



## NOTE

Internal Medicine

# The presence of tick-borne diseases in domestic dogs and cats living on Iriomote-jima and Tsushima islands

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**ABSTRACT.** The Iriomote cat and Tsushima leopard cat are endangered wildcats in Japan and inhabit only Iriomote-jima and Tsushima islands, respectively. Domestic dogs and cats living on Iriomote-jima and Tsushima islands were surveyed to clarify the interrelationship between wildcats and domestic animals regarding tick-borne disease transmission. Pathogen-derived DNA in blood samples was detected by polymerase chain reaction. *Babesia gibsoni* was detected in dogs of Iriomote-jima, and *Hepatozoon felis* and hemoplasmas were detected in domestic cats of Tsushima. Because the *H. felis* detected in this study was closely related to that isolated from wildcats, we suspect that common *H. felis* is harbored and transmitted among wildcats and domestic cats via ticks in Tsushima.

**KEY WORDS:** cat, dog, tick-borne disease, wildcat

The Iriomote cat (IC), *Prionailurus bengalensis iriomotensis*, and the Tsushima leopard cat (TLC), *Prionailurus bengalensis euptilura*, are the only two subspecies of wildcats that inhabit only Iriomote-jima and Tsushima islands in Japan, respectively (Fig. 1) [13, 18]. Their current populations are estimated to be approximately 100, and these cats are designated protected species in Japan [11]. Exposure to infectious agents may be a possible threat to Japanese wildcats. These wildcats are living in environments inhabited by many other animal species. Furthermore, the introduction of animals to islands, especially domestic dogs and cats, with the growth of the human population may increase the risk of infectious diseases in these animals. A previous study revealed interspecies transmission of feline immunodeficiency virus in TLCs [15]. This virus is commonly found in domestic cats; however, a phylogenetic analysis revealed that the feline immunodeficiency virus isolated from TLCs was closely related to that from domestic cats living in the same vicinity [15]. Therefore, domestic animals living on Iriomote-jima and Tsushima islands could be a possible source of infectious diseases. We recently showed that several pathogens, such as hemotropic mycoplasmas (hemoplasmas), *Hepatozoon*, *Ehrlichia* and *Anaplasma*, were found in ICs and/or TLCs [9, 19, 20]. In the present study, domestic animals living on Iriomote-jima and Tsushima islands were surveyed for hemoplasma, *Ehrlichia* sp., *Anaplasma* sp., *Hepatozoon* sp. and *Babesia* sp. infections to clarify the interrelationship between wildcats and domestic animals with respect to the transmission of those pathogens.

From July 2012 to August 2015, 106 blood samples were collected from 105 domestic cats and 88 blood samples were collected from 82 domestic dogs on Iriomote-jima island (blood samples were collected twice in 6 dogs and 1 cat). In addition, 284 blood samples were obtained from the same number of domestic cats on Tsushima island from September 2009 to March 2012 (kindly provided by Dr. Kazuo Nishigaki, Yamaguchi University). The blood samples were collected from domestic animals regardless of their lifestyle (indoor or outdoor), but most of the blood samples collected from the domestic cats on Tsushima island were collected from cats who went outside of the house. The collected blood samples were preserved at  $-20^{\circ}\text{C}$  until use. DNA was extracted from 200  $\mu\text{l}$  of each blood sample using a QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) with a final elution volume of 200  $\mu\text{l}$ . For polymerase chain reaction (PCR) amplification of pathogen-derived DNA, previously reported primers and procedures were used [3, 5–7, 10, 16, 22]. We used a two-step analysis (initial screening and detailed tests) for the detection of *Babesia* spp. and *Hepatozoon* spp. Briefly, a nested PCR was performed to amplify the 18S rRNA gene derived from *Babesia* spp. and/or *Hepatozoon* spp. as an initial screening [1, 3]. *Babesia* spp. and *Hepatozoon* spp. could be differentiated based on the size of the amplified DNA fragments (*Babesia* spp., 230 base pairs [bp]; and *Hepatozoon* spp., 267 bp). If the samples were positive for *Hepatozoon* spp. in the initial screening PCR, they were then subjected to an additional PCR to amplify a longer

