NOTE *Clinical Pathology*

A Case Report: a Dog with Acute Onset of *Hepatozoon canis* Infection

Masato SAKUMA[1)], Yoshitaka NAKAHARA[1,2)], Hiroyuki SUZUKI[3,4)], Masato UCHIMURA[4)], Zin SEKIYA[5)], Asuka SETOGUCHI[1)] and Yasuyuki ENDO[1)]*

[1)]*Laboratory of Veterinary Internal Medicine, Faculty of Agriculture, Kagoshima University, 1–21–24 Korimoto, Kagoshima 890–0065,* [2)]*Veterinary Medical Center, Faculty of Agriculture, the University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–8657,* [3)]*Chuzan Animal Hospital, 7–25–6 Higashi Taniyama, Kagoshima 891–0113,* [4)]*Usuki Animal Clinic, 879–2 Usuki-cho, Kagoshima 890–0074 and* [5)]*Kanoya Animal Clinic, 4536–11 Nozato-cho, Kanoya, Kagoshima 893–0055, Japan*

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ABSTRACT. We present a clinical overview of a dog with acute onset of *Hepatozoon canis* infection. A stray female beagle dog of unknown age was referred to Kagoshima University showing anemia. Blood tests revealed the presence of anemia, thrombocytopenia, hyperproteinemia, polyclonal gammopathy, hypoalbuminemia, and elevated creatine kinase and alkaline phosphatase activities. In addition, capsule-like organisms were detected in the cytoplasm of approximately 50% of neutrophils in blood smears. *H. canis* infection was confirmed by polymerase chain reaction and DNA sequencing analyses. Amplified DNA fragments revealed 100% identity to the 18S ribosomal RNA gene of *H. canis*. The clinical symptoms improved after the administration of antibiotics. Hepatozoonosis in dogs is rare, but veterinarians should be alert to its possible acute onset.

KEY WORDS: acute onset, canine, *Hepatozoon canis*.

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Hepatozoon is a genus of protozoa belonging to phylum Apicomplexa. Dogs are known to be susceptible to infection by both *Hepatozoon canis* and *H. americanum*, but only the former has been confirmed in Japan [3, 10, 15–19, 21]. *Hepatozoon* parasites are transmitted by ticks, and the infection becomes established after the ingestion of infected ticks [4]. Ingested parasites enter the blood or lymphatic vessels through the intestinal mucosa and reach the bone marrow. Most *H. canis* infected dogs show clinical latency, and several factors, such as co-infection with other infectious agents and immunosuppression, are thought to be important factors promoting the acute onset of hepatozoonosis [2, 4, 6, 9, 20]. A few reports have described the acute onset of *H. canis* infection and its epidemiological distribution in Japan [10, 15–19]. The clinical symptoms associated with severe *H. canis* infection are anemia, fever, lethargy and weight loss [5]. Ataxia and lameness are also sometimes observed in *Hepatozoon*-infected dogs, due to the development of osteomyelitis. Characteristic hematological abnormalities in *H. canis* infection include non-regenerative anemia, thrombocytopenia, neutrophilia, hyperproteinemia, hypoalbuminemia, polyclonal gammopathy, and increased serum creatine kinase (CK) and alkaline phosphatase (ALP) concentrations [5–7]. The detection of capsule-like gamonts in the cytoplasm of neutrophils in blood smears is helpful for diagnosing hepatozoonosis. Other diagnostic techniques, such as polymerase chain reaction (PCR) for *Hepatozoon*-derived genomic DNA or enzyme linked immunosorbent assay for anti-*Hepatozoon* antibodies, are also potentially useful [8, 10, 11]. Several drugs have been proposed as candidate therapeutics for *H. canis* infection, but no satisfactory results have yet been obtained and there is currently no established treatment protocol for *H. canis* infection [5, 12]. As there is limited information available regarding the clinical features of *H. canis* infection, we present clinical findings of an *H. canis*-infected dog with anemia and present an overview of this rare case.

