NOTE *Clinical Pathology*

A Case of Iriomote Cat (*Prionailurus bengalensis iriomotensis*) with *Hepatozoon felis* Parasitemia

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ABSTRACT. We herein present clinical findings of an Iriomote cat with *Hepatozoon felis* parasitemia. A male Iriomote cat was captured for ecological analyses three times from January 2010 to January 2011. Although this cat did not show any hematological abnormalities at the time of the first capture, *H. felis* parasitemia and increased serum creatine kinase levels were detected at the second and third captures. *H. felis* infection was confirmed by polymerase chain reaction, and amplified 18S ribosomal RNA gene fragments were 100% identical to those of *H. felis* in leopard cats in Korea. Although the virulence of *H. felis* in this cat was suggested to be low, this is the first report of an *H. felis*-infected Iriomote cat with parasitemia.

KEY WORDS: *Hepatozoon felis*, Iriomote cat, parasitemia.

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Hepatozoon is a genus of protozoa belonging to the phylum Apicomplexa, members of which are transmitted by ticks and infect a wide variety of mammals, birds, reptiles, and amphibians. The domestic cat and wild felids including the Iriomote cat are also susceptible to *Hepatozoon* spp. [1, 3, 16]. In general, *Hepatozoon* spp. are not highly pathogenic, with the exception of *Hepatozoon americanum* in dogs. They induce acute disease in limited populations of animals, including immune-compromised or -suppressed hosts. Dogs infected with *Hepatozoon canis* sometimes show clinical signs including anemia, fever, lethargy, weight loss, ataxia, and lameness [5]. Characteristic hematological abnormalities in *H. canis* infection include nonregenerative anemia, thrombocytopenia, neutrophilia, hyperproteinemia, hypoalbuminemia, polyclonal gammopathy, and increased serum creatine kinase (CK) and alkaline phosphatase (ALP) concentrations [5, 8, 9]. The detection of capsule-like gamonts in the cytoplasm of neutrophils in blood smears is sometimes helpful for diagnosing acute-onset hepatozoonosis, but a small percent of *H. canis*-infected dogs are reported to possibly show parasitemia [2, 4, 8, 10, 20]. On the other hand, the pathophysiology of hepatozoonosis in felids is not yet fully understood. Microscopic parasitemia of *Hepatozoon* sp. in felids is believed to be much less frequent than that in dogs [3, 14].

The Iriomote cat (*Prionailurus bengalensis iriomotensis*) is a Japanese wild felid that was discovered in 1965 [11]. However, the population of the Iriomote cat is assumed to be approximately 100 [12]. An ecological survey for conservation of the Iriomote cat was performed, and five cats were captured between January 2010 and January 2011. These five cats were clinicopathologically evaluated, and we found one Iriomote cat showing *H. felis* parasitemia. We herein present the clinical findings of this cat and the genetic characteristics of the infectious *H. felis*.

A mature male Iriomote cat (Cat ID, W129) was captured to evaluate its behavior and mode of life in January 2010. This cat weighed 4.37 kg and seemed well from a nutritional standpoint. The cat was anesthetized with medetomidine (0.05 mg/kg) and ketamine (7.5 mg/kg). The physical examination revealed large numbers of pubic lice, a thin abdominal hair coat, and a few larvae and nymphs of *Amblyomma testudinarium* and *Haemaphysalis hystricis* on the caudal aspect of the pinna. Although the absence of an upper-left dens praemolaris was detected, other body conditions seemed to be well maintained. Following an accurate measurement of body size, collection of blood and urine samples, and implantation of an identification microchip in the scapular region, this cat was released at the site of capture after awakening by the administration of atipamezole (0.125 mg/kg).