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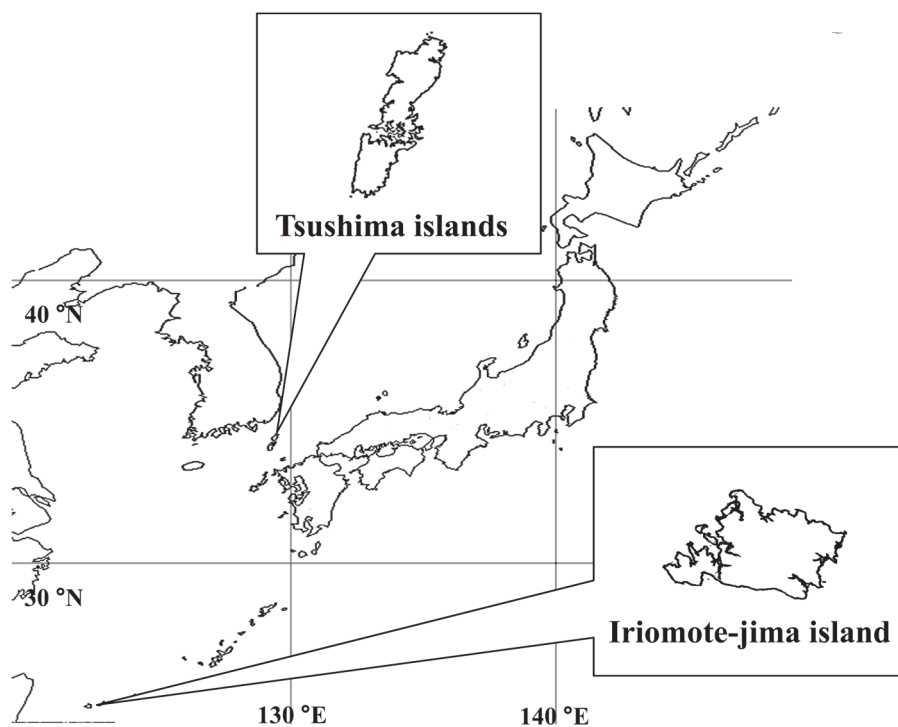


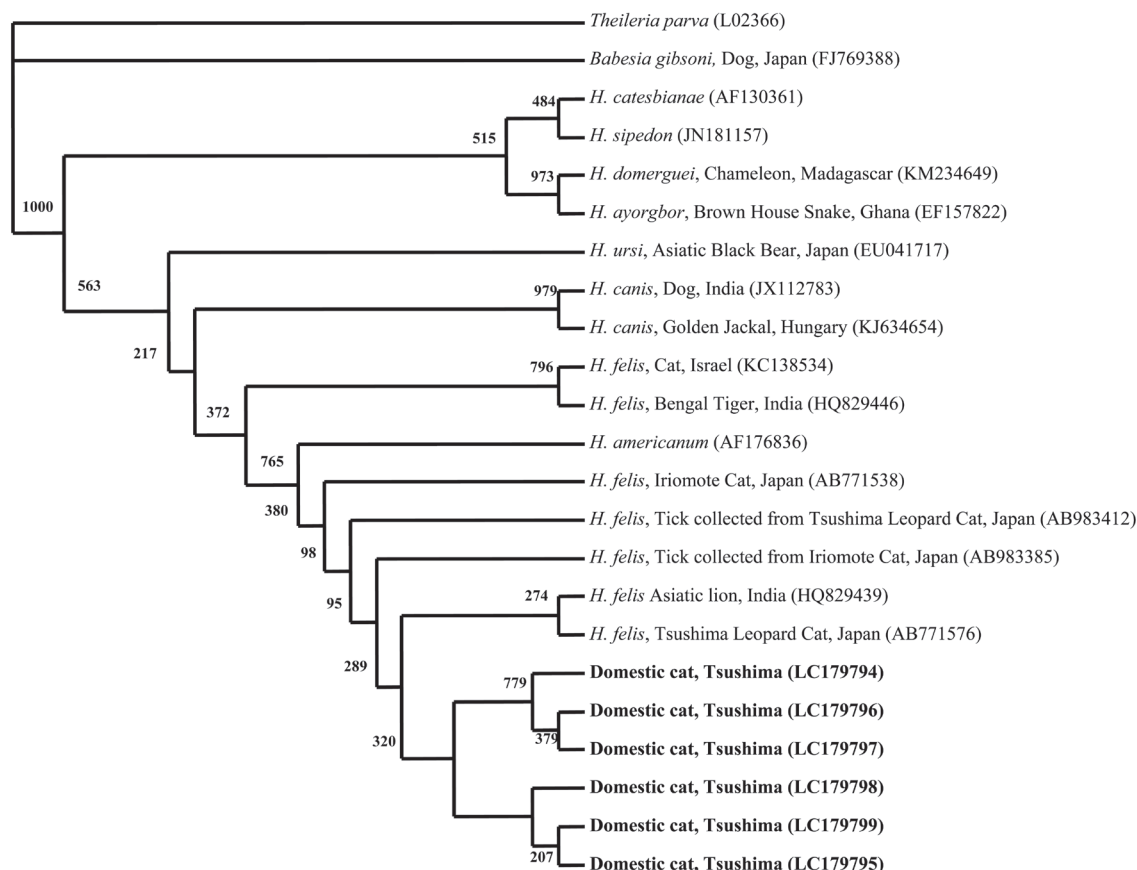
Fig. 1. Locations of Iriomote-jima and Tsushima islands [20].

