Summary of the dissertation

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The title of the doctoral dissertation

Molecular investigation of ticks and tick-borne pathogens in domestic and wild animals in Nepal

(ネパールの家畜と野生動物におけるマダニおよびマダニ媒介性病原体の分子遺伝解析)

Summary

Ticks are hematophagous ectoparasites that infest a wide host range including humans and transmit a rich diversity of pathogens. Tick-borne diseases (TBDs) are emerging infectious diseases mainly originating from wildlife. Knowledge of the ticks and tick-borne pathogens (TBPs) in Nepal, as in many developing nations, has been limited by a range of factors which have in turn hampered efforts to control ticks and TBPs. One major factor which has contributed to this knowledge gap is the difficulty in accurately identifying and monitoring ticks and TBPs in Nepal. Previous surveillance efforts have mostly employed traditional techniques such as morphological investigations using light microscopy. However, morphological diagnosis of TBPs such as protozoa often suffers from lower accuracy and sensitivity compared with molecular techniques and morphological identification of ticks is also challenging without experienced skills. This study used molecular techniques such as Sanger sequencing, molecular cloning, and amplicon sequencing for the detection of pathogens and characterization of ticks in Nepal.

Molecular cloning is the procedure of recombining numerous DNA fragments into plasmids that can be replicated in bacterial hosts, specifically *Escherichia coli*. The technique is useful in the sequencing analysis of multiple DNA sequences present in a sample. In this study, the cloning

technique was successful employed in chapters I to IV for the simultaneous detection of multiple TBPs present in the samples. Co-infection of different pathogens was detected in both domestic and wild animals. Chapters I and II provided the data on several TBPs such as *Theileria bicornis* in rhinos, tigers, and leopards, which has implication for wildlife conservation. In chapter III, multiple TBPs including *Babesia, Anaplasma*, and *Ehrlichia* were detected in dogs, indicating that community dogs are sentinels and reservoirs for some zoonotic pathogens. In chapter IV, *Anaplasma bovis, Anaplasma marginale, Theileria annulata,* and *Theileria orientalis* were detected in cattle of two different ecological zones of Nepal holding the major population of cattle in the country. Epidemiology of these pathogens is important to understand for developing control strategies and reducing the economic losses caused by the infections.

Next-generation sequencing is a powerful tool that can be used in a variety applications in diagnosis of infectious diseases. Amplicon sequencing is one of the applications that can simultaneously sequence genetic fragments from multiple species and strains within samples, demonstrating its advantage over the previous PCR-based and microscopic methods. In chapter V, amplicon sequencing approach was used for simultaneous detection of TBPs in goats. The resulting data showed that different genotypes of *Anaplasma bovis* presented within a sample. Co-infection with other TBPs such as *Anaplasma marginale*, *Anaplasma ovis*, and unclassified *Ehrlichia* sp. was also detected. The findings have a clinical significance in that the co-infections may complicate disease diagnosis and treatment in goats.

Molecular techniques are useful for precise tick identification, especially for damaged and engorged individuals and those of closely related species. In chapter VI, ticks collected from domestic and wild animals were identified by an integrative approach using both morphological and molecular methods. Morphological examination detected a total of 12 tick species, while molecular analysis revealed that *Rhipicephalus turanicus* analyzed in this study formed a distinct subclade within the *Rhipicephalus sanguineus* complex, potentially representing a new species. In this study, *Rhipicephalus haemaphysaloides* and *Haemaphysalis bispinosa* were identified in both domestic and wild animals, suggesting a possible overlap in tick population at the interface between domestic and wildlife. This overlap indicates the potential for spillover of TBPs between domestic and wild animals.

This thesis revealed that a wide range of TBPs infected both domestic and wild animals in Nepal. Using molecular methods such as cloning and amplicon sequencing made it possible to identify co-infections of different TBPs and/or different strains of the same pathogens. Presence of mixed infection may hinder the accurate diagnosis and treatment of TBPs. Future studies are required to evaluate clinical significance of mixed infections. This study could serve as a pioneering approach for screening TBPs in domestic and wild animals in Nepal and will support improving the diagnosis of TBDs in Nepal.