NOTE *Internal Medicine*

Astasia and Pyrexia Related to *Borrelia garinii* Infection in Two Dogs in Hokkaido, Japan

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ABSTRACT. Two dogs that exhibited sudden astasia, anorexia and fever higher than 40°C were suspected of having Lyme disease in July 2011. Clinical symptoms gradually improved with antibiotic treatment in both cases. Polymerase chain reaction and sequence analysis revealed *Borrelia garinii* DNA fragments in the peripheral blood in the acute disease phase. Serological tests, including enzyme linked immunosorbent assay and Western blot analysis, showed an increased IgG antibody titer against *Borrelia* pathogens in one of the dogs. These findings suggested that diagnosis of the two dogs was Lyme disease related to *B. garinii* infection.
KEY WORDS: *Borrelia garinii*, canine, Japan, Lyme disease.

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Ticks can transmit various pathogens, including bacteria, rickettsia and protozoa. *Borrelia* is a tick-borne zoonotic pathogen that causes Lyme disease [6]. Many *Borrelia* species are known to exist worldwide. *Borrelia burgdorferi* sensu stricto is a well known Lyme disease pathogen that has been mainly isolated in North America and Europe [4]. The clinical symptoms of Lyme disease caused by *B. burgdorferi* sensu stricto infection in humans include erythema migrans, arthritis, neurological abnormalities, circulatory disturbances, conjunctivitis, muscular pain and gastrointestinal complaints [23, 24]. In areas of endemic *B. burgdorferi* sensu stricto infection, Lyme disease can also occur in dogs, with clinical signs characterized by lameness, arthritis, fever and anorexia [1, 5, 9, 21, 25].

B. burgdorferi sensu stricto has not been detected in Japan, although *Borrelia garinii* and *Borrelia afzelii* have been isolated from humans with Lyme disease [16, 17]. The chief complaint of human patients with Lyme disease in Japan is an erythema migrans skin rash followed by muscular pain, arthritis, fever and neurological abnormalities [7]. There are limited data regarding cases of canine Lyme borreliosis in Japan. Neurological abnormalities, including astasia, persistent tonic convulsions and hyper-reflexia, were the clinical symptoms of Lyme disease reported in dogs in Hokkaido, Japan in the 1990s [2, 3]. Although *B. burgdorferi* was considered to be the causative agent at the time, the actual pathogen has not yet been identified. The pathogenicity of *B. garinii* and *B. afzelii* in dogs is uncertain. The present report describes two clinical cases of sudden astasia and pyrexia related to *B. garinii* infection in dogs in Hokkaido, Japan.

Case 1 was a 12-year-old castrated male Collie that lived in Sapporo, Hokkaido, Japan. The dog was mostly kept indoors, with some outdoor exposure. A history of tick bites was unclear. The animal presented at Maetani Animal Hospital in Sapporo, Hokkaido, Japan on July 13, 2011 with complaints of sudden astasia and anorexia (day 1). The patient had a body temperature of 40.6°C and a heart rate of 144 bpm. Physical examination revealed abnormal proprioception in the hind legs. On day 1, hematological examination revealed an increased white blood cell count (WBC: 37,500/μl), and serum biochemical analysis showed increased C-reactive protein (CRP: 20 mg/dl). On day 4, increased creatine kinase activity (CPK: 1,901 IU/l) was recorded. The patient was treated for suspected Lyme disease with ampicillin (from day 1 to 60). Clindamycin was also administered from day 4 to day 30. The general condition of the patient gradually improved. Astasia was absent on day 7, and the dog was discharged from the hospital on day 8. On day 60, the WBC and CRP values were normal at 7,400/μl and 0.15 mg/dl, respectively (Fig. 1A).

Case 2 was a 7-year-old male Australian shepherd that lived in Sapporo. The dog was kept indoors with some outdoor exposure and an uncertain history of tick exposure. The animal presented to the Maetani Animal Hospital on July 18, 2011 with complaints of sudden astasia, anorexia and lethargy (day 1). The patient showed prostration with a body temperature of 40.3°C. Increased WBC count (18,700/μl) with neutrophilia and CRP (7.1 mg/dl) were recorded on day 1. There was increased CPK activity of 2,000 IU/l on day 2. Thoracic radiograph revealed concurrent pneumonia.

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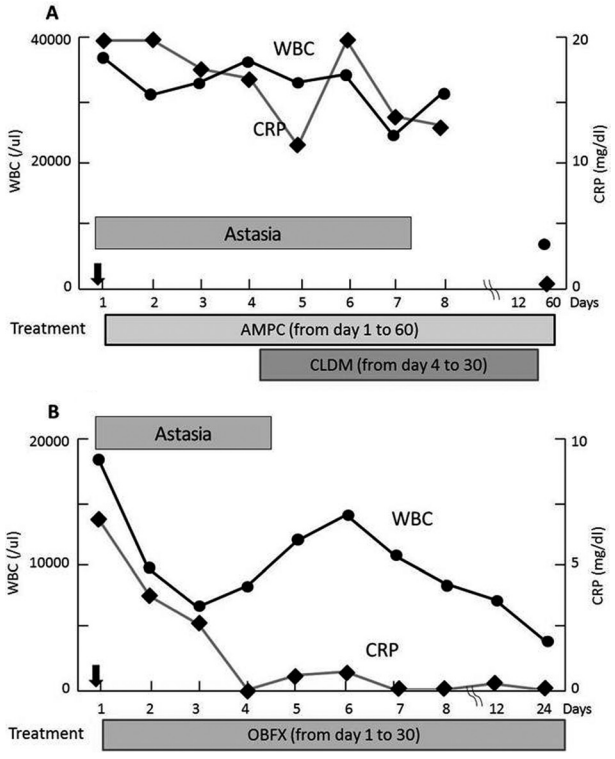