region of the 18S rRNA gene [1, 6, 7, 10]. The product length of the PCR was approximately 900 bp. Primers BT-1F (5'-GGT TGA TCC TGC CAG TAG T-3') and HEP2P (5'-GAC TTC TCC TTC TTT AAG TGA TAA G-3') were used for the first round of PCR [6, 7]. Primers HepF (5'-ATA CAT GAG CAA AAT CTC AAC-3') and RLB-R (5'-TCT TCG ATC CCC TAA CTT TC-3') were used for the second round of PCR [1, 10]. PCR for *Ehrlichia* spp. and *Anaplasma* spp. was conducted based on the nested PCR strategy reported by Sakamoto *et al.* [16]. The expected length of the nested PCR fragments was approximately 340 bp. To amplify the 16S rRNA gene from a broad range of hemoplasma species, a universal primer set consisting of one forward primer (MY-F, 5'-AGC AAT RCC ATG TGA ACG ATG AA-3') and two reverse primers (MY-R1, 5'-TGG CAC ATA GTT TGC TGT CAC TT-3'; and MY-R2, 5'-GCT GGC ACA TAG TTA GCT GTC ACT-3') was used according to Willi *et al.* [22].

The nucleotide sequences of the amplified DNA fragments were inserted into a pCR2.1 plasmid vector (Invitrogen, Carlsbad, CA, U.S.A.), and the nucleotide sequence of the inserted DNA fragments was determined by the dideoxy chain termination method (ABI Prism BigDye Primer Cycle Sequencing Ready Reaction Kit; Applied Biosystems, Foster City, CA, U.S.A.). Nucleotide sequence data from each sample were subjected to Basic Local Alignment Search Tool (BLAST) analysis in the DNA Data Bank of Japan [2] (<http://www.ddbj.nig.ac.jp/Welcome-j.html>) to identify closely related species of pathogens. Phylogenetic analyses were performed for *Hepatozoon* spp. to determine the genetic relationship among those pathogens by a neighbor-joining method in the DNADIST program from the PHYLIP software package (<http://evolution.genetics.washington.edu/phylip.html>), as previously described [8].

