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

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学位論文題名
The title of the doctoral dissertation

Studies on molecular detection of *Leishmania* infection in stray dogs from Bangladesh

(バングラデシュ人民共和国の野犬におけるリーシュマニア感染に関する研究)

Visceral leishmaniasis (VL) is a fatal vector-borne parasitic disease. This disease is zoonotic in Mediterranean countries, the Middle East, Asia, and South America, where it is caused by *Leishmania infantum*, with dogs as the main reservoir host whereas in the Indian subcontinent, transmission of VL caused by *L. donovani* is thought to be anthroponotic with the transmission of infection from human to human by phlebotomine sand fly bites. However, the importance of dogs as reservoirs in *L. donovani* transmission is still not well investigated in the Indian subcontinent as well as in Bangladesh which is one of the most highly endemic countries for VL. In our previous study, *L. donovani* DNA was detected in one stray dog (1.2%) from VL-endemic areas of Bangladesh and recommended further verification to better understand the possible role of stray dog as a reservoir for VL transmission in that area. Considering that stray dog's population in Bangladesh is high and they are living close to humans, there is a possibility of disease transmission from dogs to humans. Therefore, it is important to understand the parasite diversity in dogs to minimize the potential risk to humans. In this study, a total of 50 stray dogs were captured in VL-endemic areas of Bangladesh and tested for the presence of parasites by using serological and molecular methods.

In Chapter I, 50 stray dogs were screened for serological and molecular evidence of *Leishmania* infection. Anti-*Leishmania* antibodies were detected in 6 dogs using rK39 immunochromatographic tests. *Leishmania* kinetoplast DNA was observed in ten buffy coat DNA samples by real-time PCR, five of which were positive based on internal transcribed spacer 1-PCR. Sequencing analysis of the amplified products revealed 100% identity to *L. donovani* DNA sequences reported from human VL patient in Bangladesh. These findings strengthen the assumption that stray dogs in this endemic area could play a potential role in VL transmission as an animal reservoir.

In Chapter II, a metagenomic approach was employed to identify the parasite-derived cell-free DNA (cfDNA) sequences in the plasma of 14 stray dogs. An average of 2.3 million reads per sample were obtained from two independent Illumina MiSeq runs. After removal of the reads derived from the dog genome, the reads were screened for homology to known parasites by local BLASTn searches against the NCBI NCBI nt (non-redundant nucleotide sequences) database. After quality filtering, a total of 150 reads were identified to show high similarity with the parasite sequences including *Leishmania* and *Babesia*, which were previously detected by molecular methods from the same dog group. The results suggest that the present approach of analyzing cfDNA would allow us to investigate the parasite diversity in a high throughput manner.

Further elaborate studies are required to analyze the *Leishmania* infection in dogs in a larger scale and to reveal the precise role of dogs in transmitting the parasite to vector sand fly in nature. The findings also implicate that cfDNA in plasma could be a potential diagnostic marker to assess the parasite communities prevailing in dogs.