学位論文内容の要旨
Abstract of the dissertation

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学位論文題名
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Studies on Genetic Diversity of Spotted Fever Group Rickettsiae in Ixodid Ticks in Japan

（日本産マダニが保有する紅斑熱群リケッチアの遺伝的多様性に関する研究）

Rickettsiae are obligate intracellular Gram-negative bacteria that cause rickettsioses in humans throughout the world. Ticks harbour most of the members of spotted fever group (SFG) rickettsiae and transmit them to humans and animals. In Japan, *Rickettsia japonica*, a causative agent of Japanese spotted fever (JSF), was firstly identified as a human pathogen. Several other SFG rickettsiae have also been reported from both animals and ticks; however, there is scanty information on their pathogenic potential and the overall diversity of *Rickettsia* species circulating in Japan. This thesis aimed to characterise a wide range of SFG rickettsiae in vector ticks in Japan by analysing multiple rickettsial genes which enables the detailed phylogenetic classification of SFG rickettsiae and to isolate *Rickettsia* using arthropod cell lines.

In chapter I, a nationwide cross-sectional survey was conducted on questing ticks to understand the overall diversity of SFG rickettsiae in Japan. Out of 2,189 individuals (19 tick species in 4 genera), 373 (17.0%) samples were positive for *Rickettsia* spp. by *gltA* real-time PCR. Conventional PCR and sequencing analyses of *gltA* indicated the presence of 15 different genotypes of SFG rickettsiae. Based on the multiple gene sequence analysis, five *Rickettsia* species, namely *R. asiatica*, *R. helvetica*, *R. monacensis* (formerly reported as *Rickettsia* sp. In56 in Japan), *R. tamurae*, and *Candidatus* R. tarasevichiae, and several

unclassified SFG rickettsiae were identified. A strong association between rickettsial genotypes and their host tick species was observed, while there was little association between rickettsial genotypes and their geographical origins. These observations may indicate that most of the SFG rickettsiae have a limited host range and are maintained in certain ticks in the natural environment.

In chapter II, two arthropod cell lines (ISE6 derived from *Ixodes scapularis* tick and C6/36 derived from *Aedes albopictus* mosquito) were used to isolate microorganisms from questing ticks. A total of 170 tick homogenates were inoculated into each cell line. Bacterial growth was confirmed by PCR amplifying 16S ribosomal DNA (rDNA) of eubacteria. During the 16 weeks of observation period, bacterial isolation was confirmed in 14 and 4 samples using ISE6 and C6/36 cells, respectively. These included 4 previously validated rickettsial species namely *R. asiatica*, *R. helvetica*, *R. monacensis*, and *R. tamurae* and one uncharacterised rickettsial genotype *Rickettsia* sp. LON and two tick symbionts, *Spiroplasma* sp. and *Rickettsiella* sp. The use of arthropod cell lines seems promising to expand the knowledge on microorganisms in ticks.

In conclusion, the present study highlights the wide distribution and high frequency of SFG rickettsiae in ixodid ticks and provides basic information essential to understand epidemiology of rickettsiosis in Japan. The genetic information obtained from this study is useful for future development of diagnostic methods for *Rickettsia* infections. The bacterial isolates are important to further analyse their pathogenic potential in vertebrate animals and their roles as symbionts in ticks.