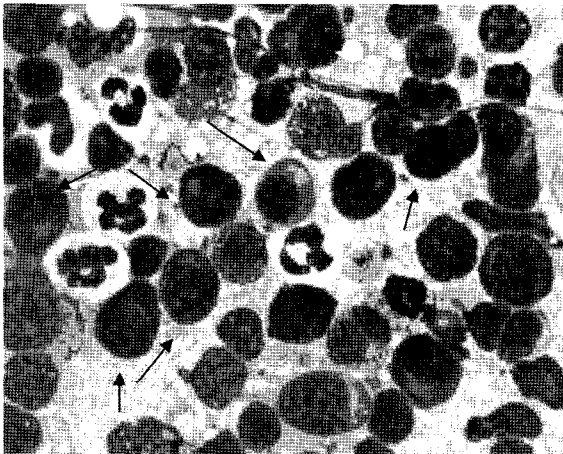


Fig 2. Lymph node cytology. Predominant cells were small lymphocytes with increased number of plasma cells (arrow) and neutrophils (Diff-Quik, x1000).

doxycycline (doxycycline hyclate capsules; Jinro Pharmaceutical) for 4 weeks with restricted diet. The response was uneventful and the general condition of the patient improved. The enlarged superficial lymph nodes returned to normal.

Discussion

Ehrlichia canis infection develops a wide variety of clinical signs which include depression, lethargy, weight loss, anorexia, pyrexia, lymphadenopathy, splenomegaly and a tendency to hemorrhage are the most common (2, 11, 15). Thrombocytopenia, pancytopenia, hyperglobulinemia is one of the main hematological abnormalities of *Ehrlichia canis* infection (5, 9). Hyperglobulinemia is usually polyclonal, and monoclonal gammopathy is rare (8, 10). This dog was depressed and anorexic, and had diarrhea, lymphadenopathy and fever, but these clinical signs were non-specific. It had a

moderate anemia and mild thrombocytopenia, moderate hyperproteinemia and hyperglobulinemia with polyclonal gammopathy. Although these hematological findings were consistent with an *Ehrlichia canis* infection, ehrlichiosis was not included in the first differential list. Ehrlichiosis is not endemic in Republic of Korea. Therefore, lymphocytic leukemia, plasma cell leukemia, idiopathic monoclonal gammopathy and multiple myeloma were considered as major differentials in view of the similar clinical signs and laboratory findings of these diseases (14). A serological examination including IFA tests detecting antibodies to *Ehrlichia canis* is the useful method for diagnosing *Ehrlichia canis* infection in non endemic area as well as endemic area (2, 4, 9). The median reciprocal *Ehrlichia canis* titer was $> 1 : 8192$ in 19 naturally infected case (15), whereas it was $> 1 : 5120$ in another study about 62 patients (5). In this case, the markedly high titers ($> 1 : 10240$) against *Ehrlichia canis* led to the diagnosis of *Ehrlichia canis* infection.

In PCR test a negative result was observed from peripheral blood in this case. It is possible that the organism could be sequestered in other sites of the body (e.g., spleen) without detection (6, 11). In one study, four dogs in the subclinical or chronic stages of *Ehrlichia canis* infection had positive PCR results from splenic aspirates and bone marrow samples; whereas three of the four had negative PCR results from blood samples (7). Another study shows that results of PCR seemed to be less sensitive in these chronic cases, as compared to the acute phase of *Ehrlichia canis* infections in dogs. Organisms may hide in the tissue such as lung, liver, spleen, etc (15). If PCR was done with spleen or bone marrow samples in this case, positive results might be obtained. Therefore, in chronic cases spleen or bone marrow samples as well as peripheral blood should be screened by PCR test for suspected organisms (6, 15).

It is possible that the dog was imported with the disease in that *Ehrlichia canis* infection may persist after a spontaneous clinical recovery or after ineffective treatment, and such animal may develop the subclinical form of the disease which may last several years (4, 6). Recently, positive results were reported in a study using spleen tissues of rodents at some military installations and training sites in Republic of Korea by PCR using *Ehrlichia canis* specific primer (3, 12). So it is also plausible that this dog had been infected after imported to Republic of Korea. This disease can be underrecognized and underreported because of the nonspecific clinical signs (2, 13). Nowadays, increasing numbers of dogs are imported from other endemic countries. Therefore, ehrlichiosis should also be screened during the routine quarantine, and should be considered in dogs with hyperglobulinemia, and thrombocytopenia even in Republic of Korea.