A stray female beagle dog of unknown age was cared for by a temporary owner in the suburbs of Kagoshima, Japan. The owner took her to a private veterinary hospital for a health check. Severe dehydration, numerous ticks on the skin and pale mucus membranes were detected by physical examination (day 1). Fecal examination revealed roundworm, whipworm and tapeworm infections. Blood examination demonstrated the presence of anemia [packed cell volume (PCV), 17%], leukocytosis (44,900/μl) and mild thrombocytopenia [platelet (PLT) count, $11.7 \times 10^4/\mu l$]. *Babesia gibsoni* infection was confirmed in the blood smear specimen. Treatment with diminazene (3 mg/kg, intramuscularly, on days 1–3, 8–10 and 15–17) and antiparasitics (praziquantel, 5 mg/kg; pyrantel, 14.4 mg/kg; febantel, 15 mg/kg; orally, on day 1) was given. *Babesia* and intestinal parasites became undetectable on day 13, but the anemia failed to improve. The dog was then introduced to Kagoshima University Veterinary Teaching Hospital (KUVTH) on day 35, in order to establish a diagnosis.

A complete blood count (CBC) was also performed at KUVTH, and the results indicated the presence of moderate anemia (PCV, 19%) and thrombocytopenia (PLT, $7.5 \times 10^4/\mu l$). Serum biochemistry revealed hyperproteinemia (9.4 g/dl), mild hypoalbuminemia (2.3 g/dl), and increased CK (339 U/l), ALP (234 U/l), and C-reactive protein (CRP, 13.0 mg/dl). Electrophoresis of serum proteins indicated polyclonal gammopathy and decreased albumin/globulin (A/G)

* CORRESPONDENCE TO: ENDO, Y., Laboratory of Veterinary Internal Medicine, Faculty of Agriculture, Kagoshima University, 1–21–24 Korimoto, Kagoshima 890–0065, Japan.
e-mail: yendo@agri.kagoshima-u.ac.jp