Fig. 1. Clinical courses of cases 1 (A) and 2 (B). WBC: white blood cells, CRP: C-reactive protein, AMPC: ampicillin, CLDM: clindamycin, OBFX: orbifloxacin. Arrows indicate the time of blood sampling for PCR.

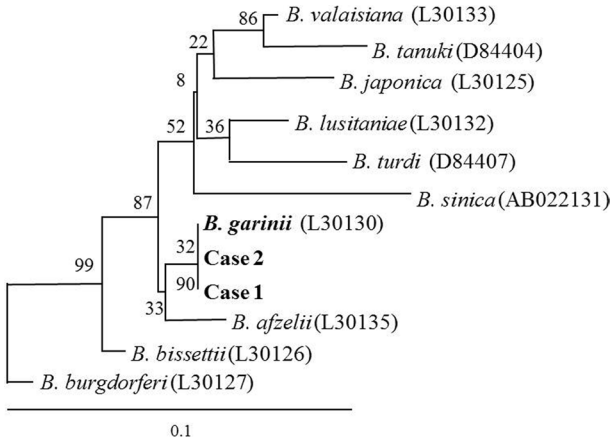

Fig. 2. Phylogenetic tree of *Borrelia* spp. based on partial sequences of the 5S-23S rRNA intergenic spacer region. *Borrelia garinii* and *Borrelia* spp. detected in cases 1 and 2 are indicated in bold type. Analysis represents a sequence divergence of 10%.

Although Lyme disease was one of the differential diagnoses, the patient was treated with orbifloxacin for concurrent pneumonia starting on day 1. The general condition of the patient gradually improved, and the dog was discharged from the hospital on day 7 (Fig. 1B).

Peripheral blood from each patient in the acute phase before treatment was collected in EDTA tubes for pathogenic analysis by polymerase chain reaction (PCR). DNA samples were extracted using the QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). Nested PCR for *Borrelia* infection was performed according to Hiraoka *et al.* [8]. The first round of PCR was performed with RIS1 and RIS2 primers [20], and the second round was performed with rrf2 and rrl2 primers [8]. Nested PCR was designed to amplify a 250-bp fragment of the 5S-23S rDNA intergenic spacer of *Borrelia*. Positive results were obtained in case 1 and case 2. Sequence analysis of the positive products using a previously described direct sequencing method [10] revealed that *Borrelia* DNA fragments from cases 1 and 2 were most closely related to *B. garinii,* with percent identities of 99.5% (198/199) and 99.6% (246/247), respectively. *Borrelia* gene sequences were deposited in the GenBank database under accession numbers AB775651 (case 1) and AB775652 (case 2). Multiple alignment analysis and phylogenetic tree construction were performed as previously described [8]. The GenBank accession numbers of the *Borrelia* 5S-23S rDNA intergenic spacer sequences used to construct the phylogenetic tree are as follows: *B. burgdorferi* strain B31, L30127; *B. garinii* strain NT29, L30130; *B. afzellii* strain VS461, L30135; *B. japonica* strain Cow611C, L30125; *B. lusitaniae* strain Poti B3, L30132; *B. turdi* strain Ya501, D84407; *B. tanukii* strain HK501, D84404; and *B. valaisiana* strain UK, L30133. *Borrelia* spp. detected in cases 1 and 2 both belonged to the same clade as *B. garinii* (Fig. 2). Molecular methods suggest that the two dogs were infected with *B. garinii* and that the clinical symptoms are strongly related to *Borrelia* infection.

Paired serum samples were collected for serological evaluation of *Borrelia* infection. In case 1, serum samples collected on days 10 and 29 were examined for sub-acute and recovery phases, respectively. In case 2, serum samples collected on days 6 and 24 were tested for acute and recovery phases, respectively. An enzyme immunoassay kit (IDEXX canine snap 4D test®, IDEXX Laboratories, Inc., Westbrook, Maine, U.S.A.) was used to detect antibodies against *B. burgdorferi* sensu stricto based on specific recombinant major surface proteins. All of the samples were negative. *Borrelia* enzyme linked immunosorbent assay (ELISA) kits (*recom*Well Borrelia IgG® and *recom*Well Borrelia IgM®, Mikrogen GMBH, Neuried, Germany) were used to detect IgG and IgM antibodies against *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii* in serum samples. Only IgG in the recovery phase in case 2 showed positive results. Results of serum analysis suggest that the dog in case 2 was exposed to a *Borrelia* spp., such as *B. garinii* or *B. afzelii.*