References

1. Breitschwerdt EB, Hegarty BC, Hancock SI. Doxycycline Hyclate Treatment of Experimental Canine Ehrlichiosis

- Followed by Challenge Inoculation with Two Ehrlichia canis Strains. Antimicrob Agents and Chemother 1998; 42: 362.
2. Burghen GA, Beisel WR, Walker JS, Nims RM, Huxsoll DL, Hildebrandt PK. Development of hypergammaglobulinemia in tropical canine pancytopenia. Am J Vet Res 1971; 32: 749-756.
 3. Chae JS, Kim C, Kim E, Hur E, Klein TA, Kang T, Lee H, Song J. Molecular Epidemiological Study for Tick-Borne Disease (Ehrlichia and Anaplasma spp.) Surveillance at Selected US Military Training Sites/Installations in Korea. Ann N Y Acad Sci 2003; 990: 118-125.
 4. Codner EC, Farris-Smith LL. Characterization of the subclinical phase of ehrlichiosis in dogs. J Am Vet Med Assoc 1986; 189: 47-50.
 5. Frank JR, Breitschwerdt EB. A Retrospective Study of Ehrlichiosis in 62 Dogs from North Carolina and Virginia. J Vet Intern Med 1999; 13: 194-201.
 6. Harrus S, Waner T, Aizenberg I, Bark H. Therapeutic effect of doxycycline in experimental subclinical canine monocytic ehrlichiosis: evaluation of a 6-week course. J Clin Microbiol 1998; 36: 2140-2142.
 7. Harrus S, Waner T, Aizenberg I, Foley JE, Poland AM, Bark H. Amplification of Ehrlichial DNA from Dogs 34 Months after Infection with Ehrlichia canis. J Clin Microbiol 1998; 36: 73.
 8. Harrus S, Waner T, Avidar Y, Bogin E, Peh H, Bark H. Serum protein alterations in canine ehrlichiosis. Vet Parasitol 1996; 66: 241-249.
 9. Harrus S, Waner T, Bark H. Canine monocytic ehrlichiosis: an update. Compend Cont Educ Pract Vet 1997; 19: 431-447.
 10. Harrus S, Waner T, Bark H, Jongejan F, Cornelissen AW. Recent advances in determining the pathogenesis of canine monocytic ehrlichiosis. J Clin Microbiol 1999; 37: 2745-2749.
 11. Heeb HL, Wilkerson MJ, Chun R, Ganta RR. Large Granular Lymphocytosis, Lymphocyte Subset Inversion, Thrombocytopenia, Dysproteinemia, and Positive Ehrlichia Serology in a Dog. J Am Anim Hosp Assoc 2003; 39: 379-384.
 12. Kim CM, Yi YH, Yu DH, Lee MJ, Cho MR, Desai AR, Shringi S, Klein TA, Kim HC, Song JW. Tick-Borne Rickettsial Pathogens in Ticks and Small Mammals in Korea. Appl Environ Microbiol 2006; 72: 5766-5776.
 13. McQuiston JH, McCall CL, Nicholson WL. Zoonosis Update: Ehrlichiosis and related infections. J Am Vet Med Assoc 2003; 223: 1750-1756.
 14. Michels GM, Boon GD, Jones BD, Puget B. Hypergammaglobulinemia in a dog. J Am Vet Med Assoc 1995; 207: 567-568.
 15. Mylonakis ME, Koutinas AF, Breitschwerdt EB, Hegarty BC, Billinis CD, Leontides LS, Kontos VS. Chronic Canine Ehrlichiosis (Ehrlichia canis): A Retrospective Study of 19 Natural Cases. J Am Anim Hosp Assoc 2004; 40: 174-184.
 16. Pan HUA, Liu S, Ma Y, Tong S, Sun Y. Ehrlichia-like Organism Gene Found in Small Mammals in the Suburban District of Guangzhou of China. Ann N Y Acad Sci 2003; 990: 107-111.
 17. Suto Y, Suto A, Inokuma H, Obayashi H, Hayashi T. First confirmed canine case of Ehrlichia canis infection in Japan. Vet Rec 2001; 148: 809-811.
 18. Waner T, Harrus S, Jongejan F, Bark H, Keysary A, Cornelissen A. Significance of serological testing for ehrlichial diseases in dogs with special emphasis on the diagnosis of canine monocytic ehrlichiosis caused by Ehrlichia canis. Vet Parasitol 2001; 95: 1-15.

개 엘리키아 감염 1 증례

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요 약 : 한국으로 이주한 3년령의 암컷 핏볼테리어가 엘리키아 증으로 진단되었다. 혈액검사상 중등도의 빈혈, 미약한 혈소판감소증과 고단백혈증, 고글로불린혈증을 보였다. 혈청검사서 엘리키아 키트에 양성이었으며, 면역형광 항체법에 의한 항체가는 1:10240 이었다. 백혈구 연중 도말상에서는 엘리키아 감염체가 관찰되지 않았고 말초혈액을 이용하여 PCR 검사를 하였으나 음성이었다. 환자는 doxycycline에 잘 반응하였으며 치료 후 건강을 회복하였다.

주요어 : 개, 엘리키아, 한국