Neutropenia Associated with Osteomyelitis due to *Hepatozoon canis* Infection in a Dog

Takako SHIMOKAWA MIYAMA¹⁾, Saori UMEKI¹⁾, Kenji BABA²⁾, Kumiko SADA¹⁾, Hiroko HIRAOKA³⁾, Yasuyuki ENDO⁴⁾, Hisashi INOKUMA⁵⁾, Masaharu HISASUE⁶⁾, Masaru OKUDA¹⁾ and Takuya MIZUNO^{1)*}

Laboratories of ¹⁾Veterinary Internal Medicine, ²⁾Veterinary Parasitology and ³⁾Veterinary Clinical Pathology, Department of Veterinary Medicine, Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, ⁴⁾Laboratory of Veterinary Internal Medicine, Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, ⁵⁾Department of Clinical Veterinary Science, Obihiro University of Agriculture and Veterinary Medicine, Inada, Obihiro, Hokkaido 080-8555 and ⁶⁾Laboratory of Internal Medicine II, School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Sagami-hara, Kanagawa 229-8501, Japan

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ABSTRACT. A 4-year-old, intact male Shiba dog was referred to Yamaguchi University Animal Medical Center, Yamaguchi, Japan, for the following complaints: anorexia, lethargy, intermittent fever, gingival bleeding and abdominal purpura. The dog presented with persistent neutropenia. Histopathological examination of a bone marrow sample revealed round to oval structures that resembled *Hepatozoon* micromerozoites and formed a “wheel-spoke” pattern. Furthermore, mature neutrophils were observed around these structures. PCR and sequencing using bone marrow aspirate confirmed *Hepatozoon canis* (*H. canis*) infection. These findings suggest that the neutropenia observed in this case was associated with osteomyelitis due to *H. canis* infection. This is the first report of neutropenia associated with *H. canis* infection. *H. canis* infection can be included in the differential diagnosis in canine cases of neutropenia in areas where the disease is endemic.

KEY WORDS: canine, *Hepatozoon canis*, neutropenia.

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Hepatozoon canis (*H. canis*) is a tick-borne protozoan reported from across the world, including Japan [1, 9, 10, 14–16, 18]. The reported clinical signs associated with *H. canis* infection include anemia, fever, lethargy and weight loss [1, 4]. Ataxia and lameness due to the development of osteomyelitis have also been reported [1, 9, 11]. The common hematological abnormalities are anemia, thrombocytopenia and neutrophilia [1, 13]. The neutrophil count is usually normal when parasitemia is low and is elevated in dogs with high parasitemia [1, 4]. However, there are no reports of neutropenia associated with *H. canis* infection. The current case report describes a dog with *H. canis* infection that presented as not the commonly observed neutrophilia but neutropenia.

A 4-year-old, intact male Shiba dog weighing 8.4 kg with a 1-month history of decreased appetite, lethargy, intermittent fever, gingival bleeding and abdominal purpura was referred to Yamaguchi University Animal Medical Center.

The dog had been housed outdoors and vaccinated, but the booster interval was >4 years. He was protected against heartworm by monthly prophylaxis. Complete blood count (CBC) disclosed repeated leucopenia (1,700–3,600/ μ l; reference range, 6,000–17,000/ μ l) and thrombocytopenia (70,000–136,000/ μ l; reference range, 200,000–500,000/ μ l) (Days 1 to 21). The dog was treated with prednisolone (Day 1), methylprednisolone acetate (Day 15) and amoxicillin

(Days 1 to 23) by the referring veterinarian; this treatment led to slight clinical improvement.

At the time of presentation to Yamaguchi University (Day 23), the CBC and serum biochemical findings included mild anemia (hematocrit, 31%; reference range, 37–55%), severe neutropenia (1,056/ μ l), thrombocytopenia (54,000/ μ l) and increased levels of alkaline phosphatase (ALP, 385 U/l; reference range, 47–254) and C-reactive protein (CRP, 3.95 mg/dl; reference range, 0.00–1.00 mg/dl). Physical examination revealed lethargy and anorexia with a normal rectal temperature (38.7°C). The findings of thoracic and abdominal radiography and abdominal ultrasonography were unremarkable. Bone marrow aspiration and core biopsy were performed on Day 29 in order to determine the cause of continuous neutropenia and thrombocytopenia.

