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Molecular survey of arthropod-borne pathogens in ticks obtained from Japanese wildcats

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ABSTRACT

The Iriomote cat (IC), *Prionailurus bengalensis iriomotensis*, and the Tsushima leopard cat (TLC), *Prionailurus bengalensis euptilurus*, are endangered subspecies of leopard cats in Japan. In addition to habitat destruction and road kills, infectious diseases may threaten their populations, and infection with arthropod-borne pathogens has been reported in both subspecies. Infestations with ectoparasites, especially ticks, have frequently been observed in ICs and TLCs. In the present study, ticks collected from captured ICs and TLCs between November 2011 and January 2012 were morphologically identified and the prevalence of the pathogens *Bartonella* sp., *Babesia* sp., *Ehrlichia* sp., *Anaplasma* sp., *Hepatozoon* sp., and hemoplasmas in the ticks was molecularly evaluated. The ticks *Haemaphysalis longicornis*, *H. hystricis*, and *Amblyomma testudinarium* were obtained from ICs, and *H. megaspinosa*, *Ixodes tanuki*, *H. campanulata*, and *A. testudinarium* were collected from TLCs. The pathogens *Hepatozoon felis*, *Babesia* sp., and *Anaplasma bovis* were detected in ticks obtained from ICs, while *H. felis. Babesia* sp., *Ehrlichia* sp., *E. muris*, '*Candidatus* Mycoplasma haemominutum', and *Bartonella henselae* were found in ticks from TLCs. To protect and conserve these endangered animals, continuous monitoring and additional surveys will be necessary to understand the role of ticks as disease vectors in Japanese wildcats.

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Introduction

Tick species play important roles in transmitting numerous vector-borne pathogens, such as *Bartonella*, *Ehrlichia*, *Anaplasma*, *Hepatozoon*, and *Babesia*, in felids (Baneth, 2011; Cotté et al., 2008; Little, 2010; Ayoob et al., 2010). Wild felids, except those living in captivity, have a high risk of exposure to these pathogens because of the presence of ticks in their habitats and the difficulties associated with applying periodic prophylactics against ectoparasite infestations. Arthropod-borne diseases have been confirmed in many wild felid species worldwide (Chomel et al., 2006; Millán et al., 2009; André et al., 2010). Although most felids infected with these pathogens generally show only mild symptoms, in situations such as immune compromise caused by other diseases or by trauma (Baneth, 2011; Guptill, 2010; Little, 2010; Ayoob et al., 2010), arthropod-borne diseases can be life threatening.

The Iriomote cat (IC), *Prionailurus bengalensis iriomotensis*, and the Tsushima leopard cat (TLC), *Prionailurus bengalensis euptilurus*, are the only two subspecies of leopard cats in Japan (Masuda and Yoshida, 1995). They inhabit only Iriomote Island (N 24°17′, E 123°51′) and the Tsushima islands (N 34°25′, E 129°20′), which are located in the Subtropical and the Temperate Zones, respectively (Fig. 1). It is currently estimated that their populations are approximately 100 each, and there is a great fear that they will go extinct (Izawa et al., 2009). Certainly, infectious diseases could threaten such small populations. Our previous studies revealed that ICs and TLCs were infected with arthropod-borne pathogens including *Bartonella*, *Ehrlichia*, *Anaplasma*, *Hepatozoon*, and/or hemotrophic mycoplasma (hemoplasma) (Hirata et al., 2012; Tateno et al., 2013a,b). Infestations with ectoparasites, such as ticks and lice, have frequently been observed in ICs and TLCs; however, no surveys have been conducted to evaluate the tick species infesting these wildcats or the presence of arthropod-borne pathogens in those ticks.

