



# HOKKAIDO UNIVERSITY

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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（感染症学）

氏名：DELA RIA NESTI

Name

学位論文題名  
The title of the doctoral dissertation

Development of a Comprehensive Detection Method for  
Paramyxoviruses Using Indonesian Bats

（インドネシア由来コウモリを用いたパラミクソウイルス網羅的検出法の開発）

Zoonotic diseases remain a major global health threat. Indonesia faces a high risk of bat-borne virus spillover to humans due to its rich bat diversity and ongoing wildlife trade. Effective surveillance requires a comprehensive understanding of both bat viromes and host species. In this study, we developed a semi-comprehensive viral detection system that integrates RT-PCR with multiplex next-generation sequencing (NGS) to detect paramyxoviruses from bat samples. Three degenerate primer sets targeting major paramyxovirus groups (RMH, ARb, and PNE) were modified by adding 8-base index sequences to enable multiplex analysis and by replacing inosine with mixed nucleotides to improve sensitivity. Although a simplified one-step multiplex PCR was feasible, semi-nested PCR yielded the highest detection sensitivity. Using tracheal and rectal swabs from 135 bats collected in Indonesia, we validated the method and identified seven novel paramyxovirus-like sequences from ARb PCR amplicons showing 70.88–82.44% similarity to known pararubulavirus, orthorubulavirus, and henipavirus genomes. These findings were confirmed by PAR PCR, which has lower sensitivity but can broadly detect major paramyxovirus groups, along with PCR linking full-length ARb and PAR amplicons, and targeted RT-PCR sequencing. Overall, the results demonstrate that this highly sensitive and low-cost method is effective for monitoring bat viruses. Accurate species identification is essential for understanding host–virus ecological dynamics. To improve taxonomic resolution, we applied mitochondrial *cox1* barcoding to bat samples collected from six locations across four Indonesian islands. Amplification was successful for all samples, and when homologous sequences were absent from GenBank or showed low similarity, species identification was supplemented using *cytb*, *BDNF*, and *RAG2* sequences. Among 54 samples from 48 bats, representing *Pteropodidae*, *Rhinolophidae*, and *Hipposideridae*, 44 bats were classified into 15 species, whereas 4 bats remained unclassified. New *cox1* sequences of *Cynopterus nusatenggara*, *Rhinolophus celebensis*, and *Dobsonia peronii* were obtained, contributing to the expansion of reference databases. The consistent amplification of the *cox1* barcode across all samples

confirmed its reliability for non-invasive bat identification. Subsequent molecular analyses of *cox1* sequences revealed genetic diversity within *Cynopterus brachyotis*, *Rhinolophus borneensis*, and *Rhinolophus affinis*, suggesting geographic influences on intraspecific variation. These results indicate that the method provides a reliable and non-invasive approach for accurate bat species identification. In this study, we developed and evaluated a highly sensitive, cost-efficient viral detection system and a robust host species identification framework. Large-scale field applications of these methods are expected to enhance our understanding of host–virus ecosystems and support efforts to control zoonotic diseases.