A complete blood count indicated no obvious abnormalities on erythrocyte and leukocyte counts (Table 1). Serum biochemistry revealed hyperglycemia (140 mg/d*l*), which was probably due to agitation prior to the anesthesia. An elevated blood urea nitrogen (BUN) was also observed (42.7 mg/d*l*); however, there was no elevation of the creatinine concentration (Table 1). The elevated BUN was likely due to the ingestion of bait at the time of capture. On the blood smear, no abnormal findings were observed for erythrocytes, leukocytes, or platelets. Serum anti-feline immunodeficiency virus antibody and feline leukemia virus antigen were analyzed using a commercially available test kit (SNAP FeLV/FIV combo kit; IDEXX Laboratories Inc.,

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Table 1. Hematological findings in the captured W129 Iriomote cat

	Date of capture			Reference ranges***
	Jan 2010	Dec 2010	Jan 2011	
CBC				
RBC (× $10^4/\mu l$)	714	690	797	500–1,000
Ht (%)	37	31	35	24–45
Hb (g/d*l*)	11.8	11.5	12.5	8.0–15.0
WBC (/μl)	13,200	16,100	22,300	5,500–19,500
Band	0	0	100	0 –300
Seg	9,100	13,000	18,700	2,500–12,500
Lym	3,600	2,200	2,600	1,500–7,000
Mono	500	600	800	0–850
Eos	0	300	100	0 –1,500
Platelet (× $10^3/\mu l$)*	Normal	Normal	Normal	200–600
TP (g/d*l*)	7.8	7.8	7.7	6.0–8.0
Parasitemia (%)	0.0	0.9	3.0	No parasitemia
Chemistry				
ALP (U/*l*)	<100	<100	<100	38–165
ALT (U/*l*)	56	98	87	22–84
AST (U/*l*)	42	38	50	18–51
ALB (g/d*l*)	NT**	3.3	3.5	2.3–3.5
GGT (U/*l*)	NT	6	8	1–10
T-Bil (mg/d*l*)	NT	0.2	0.3	0.1–0.4
T-Cho (mg/d*l*)	151	127	152	89–176
Glu (mg/d*l*)	140	180	180	71–148
BUN (mg/d*l*)	42.7	20.0	46.4	18–33
Cre (mg/d*l*)	0.9	0.9	0.7	0.8–1.8
Ca (mg/d*l*)	NT	10.7	10.0	8.8–11.9
P (mg/d*l*)	NT	4.5	5.0	2.6–6.0
CK (U/*l*)	NT	788	389	87–309
Hepatozoon PCR	Positive	Positive	Positive	Negative

* Platelet counts were calculated on blood smear specimens as a relative count against WBC. **NT, not tested. ***Ranges refer to those in healthy domestic cats.

Westbrook, MA, U.S.A.); both were negative. Urinalysis revealed mild proteinuria and hematuria. Nematode eggs of an unknown origin were also obtained from the urine.

This W129 cat was recaptured two more times (December 2010 and January 2011). The captured cat was identified as W129 by scanning of the implanted microchip. The cat was anesthetized as mentioned above, and physical and blood examinations were carried out in the same ways. No serious problems were found on either physical examination; however, hematological findings were slightly different at the December 2010 capture. The erythrocyte and leukocyte counts were normal, and the platelet number was confirmed to be adequate on a blood smear. However, enlarged neutrophils with capsule-like structures in their cytoplasm were detected in the same specimen. This capsule-like structure was morphologically similar to the gamont of *Hepatozoon* spp. (Fig. 1A). This gamont-like structure was observed in 0.9% of neutrophils (Table 1). In January 2011, no abnormal erythrocyte, leukocyte, or platelet counts were detected, and this gamont-like structure was found in 3.0% of neutrophils (Table 1, Fig. 1B). Serum chemistry results showed no obvious abnormal findings, with the exception of increased CK levels at both captures and increased BUN in January 2011 (Table 1).

Next, we carried out polymerase chain reaction analysis

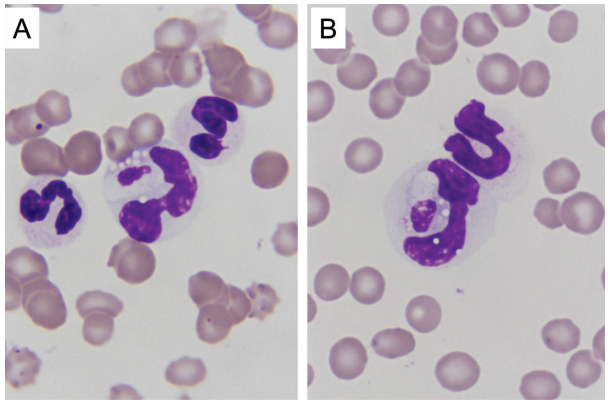

Fig. 1. Enlarged segmented neutrophils with *Hepatozoon* gamonts in the cytoplasm were found on blood smears obtained in December 2010 (A) and January 2011 (B). Wright-Giemsa staining, × 1000.

