



HOKKAIDO UNIVERSITY

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Molecular investigation of tick-borne diseases in Hokkaido wildlife
(北海道の野生動物におけるダニ媒介性感染症の分子疫学研究)

Ticks are considered one of the most important vectors for zoonotic diseases in the world. In this study, my main goal was to understand the ecology of potentially zoonotic TBP in Hokkaido wildlife. In chapter 1, I investigated a previously undescribed *Anaplasma* species (AP^{sd}), which has been detected in sika deer, cattle and ticks in Japan. I developed a method for specific detection, and examined AP^{sd} prevalence in Hokkaido wildlife. My study included 250 sika deer (*Cervus nippon yezoensis*), 13 brown bears (*Ursus arctos yezoensis*) and 252 rodents including 138 (*Apodemus speciosus*), 45 (*Apodemus argenteus*), 42 (*Myodes rufocanus*) and 27 (*Myodes rutilus*) were collected from Hokkaido island, northern Japan, during 2010 to 2015. A 770 bp and 382 bp segment of the 16S rRNA and *gltA* genes, respectively, were amplified by nested PCR. A reverse line blot hybridization (RLB) based on the 16S rRNA gene was then developed for the specific detection of AP^{sd}. The prevalence of AP^{sd} by nested PCR in sika deer was 51% (128/250). We detected this *Anaplasma* sp. for the first time in wild brown bears and rodents with a prevalence of 15% (2/13) and 2.4% (6/252), respectively. The sequencing results of the 16S rRNA and *gltA* gene amplicons were divergent from the selected

A. phagocytophilum sequences in GenBank. This study provided a high throughput molecular method that specifically detects AP-sd, and which can be used to investigate its ecology and its potential as a threat to humans in Japan. In chapter 2, a combination of newly designed oligonucleotide probes and previously published ones were used to investigate the prevalence of 13 potentially zoonotic TBPs in 6 small mammal species in Hokkaido ecosystem. The objective of this study was to examine the prevalence of potentially zoonotic TBPs in small mammal populations in Hokkaido, in relation to variations in many extrinsic factors that might affect the infection rates of these pathogens. In this study, we examined 459 small mammals including 219 (*Apodemus speciosus*), 86 (*Apodemus argenteus*), 85 (*Myodes rufocanus*), 51 (*Myodes rutilus*), 11 (*Myodes rex*) and 7 (*Sorex unguiculatus*) from Furano and Shari sites in Hokkaido, Japan, during 2010 to 2012. A fragment of 460–520 bp from the 16S rRNA gene of *Anaplasma* and *Ehrlichia* and 460–540 bp from the 18S rRNA gene of *Babesia* species was amplified by multiplex PCR and then screened by RLB hybridization. The prevalence of *Anaplasma* sp. AP-sd, *E. muris*, *C. N. mikurensis* and *B. microti* was 19 (4.1%), 5 (1.1%), 56 (12.2%) and 95 (20.7%), respectively. The infection rates of *B. microti* and *C. N. mikurensis* were significantly higher in males and adults. The prevalence of the detected TBPs was significantly correlated with the small mammal species variation. A total of 24, two triple and 22 double, co-infection cases were detected in this study (5.2%). The most frequent co-infection cases occurred between *C. N. mikurensis* and *B. microti* (66.7%). These results suggest that human investigations for TBPs in Hokkaido are recommended to understand their epidemiology.