In the screening PCR of *Hepatozoon* and *Babesia*, two samples from domestic dogs on Iriomote-jima island were positive for *Babesia* spp. (GenBank accession numbers LC179792 and LC179793). DNA sequence and BLAST analysis revealed that these samples showed high similarity with *B. gibsoni*. No *Babesia*-derived DNA was detected in domestic cats of either island. In domestic cats of Tsushima island, seven samples were positive for *Hepatozoon* spp. BLAST analysis revealed that all of them had high homology to *H. felis*. These blood samples were then analyzed by additional PCR to obtain the longer region of the 18S rRNA gene, and successful amplifications were obtained from six of seven samples (LC179794–LC179799). Nucleotide sequences from these six samples were subjected to phylogenetic analysis (Fig. 2). Phylogenetic analysis revealed that these samples formed one cluster with *H. felis* and species closely related to *H. felis*, all of which have been previously isolated from TLCs (AB771576) [20]. The prevalence of *B. gibsoni* was 2.4% in domestic dogs on Iriomote-jima island, and the prevalence of *H. felis* was 2.4% in domestic cats on Tsushima island (Table 1). No *Ehrlichia*- or *Anaplasma*-derived DNA was detected in domestic dogs and cats on either Iriomote-jima or Tsushima island.

Nineteen samples from domestic cats on Iriomote-jima island were positive for hemoplasma, but no positive cases were found among the dog samples. Nucleotide sequences obtained from these 19 samples showed high similarity with “*Candidatus Mycoplasma haemominutum*” (CMhm, KM275256) in the BLAST analysis, and the prevalence of CMhm was 17.9% in domestic cats on Iriomote-jima island. On the other hand, 74 samples from domestic cats on Tsushima island were positive for hemoplasma, and 12 of these 74 samples showed dual bands. BLAST analysis revealed that 18 (6.1%), 61 (20.7%) and 7 (2.4%) samples had



**Fig. 2.** Phylogenetic relationship of the partial 18S rRNA gene sequences of *Hepatozoon* spp. isolated from domestic cats, Iriomote cats, Tsushima leopard cats and ticks collected from Japanese wildcats and other animals. Six clones of the *H. felis* 18S rRNA gene from domestic cats on Iriomote-jima island (LC179794–LC179799) were analyzed. The pathogen names, host species (if available), country of isolation (if available) and GenBank accession numbers (in parentheses) of the compared sequences are shown in the phylogenetic tree: *H. felis* from a Bengal tiger in India (HQ829446), a cat in Israel (KC138534), an Asiatic lion in India (HQ829439), an Iriomote cat in Japan (AB771538), a Tsushima leopard cat in Japan (AB771576), a tick collected from an Iriomote cat (AB983385), a tick collected from a Tsushima leopard cat (AB983412); *H. canis* from a dog in India (JX112783) and a golden jackal in Hungary (KJ634654), *H. americanum* (AF176836), *H. ursi* from an Asiatic black bear in Japan (EU041717), *H. catesbiana* (AF130361), *H. sipedon* (JN181157), *H. avorgbor* from a brown house snake in Ghana (EF157822), *H. domerguei* from a chameleon in Madagascar (KM234649), *B. gibsoni* from a dog in Taiwan (FJ769388) and *Theileria parva* (L02366). Numbers under internal nodes indicate the percentages of 1,000 bootstrap replicates that supported the branch.

**Table 1.** Prevalence of detected pathogens in domestic animals

Localization	Animal	Detected pathogen <sup>a)</sup>	Positive sample (Prevalence)(%)
Iriomote-jima	Dog	<i>Babesia gibsoni</i>	2 (2.4)
	Cat	Hemoplasma	19 (17.9)
		CMhm	19 (17.9)
Tsushima	Cat	<i>Hepatozoon felis</i>	7 (2.4)
		Hemoplasma	74 (26.0)
		Mhf	18 (6.1)
		CMhm	61 (20.7)
		CMt	7 (2.4)
		Mhf and CMhm	8 (2.8)
		CMhm and CMt	4 (1.4)

a) Mhf, *Mycoplasma hemofelis*; CMhm, “*Candidatus Mycoplasma haemominutum*”; CMt, “*Candidatus Mycoplasma turicensis*”.

high homology with *Mycoplasma hemofelis* (Mhf, KM275245), CMhm (KM275256) and “*Candidatus* *Mycoplasma turicensis*” (CMt, KM275268), respectively (Table 1). Coinfection of Mhf and CMhm was observed in eight samples, and coinfection of CMhm and CMt was detected in four samples.