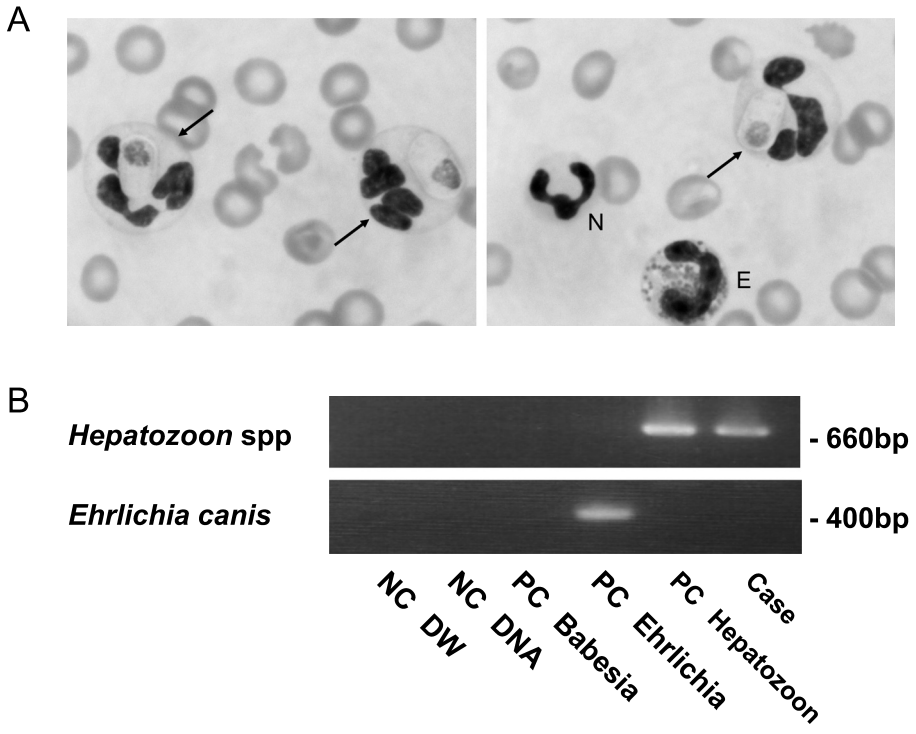

Fig. 1. Detection of *H. canis* in blood smears and of *Hepatozoon*-derived DNA by PCR. (A) Capsule-like structures of *H. canis* were detected in the cytoplasm of neutrophils in blood smear specimens prepared at day 35 (× 1000). Arrows indicate the *H. canis*-infected enlarged neutrophils. Wright-Giemsa staining (pH 6.8). N, morphologically normal neutrophil; E, eosinophil. (B) The results of PCR analyses for the detection of *Hepatozoon* spp or *E. canis*-derived DNA. *Hepatozoon* spp DNA (upper panel), but not *E. canis* DNA (lower panel), was detected in a blood sample at day 35. NC, negative control; PC, positive control.

ratio (0.26). In addition to the findings of non-regenerative anemia, enlarged neutrophils with a capsule-like structure in their cytoplasm were detected in blood smears; however, *B. gibsoni* infection in erythrocytes was not observed at this time. This capsule-like structure was morphologically similar to the gamont of *H. canis* (Fig. 1A). Forty-eight percent of neutrophils contained these capsule-like structures in their cytoplasm.

We carried out PCR analysis to determine if this capsule-like structure was of *Hepatozoon* origin [11, 22]. As shown in Fig. 1B, the dog was positive for *Hepatozoon* spp infection, but negative for *Ehrlichia canis* infection. In addition, genotypic analysis of the 18S ribosomal RNA gene revealed 100% identity to that of a Japanese isolate of *H. canis* (GenBank accession number AF418558) [11]. Based on these findings, the dog was finally diagnosed with acute onset of *H. canis* infection.

Figure 2 summarizes the treatment and clinical course of this case. We initially treated this case with trimethoprim-sulfadiazine (15 mg/kg, orally, q12h) for 1 week. After the diagnosis of hepatozoonosis, clindamycin (10 mg/kg, orally, q12h) followed by doxycycline (7 mg/kg, orally, q12h) were administered, based on previous reports [14]. PCV and PLT counts gradually recovered and CRP levels decreased, concurrent with the recovery of other hematological parameters (Fig. 2). On day 86, gamonts in neutrophils became undetectable in blood smears. Doxycycline was discontinued, and no clinical signs of relapse were detected, although PCR analyses continued to show positive results (Fig. 2).

The acute onset of hepatozoonosis in a Japanese dog was first reported by Murata *et al.* in 1991 [16]. Since then, several reports concerning clinical and subclinical cases of *H. canis* infection have been published [10, 15, 17–19]. The onset of hepatozoonosis in dogs is still rare, though a previous epidemiological study suggested a relatively high serological prevalence in Japanese dogs [10]. *H. canis* infection should therefore be included in the differential diagnosis for anemia in areas containing *Hepatozoon*-bearing ticks, especially in western Japan. In this study, we successfully diagnosed and treated a case of acute onset *H. canis* infection. Because there is presently limited information about the therapeutics available for use against *H. canis* infection, the clinical findings from this case should be useful to determine the future directions for the establishment of a treatment strategy [5, 12–14].

This case raises three points of particular interest: The first point is what caused or triggered disease onset. It has been shown that most infected dogs show clinical latency and that

