Form No.5

Abstract of Thesis/Dissertation

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Title: Surveillance of avian paramyxoviruses (APMVs) in wild bird population in Hokkaido and characterization of the APMV isolates
(北海道に飛来する野鳥における鳥パラミクソウイルスのサーベイランスと分離ウイルスの特徴付け)

Abstract

Avian paramyxoviruses (APMVs) are single-stranded, nonsegmented, negative-sense, enveloped RNA viruses belonging to the family *Paramyxoviridae.* All APMVs belong to the genus *Avulavirus* that are now classified into 13 serotypes (APMV-1 to APMV-13) based on antigenic and/or genetic analysis, so far. APMV is one of the important viruses in bird species. Research on APMVs has been exclusively focused on APMV-1, because virulent APMV-1 causes serious economic loss in poultry industry. However, very little is known about virological properties or ecology of other APMV serotypes in nature. Migratory wild birds are well known as natural reservoir for APMVs and may introduce virus to poultry. Therefore, to understand the ecology of APMVs circulating in wild birds, I conducted a surveillance of APMVs in wild birds flying in Hokkaido, and the characterization of APMV isolates were also described.

Chapter I describes the surveillance of APMVs in wild birds flying in eastern Hokkaido. Wild birds are of concern as a carrier of APMVs. Japan is in the East Asian Australia flyway, and Hokkaido is one of the important stopover sites for migratory birds in Japan. A total of

10,606 cloacal swab or fecal samples were collected form wild birds. The predominance of APMVs isolated from this study was APMV-4 (58 isolates), followed by APMV-1 (18), APMV-6 (13), and APMV-9 (2). The phylogenetic analysis was performed based on partial F genes covering the F protein cleavage site motif. The aa sequence at this site in all the isolates resembled low virulent APMV-1. The genetic diversity within serotypes was observed in APMV-1, APMV-6, and APMV-9, while the high similarity was found among all APVM-4 isolates. On the other hand, the geographical connection between different continents were found due to the phylogenetic analysis of APMV-1 and APMV-4, because the virus exchange between Africa, America, Asia, and Europe were noticed. This evidence emphasized the important role of wild birds in virus transmission. The results obtained in this study provided evidences of ongoing genetic changes in APMVs. Therefore, a surveillance of APMVs in wild bird populations should be continued. This is the first large-scaled surveillance study of APMV performed in Hokkaido

Chapter II describes genetic characterization of APMV-1 isolates derived from wild birds based on the hemagglutinin-neuraminidase (HN) genes and antigenic comparison between chicken and wild bird APMV-1 using monoclonal antibodies (mAbs) against the F and HN proteins of reference strains of APMV-1. So far, the F protein cleavage site of APMV-1 has been used to determine virus virulence, since the presence of multibasic amino acid (aa) motif at the cleavage site is associated with the virulence in chickens. However, it has been reported that the pathogenicity of virus in chickens did not always correlate with the aa sequence at this site. Beside the F genes, the HN genes were also considered as a virus virulence determinant. The phylogenetic tree generated from the HN genes classified APMV-1 isolates into two genetic classes, I and II as observed in the tree based on the F genes. The various length (585 and 616 aa) of the HN proteins were found

in the APMV-1 isolates in this study. Although the short length (571 aa) of the HN protein was reported to be related to the high virulence of APMV-1, the short length (585 aa) of the HN protein of the APMV-1 isolate found in this study seemed not to be related to the high virulence. The antigenicity comparison study using nine mAbs against reference APMV-1 strains showed the considerable antigenic difference between wild bird APMV-1 and poultry strains including vaccine strains. Most of the mAbs did not recognize the APMV-1 isolates in this study. Thus, the antigenic difference between field and vaccine strains may affect the efficacy of vaccination in poultry. Therefore, to monitor antigenic changes in APMV-1, the HN genes should be analyzed together with the F genes, because immune response is raised against the F and HN proteins on the viral envelope.

Chapter III describes the identification and virological characterization of a novel serotype of APMV. A hemagglutinating virus isolate designated 11OG0352, was obtained from a duck fecal sample. Genetic and virological analyses indicated that it might represent a novel serotype of APMV. Electron micrographs showed that the morphology of the virus particle was similar to that of APMV. The phylogenetic analysis of the whole genome revealed that the virus was a member of the genus *Avulavirus*, but that it was distinct from APMV-1 to APMV-13. Although the F-protein cleavage site was TREGK↓L, which resembles a low virulent strain of APMV-1, the K residue at position −1 of the cleavage site was first discovered in APMV members. The intracerebral pathogenicity index test did not detect virulence in infected chicks. The virus replicate in the respiratory tract of infected mice. In hemagglutination inhibition (HI) tests, an antiserum against this virus did not detectably react with other APMVs (serotypes 1–4, 6–9) except for a low cross-reactivity with APMV-6. From these results, I designated this isolate, as APMV-14/duck/Japan/11OG0352/2011 and propose that it is a novel APMV serotype. This is the first report on a new APMV serotype,

APMV-14. The HI test may not be widely applicable for the classification of a new serotype because of the limited availability of reference antisera against all serotypes and cross-reactivity data. The nucleotide sequence identities of the whole genome of 11OG0352 and other APMVs ranged from 46.3% to 56.1%. Such comparison may provide a useful tool for classifying new APMV isolates. However, the nucleotide sequence identity between APMV-12 and APMV-13 was higher (64%), which was nearly identical to the lowest nucleotide identity (67%) reported in subgroups within the serotype. Therefore, consensus criteria for using whole genome analysis should be established.

In conclusion, this study provides the informative information of APMVs circulating in wild birds. The results supported the evidence of intercontinental dispersal of APMV by migratory birds between different continents. The genetic and antigenic variation among each serotype and the discovery of a novel serotype, APMV-14 indicated that APMVs are undergoing evolution. The finding obtained in this study highlights the importance to continue an extensive surveillance of APMVs in wild bird populations to understand the global dispersal, genetic diversity and evolution of APMVs.

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