to determine whether this capsule-like structure was of *Hepatozoon* origin. Partial 18S ribosomal RNA gene of *Hepatozoon* spp. was amplified from blood samples by polymerase chain reaction using primers HepF (5'-ATA CAT GAG CAA AAT CTC AAC-3') and HepR (5'-CTT ATT ATT CCA TGC TGC AG-3') as described elsewhere

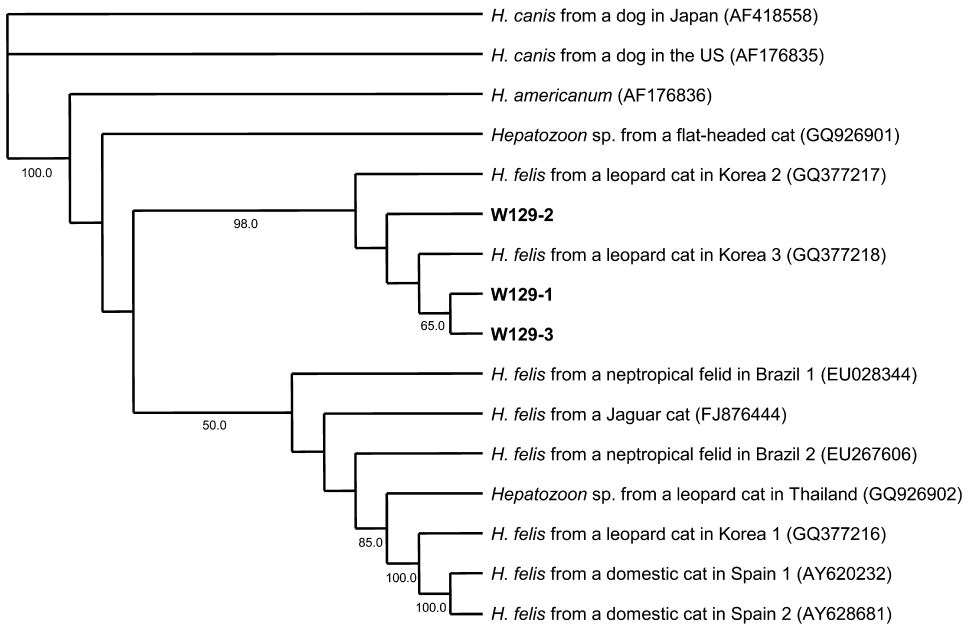

Fig. 2. Phylogenetic relationship of the partial 18S rRNA gene sequences of *Hepatozoon* isolated from Iriomote cats and other carnivores. Three clones were obtained from W129 Iriomote cat (W129–1, –2, and –3). DDBJ/EMBL/GenBank accession numbers are indicated in parentheses: AF418558, *H. canis* from a dog in Japan [13]; AF176835, *H. canis* from a dog in the US [18]; AF176836, *H. americanum* [18]; GQ926901, *Hepatozoon* sp. from a flat-headed cat [21]; GQ926902, *Hepatozoon* sp. from a leopard cat in Thailand [22]; GQ377217-GQ377219, *H. felis* from a leopard cat in Korea [15]; AB636285-AB636287, *H. felis* from W129 Iriomote cat (W129–1, –2, and –3); EU028344 and EU267606, *H. felis* in Brazil [19]; and AY620232 and AY628681, *H. felis* in Spain [6]. Bootstrap values of more than 50 are indicated on the branches.