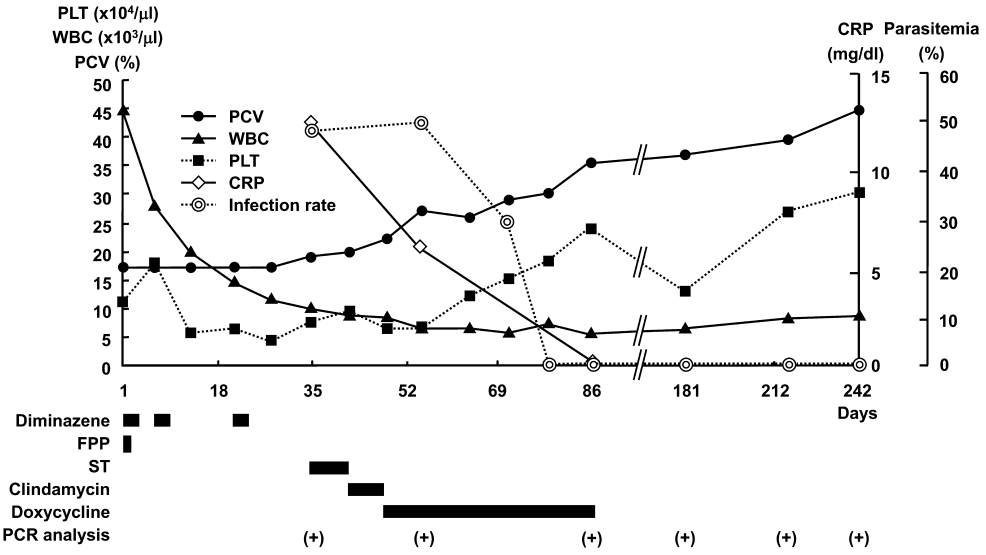

Fig. 2. Changes in clinical parameters and a schema of the treatment schedule. PCR analysis was performed using a *Hepatozoon* spp-specific primer set [11]. PCV, packed cell volume; WBC, leukocyte counts; PLT, platelet counts; CRP, C-reactive protein; FPP, antiparasitic containing febantel, pyrantel and praziquantel; ST, trimethoprim-sulfadiazine.

only immunosuppressed cases tend to show clinical signs [2, 4, 6, 9, 20]. In this case, *Babesia* infection was detected at the time of the first hospital visit, and the dog might have been stressed as a result of this. In addition, the dog was a stray and was in poor condition and suffering from severe dehydration and several intestinal parasitic infections. This might also have induced an immunocompromised state, although intestinal parasitic infections might have been produced as a result. It therefore seems likely that the *Babesia* infection and malnutrition were the major factors responsible for the acute onset of hepatozoonosis in this dog. We do not know when this dog became infected with *H. canis* or how long the development of clinical symptoms took, but these factors could possibly be determined if the antibody response against *H. canis* could be evaluated. *H. canis* was not completely eliminated in this dog, as shown by PCR analyses, and the dog therefore remained at risk of relapse. Close monitoring is required to detect any signs of relapse.

The second point is what caused the hematological and biochemical abnormalities: Four factors including babesiosis, intestinal parasites, hepatozoonosis, and chronic inflammation were candidate causes for anemia in this dog. Babesiosis and intestinal parasites could be excluded because they became undetectable after the administration of specific treatments. Myelosuppression due to *H. canis* and dysfunction of transferrin caused by chronic inflammation are possible causes. Evaluation of bone marrow and measurements of total iron binding capacity and serum iron would be necessary to distinguish between these. The direct effect of *H. canis* on thrombocytopenia is suspicious, but the detailed mechanism of this process is unknown, as described previously [1]. In addition, biochemical abnormalities, including increased ALP, CK and CRP levels, decreased A/G ratio, and polyclonal gammopathy, were detected. These changes are typical in *H. canis* infected dogs and are therefore likely due to the direct effects of the infection [5–7]. Although we were unable to identify any signs of infectious myositis or osteomyelitis, this dog might have been suffering from subclinical inflammatory lesions, because parameters characteristic of muscle and bone lesions, including ALP and CK, were elevated.

The third point is the treatment strategy: The anemia and thrombocytopenia improved after the administration of clindamycin and doxycycline. No effective therapeutic strategy has yet been established [5, 12], and these two drugs might therefore be suitable candidates as specific drugs for the treatment of canine hepatozoonosis. However, they have previously only been reported to be effective against *H. americanum* infection [13, 14]. It is also possible that the dog's condition improved spontaneously following improved nutritional conditions or the elimination of the other factors responsible for its immunosuppressed state. Further studies are required to establish a specific treatment protocol for dogs with hepatozoonosis, especially those cases caused by *H. canis* infection.

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