To confirm the ELISA results, serum samples from case 2 were analyzed using the *recom*Blot Borrelia canis IgG® kit (Mikrogen GMBH), which includes several recombinant proteins for *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*. Results are shown in Fig. 3. A total of five positive bands, including p41 of *B. burgdorferi* sensu stricto, OspC of *B. burgdorferi* sensu stricto + *B. afzelii*, OspC of *B. gari-*

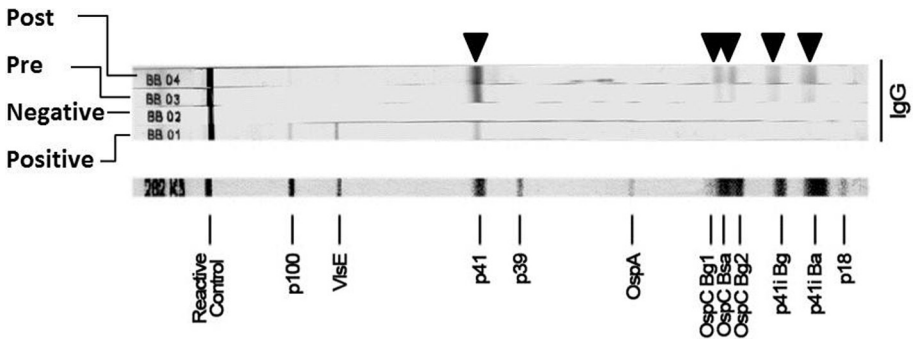

Fig. 3. Western blot analysis of serum samples from case 2 using several recombinant proteins in pathogenic *Borrelia*. A total of five positive bands (arrow heads) were observed. Pre and Post indicate serum reactions in the acute and recovery phases, respectively. Negative and positive controls were supplied in the kit. All of the proteins supplied in the kit are shown on the test strip, including the lowest band.

nii, p41/internal of *B. garinii* and p41/internal of *B. afzelii*, were observed in the serum from both the acute and recovery phases. Serum from the recovery phase showed a stronger reaction compared to serum from the acute phase. Although the Western blot results were not specific for *B. garinii*, they proved that case 2 was exposed to *Borrelia* spp.

In the present study, two dogs with astasia and fever were suspected of having early stage Lyme disease. Molecular analysis revealed *B. garinii* DNA fragments in the peripheral blood of both dogs in the acute disease phase. These findings indicate probable *B. garinii* infection in both cases. Although serological tests proved infection with *Borrelia* in case 2, a serological diagnosis of Lyme disease was not confirmed in case 1. Case 2 represents the first confirmed clinical case of canine Lyme disease caused by *B. garinii* infection in Japan demonstrated using molecular and serologic methods. The lack of a positive antibody reaction in case 1 is unknown. Because IgG seroconversion generally occurs in most canine Lyme disease patients after the recovery phase [1, 11], the diagnosis in case 1 should be carefully defined. However, a previous study reported that only 4.3% of sera from dogs clinically diagnosed with borreliosis have significantly high *Borrelia* antibody titers [9]. Case 1 was suspected of having Lyme disease related to *B. garinii*.

A positive reaction to antibiotic treatment would support a Lyme disease diagnosis. Improvements in the general health of both animals were observed. Because beta-lactams, macrolides and tetracyclines are generally recommended for treating *Borrelia* infections [14], administration of ampicillin and clindamycin in case 1 was reasonable. Orbifloxacin was selected in case 2 due to concurrent pneumonia. Several studies have reported that *Borrelia* spp. are resistant to quinolone antibiotics [12, 13, 19]. However, some of new quinolones have shown *in vitro* activity against the *B. burgdorferi* sensu lato complex [15]. Orbifloxacin is a new quinolone recently developed for use in animals, although its effects against *Borrelia* have not been evaluated. The actual efficacy of orbifloxacin against *Borrelia* infection in case 2 cannot be determined, because natural recovery may have occurred after the pneumonia improved following orbifloxacin treatment.

Although many human cases of *B. garinii* infection have been reported, including in Hokkaido, Japan [7], there have been few reports of canine Lyme disease since the first clinical case was described in 1993 [2]. Laboratory examination procedures for Lyme disease, including molecular and serological methods, are not readily available in veterinary clinics in Japan, which may be a key reason for the lack of canine Lyme disease case reports. Because dogs do not exhibit pathognomonic signs of infection, such as the characteristic erythema migrans rash in humans [6], a diagnostic system to detect laboratory evidence for *Borrelia* infection is important in canine medicine.

The tick *Ixodes persulcatus*, which is distributed in the northern part of Japan [26], is a known vector of *B. garinii* [16]. As *I. persulcatus* shows higher activity from May to July in Hokkaido [18], it is reasonable that the both cases were found in July. This tick has been reported as a dominant tick species in dogs in Hokkaido, Japan [22]. Canine Lyme disease caused by *B. garinii* may be widely distributed in the Hokkaido area. Because Lyme disease is zoonotic tick-borne infection, further epidemiological studies on canine Lyme disease are necessary from both public and animal health perspectives.

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