The bone marrow aspirate smears revealed normocellularity and a decreased myeloid to erythroid (M:E) ratio (0.65; Fig. 1A and Table 1). The maturation and morphology of the erythrocytes and granulocytes were normal; however, the number of mature neutrophils was considerably decreased (Fig. 1A). Megakaryocytic cells were seen occasionally. Monocytic hyperplasia and increased phagocytosis were also observed. Some macrophages had capsule-like structures containing a round eosinophilic core in their cytoplasm (Fig. 1B and 1C). Immune-mediated neutropenia (IMN) was suspected on the basis of selective neutrophilic hypoplasia.

While the histopathological results of the bone marrow core biopsy were awaited, the dog was treated with prednisolone (2 mg/kg, q12 h, PO for 7 days; the dose was then

* CORRESPONDENCE TO: MIZUNO, T., Laboratory of Veterinary Internal Medicine, Department of Veterinary Medicine, Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan.
e-mail: mizutaku@yamaguchi-u.ac.jp

Fig. 1. (A–C) Bone marrow aspirate cytology (Wright-Giemsa stain). (A) Slightly decreased M:E ratio (0.65) and decreased mature neutrophil count. (B and C) Macrophages containing capsule-like structures with a round eosinophilic core in their cytoplasm (white arrowheads). (D–F) Photomicrographs of bone marrow histopathology specimens (hematoxylin and eosin stain). *H. canis* meront containing micromerozoites forming a “wheel-spoke” pattern (arrow) and mature neutrophils with clastic nuclei (black arrowheads).

tapered to 1.5 mg/kg, q12h), cyclosporine (6 mg/kg, q24 h, PO) and amoxicillin (30 mg/kg, q12h, PO) on the basis of the provisional diagnosis of IMN (Day 29). Contrary to our expectations, the results of the bone marrow core biopsy indicated osteomyelitis probably associated with hepatozoon infection. Histopathological examination of the bone marrow sample stained with hematoxylin and eosin revealed round to oval structures (approximately 50–80 µm) containing a hematoxylin-positive organism that resembled *Hepatozoon* micromerozoites and formed a “wheel-spoke”

pattern (Fig. 1D). Mature neutrophils with clastic nuclei were observed around these structures (Fig. 1E and 1F). Polymerase chain reaction (PCR) was performed to confirm hepatozoon infection by using bone marrow aspirate and peripheral blood samples (obtained on Day 29 and Day 43, respectively) [9]. In addition, PCR for *Babesia* spp. and *Ehrlichia* spp. were performed [8, 12]. The dog was positive for *Hepatozoon* spp. infection but negative for *Babesia* spp. and *Ehrlichia* spp. infection. Sequence analysis of the partial 18S ribosomal RNA gene revealed 100% identity to

Table 1. Myelogram of this case

Cell type	Percentage (%)
Rubriblast	0.6
Prorubricyte	0.8
Rubricyte	35.1
Metarubricyte	10.5
Myeloblast	0.4
Promyelocyte	1.2
Myelocyte (Neutrophilic)	0.8
Myelocyte (Eosinophilic)	1.2
Metamyelocyte (Neutrophilic)	1.6
Metamyelocyte (Eosinophilic)	0.6
Band (Neutrophilic)	20.5
Band (Eosinophilic)	1
Neutrophil	3.6
Eosinophil	0.8
Monoblast	1.8
Promonocyte	4.1
Monocyte	8.9
Macrophage	1
Lymphoblast	0
Lymphocyte	6.1
Plasma cell	0.6
M/E ratio	0.65

that of a Japanese isolate of *H. canis* (GenBank accession number: AF418558) [10]. The dog was finally diagnosed with *H. canis* infection.

The treatment and clinical course are summarized in Fig. 2. By 14 days after the initiation of immunosuppressive agents (Day 43), the WBC count had returned to normal and the clinical signs had improved although mild thrombocytopenia persisted. An intracellular gamont was observed in the cytoplasm of the neutrophils derived from the peripheral blood smear on the same day (Fig. 3). However, based on the definitive diagnosis of *H. canis* infection, the treatment strategy was changed. Cyclosporine and enrofloxacin were discontinued, and prednisolone was tapered to prevent a rebound. Trimethoprim-sulfadiazine (15 mg/kg, q12h, PO) and clindamycin (18 mg/kg, q12h, PO) were added as anti-protozoal agents. The dog gradually recovered, and almost all the clinical signs resolved by Day 64. Regular follow-ups have been conducted over the phone since the last clinicopathological evaluation (Day 64); currently, after 1 year, the dog is clinically healthy.