[13, 23]. This primer set amplifies a 625-bp-long DNA fragment derived from the partial 18S ribosomal RNA gene. As shown in Table 1, the cat was positive for *Hepatozoon* sp. infection in January 2010 even though the parasitemia had not been detected in addition to the December 2010 and January 2011 captures. Furthermore, genotypic analysis of the partial 18S ribosomal RNA gene revealed 98 to 99% identity to those of *H. felis* isolates in leopard cats in Korea (GenBank accession numbers GQ377216–GQ377218) [15]. No information is currently available for Japanese isolates of *H. felis*. Phylogenetic analysis was also performed by a neighbor-joining method in the DNADIST program from the PHYLIP software package [7]. This analysis revealed that the *Hepatozoon* sp. isolated from W129 was closely related to Korean isolates from a leopard cat (Fig. 2). Based on these findings, the W129 Iriomote cat was diagnosed with a persistent *H. felis* infection and had just begun to show signs of an acute onset of microscopic parasitemia beginning in January 2010 at the latest.

As mentioned above, we incidentally found an Iriomote cat with *H. felis* parasitemia. To our knowledge, this is the first report of *Hepatozoon* parasitemia in an Iriomote cat, although *Hepatozoon* infection in this cat has already been reported [16]. *Hepatozoon* infections in other mammals, especially in dogs, occasionally result in symptomatic disease. In addition, parasitemia and acute onset of disease often coincide in *Hepatozoon*-infected animals. Anemia and thrombocytopenia, and sometimes lameness and ataxia, are found in typical cases showing acute onset of *H. canis* infection. Additional hematological findings include increases in ALP and CK activities, hyperproteinemia, hypoalbuminemia, and polyclonal gammopathy [5, 8, 9]. However, no clinical signs or hematological abnormalities with the exception of increased CK activity were found in the W129 Iriomote cat even though this cat was showing parasitemia at the time of examination. A possible explanation for the increased CK activity is that cardiac and/or skeletal muscles were affected. A previous report on *Hepatozoon* infection in Japanese wildcats revealed that the cardiac muscle was the most common site of schizont formation [16]. In addition, skeletal muscle in the tongue, masseter, thigh, and diaphragm was also parasitized. Therefore, schizont formation might have occurred in cardiac and/or skeletal muscle in the W129 Iriomote cat. Although we could not evaluate the isozyme of CK, its identification will clarify the origin of the increased CK level.

Fortunately, the virulence of the *Hepatozoon* sp. in the Iriomote cat was assumed to be low based on the hematological findings. However, we should consider the immunological status of affected cats. Based on the findings in *Hepatozoon* infections in other species, parasitemia may be related to immunodeficiency or immune-compromised conditions such as the coexistence of other infections and the administration of immunosuppressants [2, 4, 8, 10, 20].

This point seems to be important for wild animals because they have many opportunities to be exposed to other infectious agents. The W129 cat had adequate counts of lymphocytes and other leukocytes; accordingly, he is expected to have had adequate immunological function. However, it will be necessary to carefully survey the status of other infectious agents (viruses, protozoa, and parasites).

Phylogenetic analysis revealed that *Hepatozoon* spp. in felids are genetically closely related. Isolates from Iriomote cat W129 were localized in the same group as the *H. felis* from leopard cats in Korea. In addition, isolates from domestic cats and wild felids in Europe, South America, and Southeast Asia formed one group. An interesting finding is that the isolates from felids in Far East Asia formed one cluster. This finding suggests that the "Far East genotype" of *H. felis* is spreading in this area. However, Korean leopard cats already possess a non-"Far East genotype" of *H. felis* [15]. The Iriomote cat was estimated to have diverged from the continental leopard cat approximately 200,000 to 100,000 years ago, based on DNA analysis results [17]. Although island *Hepatozoon* spp. might have diverged from continental *Hepatozoon* spp. at the same time, further analyses using many samples obtained from ticks, wildlife, and domestic animals in Asian countries, as well as from Iriomote cats in the Ryukyu Archipelago in Japan, are needed. The difference in virulence between Far East isolates and others is unknown; however, we must consider the incursion of other types or species of *Hepatozoon* to the island because Iriomote-jima Island is isolated and surrounded by the sea.

In this study, we incidentally found an Iriomote cat showing *H. felis* parasitemia. Although this pathogen did not induce severe clinical symptoms in infected cats, infectious diseases might threaten the continued existence of endangered species. Therefore, it is essential for conservation to monitor infectious diseases not only in Iriomote cats, but in domestic animals and arthropods on Iriomote-jima Island.

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