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A study on emerging viral infections and associated risks in wildlife of Hokkaido, Japan
(北海道の野生動物における新興ウイルスの感染状況および感染リスクの評価)

The number of emerging infectious diseases (EIDs) caused by pathogens that can infect both humans and animals (zoonotic pathogens), such as COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is increasing worldwide. Many such pathogens originate in wildlife, and viruses are of particular concern due to their ability to spread rapidly and mutate to infect multiple animal species. Investigating which pathogens infect wild animals, including recently identified EIDs, can help identify competent hosts, facilitate monitoring of virus circulation in nature, and is therefore important for better preparedness against potential pathogen transmission from susceptible wildlife species to humans. Importantly, many viruses can circulate in wildlife without causing disease, later mutating to infect different species through transmission cycles. Therefore, it is important to identify pathogens carried by wildlife and assess their zoonotic potential. In Hokkaido, the northernmost island of Japan, past land-use changes and agricultural expansion followed by abandonment have created suitable wildlife habitats. In recent decades, increasing populations of sika deer, brown bears, and raccoons have frequently invaded urban and peri-urban areas, leading to human–wildlife conflicts and concerns about zoonotic pathogen transmission. In this study, surveillance of EIDs in local wildlife in Hokkaido was conducted, focusing on species that frequently enter human-dominated landscapes. This thesis consists of three chapters. Chapter I describes surveillance of SARS-CoV-2 among raccoons, sika deer, brown bears, and raccoon dogs in Hokkaido. Among more than 500 animals tested, none were PCR- or antibody-positive, suggesting the absence of infection. This study is the first report of SARS-CoV-2 surveillance in wildlife in Japan and provides important evidence of the lack of virus transmission in Hokkaido, informing prefectural wildlife health management strategies. Given that cervids are known to be susceptible to SARS-CoV-2, Chapter II investigated possible reasons for the absence of SARS-CoV-2 transmission in sika deer through molecular and functional characterization of ACE2, the cellular receptor mediating viral entry. Sika deer ACE2 supported viral infection, indicating its functionality as a SARS-CoV-2 receptor. These findings suggest that factors beyond receptor compatibility, such as tissue-specific ACE2 expression, host immune responses, and human–deer interaction patterns, may explain the lack of transmission in sika deer

populations in Hokkaido. Chapter III investigated other wildlife pathogens with zoonotic potential, focusing on tick-borne viruses in the family Phenuiviridae. Toyo virus (TOYV) infection was identified in brown bears on the Shiretoko Peninsula, and the virus was successfully isolated from bear samples. This represents the first report of TOYV infection in mammals and the first isolation of a tick-borne virus from ursids. Serological surveillance of wildlife in Hokkaido, including brown bears, raccoons, sika deer, and rodents, revealed endemic TOYV circulation in the region. Overall, this thesis improves understanding of viral EID exposure in wildlife in Hokkaido and clarifies the role of these species as potential reservoirs, supporting efforts to protect animal and human health.