

HOKKAIDO UNIVERSITY

Title	Analysis of population genetic structure of Echinococcus multilocularis in Hokkaido [an abstract of dissertation and a summary of dissertation review]
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学位論文内容の要旨 Abstract of the dissertation

博士の専攻分野の名称:博士(獣医学)

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学位論文題名

The title of the doctoral dissertation

Analysis of population genetic structure of *Echinococcus multilocularis* in Hokkaido (北海道における多包条虫 *Echinococcus multilocularis*の集団遺伝構造の解析)

< abstract >

The tapeworm *Echinococcus multilocularis*, a species within the genus *Echinococcus* of the family Taeniidae, maintains its life cycle using canids, including dogs and foxes, as definitive hosts and wild rodents as intermediate hosts. Humans become infected by ingesting eggs excreted in the feces of definitive hosts, resulting in a severe zoonotic disease, alveolar echinococcosis (AE). Globally, over 18,000 new AE cases are estimated each year. In Japan, Hokkaido is a highly endemic area for *E. multilocularis*, with a prevalence rate of about 30–40% in red foxes and around 20 new human cases annually.

Genetic information of pathogen populations is fundamental for a better understanding of their prevalence, diffusion process, and differences in biological characteristics among genetic variants. All these aspects are essential for controlling infectious diseases. Globally, genetic studies have been performed on *E. multilocularis*, providing some knowledge on their transmission dynamics and the origins of introduction. However, there is a significant lack of genetic information regarding *E. multilocularis* in Hokkaido, Japan.

In Chapter I, the origins of *E. multilocularis* in Hokkaido were investigated through the analysis of the mitogenome sequences from 60 *E. multilocularis* parasites. The analysis identified two haplogroups composed of 16 haplotypes in Hokkaido. Comparisons of the genetic data with other endemic areas showed that a major haplogroup (Haplogroup 1) originated from St. Lawrence Island, USA, which supports the historical hypothesis of the introduction of *E. multilocularis*. Furthermore, a minor haplogroup (Haplogroup 2) was closely related to the parasites in Sichuan, China, implying that parasite invasion into Hokkaido may have resulted from complex and inadvertent animal translocations. These findings highlight the anthropogenic impacts on the spread of the zoonotic parasite and provide a crucial perspective for preventing future potential epidemics.

In Chapter II, a deep amplicon sequencing assay and the MIG-seq analysis were performed on the *E. multilocularis* samples in Hokkaido to gain insights into interactions between two distinct haplogroups. The deep amplicon sequencing assay targeting mitogenome revealed a co-existence of two haplogroups on a local scale, with certain levels of co-detection in the feces and intestine samples. The co-detections were found exclusively in Eastern Hokkaido. These results indicate that the two haplogroups share the same niche. Genome-wide SNP analysis by the MIG-seq method revealed a discrepancy in genetic profiling results between mitochondrial and nuclear DNA markers. This suggests that a cross-hybridization between the two haplogroups has occurred, giving rise to parasites with unique genetic backgrounds. Further investigations will be required to determine whether the differences in genetic backgrounds affect phenotypic variations of the parasites.

Through Chapters I and II, the population genetic structure of *E. multilocularis* in Hokkaido has been revealed. This study has provided insights into the diffusion process of *E. multilocularis*, offering implications for preventing the global epidemic of AE. Furthermore, the genetic diversity found in this study would facilitate the understanding of biological differences among individuals with different backgrounds. Additionally, the genetic data and analytic methods developed in this study will serve as valuable tools for molecular epidemiological surveys of *E. multilocularis*.