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Abstract of the dissertation

博士の専攻分野の名称：博士（感染症学）

氏名：Gita Pandey Sadaula

Name

学位論文題名
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)

Ticks are hematophagous ectoparasites that infest a wide range of hosts and transmit various pathogens. Tick-borne diseases are emerging infectious diseases mainly originating from wildlife. In Nepal, the documentation of ticks and tick-borne pathogens (TBPs) remains limited and has been primarily based on microscopic examination. However, morphological diagnosis of TBP infections 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 technique is useful in the sequencing analysis of multiple DNA sequences present in a sample. In this study, the cloning technique was successfully 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 provide 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. Understanding the epidemiology of these pathogens is important for developing control

strategies. Next-generation sequencing is a powerful tool that can be used in a various 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. 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 also useful for precise tick identification of tick species. In chapter VI, ticks collected from domestic and wild animals were identified by both morphological and molecular methods. Morphological examination detected a total of 12 tick species, while molecular analysis revealed that *Rhipicephalus turanicus* detected in study potentially representing a new species within the *Rhipicephalus sanguineus* complex. 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. Overall, this thesis revealed a wide range of TBPs infecting both domestic and wild animals in Nepal. 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 TBPs in Nepal.