The reported presence of *H. felis* in ICs and TLCs was 72.0 and 100.0%, respectively [20]. In this study, *Hepatozoon*-derived DNA was detected in domestic cats on Tsushima island, although the presence was much lower than that in TLCs. In addition, no positive cases were found among domestic dogs and cats of Iriomote-jima island. This difference in the prevalence of *H. felis* between domestic cats and wildcats might be due to the differences in the frequency and numbers of tick infestations [15]. Ticks have a very important role in the transmission of *Hepatozoon* spp., and the main infection route of *Hepatozoon* spp. into the final host is ingestion of a *Hepatozoon*-positive tick [4]. Infestation of high numbers of ticks is observed in most ICs and TLCs, but not in domestic cats [20]. Heavy tick infestations in Japanese wildcats might have increased the risk of infection by *H. felis*.

Phylogenetic analysis revealed that the *H. felis* detected in domestic cats on Tsushima island was closely related to *H. felis* isolated from TLCs (Fig. 2). This finding indicates that the strain of *H. felis* detected in domestic cats is very similar to that detected in TLCs; thus, interspecies transmission of *H. felis* between wild and domestic cats was suspected. However, because the prevalence of *H. felis* in domestic cats was much lower than that in TLCs, frequent interspecies transmission seems unlikely to have occurred [20]. In our previous epidemiological survey using the same PCR system as used in the present study, *Hepatozoon*-derived DNA was not detected in 1,770 domestic cats living on the mainland of Japan (unpublished data). This is the first time that *H. felis* has been detected in domestic cats in Japan.

A previous molecular epidemiological study of *Babesia* spp. in Japan showed that 2.4% of domestic dogs were positive for *B. gibsoni* [12]. In the present study, 2.4% of domestic dogs on Iriomote-jima island were positive for *B. gibsoni*, and the prevalence of *B. gibsoni* in dogs on Iriomote-jima island was comparable with that on the mainland of Japan (2.4%) [12]. On the other hand, *Babesia*-derived DNA has not been detected in domestic cats or wildcats in Japan [20]. The present study also failed to detect *Babesia* spp. in domestic cats on either island. Therefore, it is likely that feline *Babesia* spp. have not yet invaded Japan, including Iriomote-jima and Tsushima islands.

A previous epidemiological study revealed that the prevalence of *Ehrlichia canis* in ICs and TLCs was 9.3 and 7.1%, respectively, and that of *Anaplasma bovis* in TLCs was 21.4% [19]. In the present study, there was no positive case of *Ehrlichia* or *Anaplasma* infection in domestic dogs and cats. Therefore, the transmission of *Ehrlichia* and/or *Anaplasma* spp. between wild and domestic animals seems unlikely to have occurred.

Mhf, CMhm and CMt are recognized as important hemoplasmas in feline practice [14, 21]. Although the transmission route of hemoplasma is not completely understood, a major transmission route is thought to be direct transmission, as in fight wounds [14]. The reported prevalence of hemoplasma in Japanese domestic cats is 26.3% [17]. Although the prevalence in domestic cats on Iriomote-jima island (17.9%) is lower than that, the overall prevalence of hemoplasma on both islands was as high as that in domestic cats on the mainland of Japan [17]. In a study on the prevalence of hemoplasma in Japanese wildcats, Mhf and CMt were detected in 6.9 and 2.3% of ICs, and Mhf and CMhm were detected in 7.1% of TLCs [9]. However, only CMhm was detected in 17.9% of domestic cats on Iriomote-jima island, and Mhf, CMhm and CMt were detected in 6.1, 20.7 and 2.4% of domestic cats on Tsushima island. The differences in the detected hemoplasma species and their prevalence suggest that direct and frequent interspecies transmission of hemoplasma was not likely to occur.

The present epidemiological study was performed to evaluate *Hepatozoon*, *Babesia*, *Ehrlichia*, *Anaplasma* and hemoplasma in domestic dogs and cats on Iriomote-jima and Tsushima islands. However, the transmission of pathogens between wild and domestic animals requires a continuous study to conserve endangered animal species.

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