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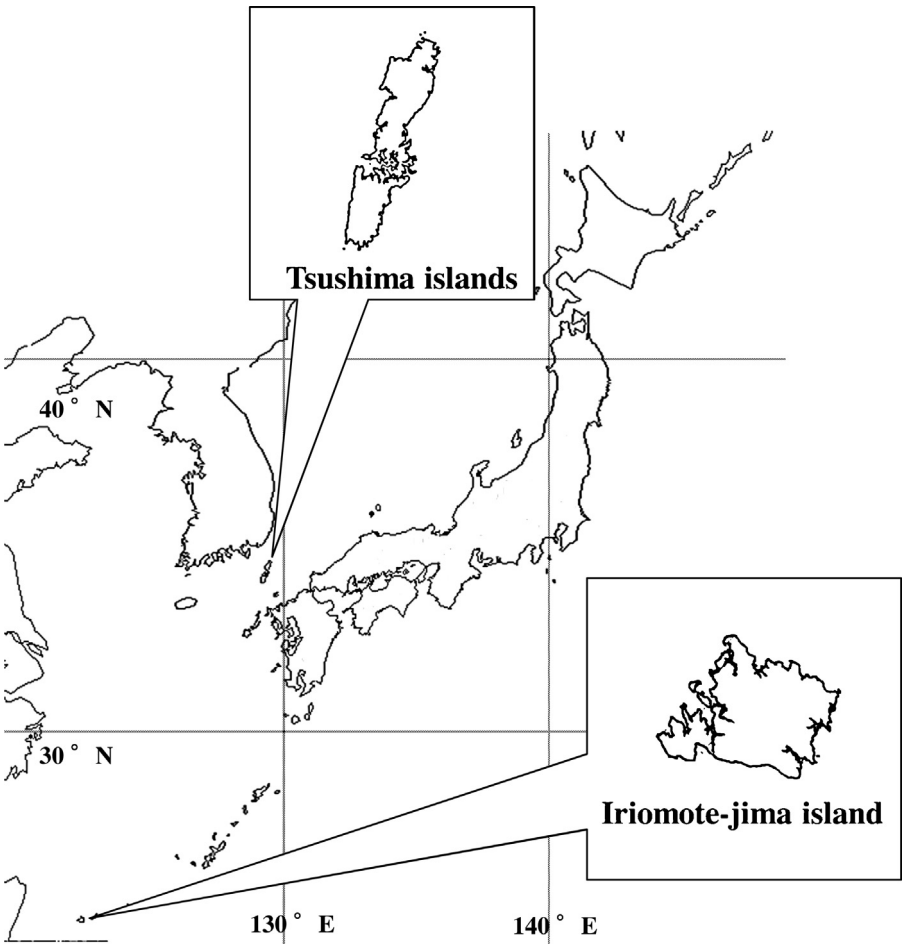

Fig. 1. Locations of Iriomote-jima and Tsushima islands.

In the present study, as part of ongoing conservation activities, we morphologically identified ticks collected from the two endangered subspecies of Japanese wildcats. We then performed molecular screening for *Bartonella* sp., *Babesia* sp., *Ehrlichia* sp., *Anaplasma* sp., *Hepatozoon* sp. and hemoplasmas to understand their prevalence in these ticks.

Materials and methods

Tick collection

As a part of an ongoing ecological survey, 13 ICs and eight TLCs were captured between November 2011 and January 2012. Ticks were collected from the captured animals and tick species, stage, and sex were morphologically identified using a dissecting microscope. The ticks were preserved in ethanol at −20 °C until use. If multiple larvae and nymphs of the same species were obtained from a wildcat at the time of capture, they were gathered and pooled in one tube for subsequent DNA extraction. Each adult tick was individually stored in a separate tube. Wildcats were captured with permission from the Ministry of the Environment and the Agency for Cultural Affairs in Japan. Capture was conducted in cooperation with the Iriomote Wildlife Conservation Center and the Tsushima Wildlife Conservation Center of the Ministry of the Environment. All captures were done at night, and the captured wildcats were released during the afternoon of the next day after physical examination and blood collection under anesthesia with medetomidine and ketamine.

DNA extraction and internal controls

The ticks were frozen in liquid nitrogen and mechanically disrupted, then extraction buffer (100 mM Tris [pH 8.0], 100 mM NaCl, 10 mM EDTA, 0.5% sodium dodecyl sulfate) and protease K (20 μg/μL) were added and the mixture was incubated at 55 °C for more than 12 h. DNA was extracted using a QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. As an internal control to confirm the presence of amplifiable DNA, the tick 16S ribosomal RNA (rRNA) gene was amplified in each sample using the primers 16S-1 (5′-CCG GTC TGA ACT CAG ATC AAG T-3) and 16 + 1 (5′-CTG CTC AAT GAT TTT TTA AAT TGC TGT GG-3′) (Black and Piesman, 1994). Each reaction mixture (50 μL) contained primers (0.5 μM each), 0.2 mM of dNTP, *Taq* DNA polymerase (1.25 U), 2 μL of template DNA, and reaction buffer (Takara, Kyoto, Japan) as recommended by the manufacturer. PCR amplification was performed with an initial heating at 94 °C for 10 min; 40 cycles of denaturation (94 °C for 1 min), annealing (56 °C for 1 min), and polymerization (72 °C for 1 min); and a final extension at 72 °C for 10 min. PCR products were then electrophoresed in 2% agarose gel.

Following complete blood counts and biochemical analyses, DNA was also extracted from blood samples collected from the

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