Most *H. canis*-infected dogs show clinical latency. The pathogenesis is influenced by immune-compromised conditions, an immature immune system in young animals, a con-

Fig. 2. Changes in the clinical parameters and a schema of the treatment schedule. Bone marrow biopsy (BM) was performed on Day 29. The vertical dotted line represents the first day at our hospital.

Fig. 3. Gamonts (arrows) found in the cytoplasm of the neutrophil-like cells of the buffy coat of the peripheral blood obtained on Day 43 (Hemacolor stain).

genital defect or concurrent infections [1, 4]. Concurrent toxoplasmosis, babesiosis or ehrlichiosis predispose an animal to clinical illness [7, 9]. In the present case, the possibility of concurrent babesiosis and ehrlichiosis was ruled out by the findings of PCR examination of the peripheral blood. Anemia, fever, lethargy and weight loss have been reported as common clinical signs associated with *H. canis* infection [1, 4]. Ataxia and lameness due to the development of osteomyelitis have been reported [1, 9, 11]. Neutrophilia is one of the common hematological abnormalities in addition to anemia and thrombocytopenia [1, 13]. Microscopic detection of *H. canis* gamonts in blood smears is the most common diagnostic finding for *H. canis* infection [1, 3]. However, in the present case, the dog presented with persistent neutropenia, but the gamonts were absent in the peripheral blood until the neutrophil counts increased because of the immunosuppressive therapy. This made the diagnosis difficult. The leucocyte count is usually normal or increased in *H. canis*-infected dogs [1, 6]. A litter of Dalmatian puppies that were diagnosed with *H. canis* infection had significant neutropenia; however, they had concurrent parvoviral enteritis [2]. Canine parvovirus infection is a well-known cause of immunosuppression, which induces severe leucopenia, especially in puppies. The dog in the present case was 4 years old and did not present with gastrointestinal signs such as vomiting and diarrhea. Therefore, parvovirus infection did not seem to be the cause of neutropenia, although a diagnostic serological test was not performed.

Neutropenia is commonly caused by shifting of the neutrophils from the circulating pool to the marginal pool because of marked inflammation or ineffective granulopoiesis secondary to bone marrow injury by drugs, toxins, infectious agents or neoplasia [5]. Immune-mediated cell injury is an uncommon cause of neutropenia in dogs, and it appears as a primary (idiopathic) syndrome associated with systemic immune-mediated disease or secondary to infectious agents, neoplasia or drugs. The present dog had no history of medical therapy or ingestion of toxins before the onset of the ill-

ness. At the time of bone marrow biopsy, IMN was suspected on the basis of the selective neutrophilic hypoplasia seen in the cytologic specimens. Although the detection of antineutrophil antibody is required for definitive diagnosis of IMN, the tests are not widely available and are rarely performed [17]. Therefore, immunosuppressive doses of prednisolone and cyclosporine were initiated on the basis of the presumptive diagnosis of IMN. After the initiation of prednisolone, the number of neutrophils promptly increased and recovered to normal. The histopathological results of the bone marrow core biopsy indicated osteomyelitis associated with *H. canis* infection and an accumulation of mature neutrophils around the micromerozoites of *H. canis*. This finding indicated that the shifting of neutrophils from the circulating pool into target tissues such as bone marrow or the spleen might have led to neutropenia. In addition, the prompt response to corticosteroid administration indicated immune-mediated injury of the neutrophils secondary to *H. canis* infection.

Thrombocytopenia is reported to be present in approximately one-third of the dogs with *H. canis* infection, although it is not a specific finding for *H. canis* infection [1]. In the present case, mild thrombocytopenia persisted after administration of immunosuppressive agents and may have been associated with the *H. canis* infection.

Several reports concerning *H. canis* infection in dogs have been published, although the onset of hepatozoonosis is still rare in Japan [9, 10, 14–16, 18]. Serologic study of *H. canis* in Yamaguchi and neighboring prefectures suggested a relatively high serological prevalence (4.2%) in dogs [10].

In conclusion, *H. canis* infection was diagnosed on the basis of the findings of bone marrow core biopsy and PCR. The persistent neutropenia observed in this case was suspected to be associated with osteomyelitis caused by *H. canis* infection. Thus far, there are no reports of neutropenia associated with *H. canis* infection without concurrent canine parvovirus. *H. canis* infection can be included in the differential diagnosis in canine cases of neutropenia in areas where the disease is endemic.

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