別記様式第３号

学　位　論　文　要　旨

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題　　目　Molecular identification of Newcastle disease viruses and their control through vaccination and enhanced biosecurity

（ニューカッスル病ウイルスの分子的識別とワクチン接種およびバイオセキュリティ強化による防除に関する研究）

Poultry infectious diseases, especially avian influenza (AI), Newcastle disease (ND), colibacillosis, salmonellosis, diseases from fowl adenovirus (FAdV), and avian reovirus (ARV) are highly contagious and detrimental to the poultry industry. Highly pathogenic avian influenza (HPAI) or ND have high morbidity and mortality rates of up to 100% due to their incidence and rapid transmission, resulting in severe economic losses for the poultry industry not only from animal losses due to diseases but also from trade restrictions and embargoes.

ND virus (NDV) is one of the major poultry pathogens that seriously endangers the poultry industry, resulting in a highly contagious septic, a fatal and destructive disease affecting a wide variety of poultry and wild birds worldwide. The economic impact of ND is severe, for example with estimated losses of 288,49 million US dollars annually in Bangladesh, 23 million US dollars in Nigeria, and 162 million US dollars in the USA. In 2017-2019, over 20 Asian and 30 African countries reported over 5,400 ND outbreaks to the World Organization for Animal Health (OIE), a large fraction of which were from Iran (n = 492), Ghana (n = 454), Afghanistan (n = 442), and Zambia (n = 425); only 40 reported outbreaks were from Kenya. The virus is shed directly from infected birds by droplets during sneezing and coughing and through feces indirectly, thus contaminating the air, objects, and floor of the farms.

There are still many ND outbreaks worldwide. The devastating effect of NDV can be controlled with vaccines and most live vaccines are sensitive to heat and require a cold chain to maintain the quality during transport and storage. Therefore, developing thermostable vaccines that could be partially or entirely independent of a cold chain is of

great importance. Prevention and control of diseases largely depend on biosecurity, and disinfectants are very important tools for biosecurity programs designed by the poultry industry. Therefore, the objective of the present studies was to identify the NDV from Afghanisthan at the molecular level, develop a Vero cell adapted thermostable vaccine candidate, and evaluate the efficacy of some biosecurity materials for the control of NDV and other avian pathogens.

In Chapter 1, hemagglutination (HA) positive viruses from three chicken farms in Afghanistan were identified as NDV by reverse transcription-polymerase chain reaction assay and sequencing. Three isolates from each farm were sequenced to characterize the part of their fusion (F) protein gene around the cleavage site. The F gene characteristics of the three isolates shown in the phylogenic analysis indicated that the isolates were velogenic, belonged to the class II subgenotype VII 1.1, and were closely related to an identified Chinese NDV isolate. To our knowledge, this is the first time that NDV isolates from Afghanistan have been partially sequenced. Therefore, this study has provided essential information regarding the genetic nature of circulating NDV, which may help for a further and complete genome study, diagnosis, and control of the disease in Afghanistan.

In chapter 2, from field isolates of NDV in Japan, one avirulent strain, APMV/northern pintail/Japan/Aomori/2003 (dk-Aomori/03, NDV261), was selected for its excellent thermostability, and the strain was heat-treated at 56°C for 30 min during each passage into Vero cells to maintain thermostability and to adapt to Vero cells. After serial 20 passages in Vero cells, it was named NDV-Vero20. When growth curves were tested in Vero cells, NDV-Vero20 grew well in comparison to the original NDV261. Sequencing of the HN gene revealed transmembrane domains with the same amino acids as other thermostable strains. The similarity of the HN gene sequence between NDV-Vero20 and other thermostable strains suggested the thermostability of NDV-Vero20. The thermostability of the virus was confirmed by storing it at different temperatures including 37°C. When susceptible chicks were inoculated with NDV-Vero20 through eye drops, induced adequate levels of antibody were measured using the serum neutralization test. The results showed that NDV-Vero20, a candidate vaccine strain is thermostable, Vero cell adapted, and has immunogenic potential which would make it an advisable alternative to the traditional embryonated chicken eggs-based vaccine.

In Chapter 3, the microbicidal activities of mixtures of quaternary ammonium compounds (QACs) and food additive grade calcium hydroxide ($FdCa(OH)_2$) were evaluated in a suspension test at −20°C using an anti-freeze agent (AFA) containing methanol, or at 1°C, with the varying contact time, toward AI virus (AIV), NDV, FAdV, ARV, *Salmonella* Infantis (SI) and *Escherichia coli* (EC). At −20°C, the mixtures could inactivate AIV and NDV within 30 min, FAdV and ARV within 5 sec, and SI and EC within 3 min, respectively. AFA did not inactivate viruses and bacteria within 30 min and

10 min, respectively. At 1°C, the mixtures inactivated FAdV and ARV within 30 sec, AIV within 10 min, and NDV within 30 min. A mixture of slaked lime (SL) and QAC could inactivate FAdV and ARV within 30 sec, but could not inactivate AIV and NDV even after 60 min at 1°C. SL could not substitute $FdCa(OH)_2$ to exert the synergistic effects with QAC. Thus, QACs microbicidal activities were maintained or enhanced by adding $FdCa(OH)_2$. It is hence recommended to use QACs with $FdCa(OH)_2$, especially in the winter season.

In Afghanistan, virulent NDVs were isolated from vaccinated chicken farms, inferring a problem with proper vaccine storage; ND vaccination and vaccine efficacy still need improvement. Therefore, the thermostable NDV-Vero20 is the most robust vaccine candidate strain adapted to Vero cells and can withstand high temperatures, making it advantageous for use in rural areas and tropical or subtropical countries. In addition, animal cell culture offers many advantages over the traditional chicken-eggs method. It is suggested for enhancing farm biosecurity to use $FdCa(OH)_2$ for synergistic and broaden the spectrum of QACs in all seasons. Enhancement of biosecurity and vaccination are the most effetive ways to combat avian pathogens, including NDV.

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