Jutarat Lidjun 2008: Development of Reverse Genetics Derived Avian Influenza H5N1 (rgH5N1) Virus Vaccine. Master of Science (Genetic Engineering), Major Field: Genetic Engineering, Interdisciplinary Graduate Program. Thesis Advisor: Associate Professor Porntippa Lekcharoensuk, Ph.D. 77 pages.

Production of vaccine against highly virulent avian influenza (HPAI) virus (H5N1) using wild type virus growed in embryonated chicken eggs results in low virus yield because the embryo dies before 24 hours. On the other hand, most of heterologous vaccines are not able to induce highly effective immunity. A reverse genetics technique was employed to produce seed HPAI H5N1 vaccine by cloning HA and NA genes derived from the HPAI H5N1 as the remaining six genes of swine influenza A virus (SIV) H1N1. Polybasic amino acids were removed from the cleavage site of HA gene to reduce pathogenicity. Each gene was cloned into bidirectional transcription plasmid (pDZ). The reassortants were created by transfecting modified HA and NA genes, from HPAI (H5N1), as well as PB2, PB1, PA, NP, M and NS genes from SIV (H1N1) into a mixture of 293T and Madin-Darby Canine Kidney (MDCK) cells. The rescued rgH5N1 in the supernatant over the transfected cells was infectious when inoculated into allantoic cavity of 10-day-old embryonated eggs. The results of RT-PCR using RNA templates isolated from the allantoic fluid demonstrated that the rescued virus contained all 8 gene segments comprising of the modified HA and NA genes derived from HPAI H5N1, and PB2, PB1, PA, NP, M and NS genes of SIV H1N1. The titer of virus in the allantoic fluid was as high as 2×10^{9} $TCID_{50}$/ml. The rescued virus was infectious in MDCK cells as confirmed by immunofluorescent assay. In addition, plaque formation by rgH5N1 was trypsin dependent. These results show that we have successfully produced seed rgH5N1 virus vaccine for the production of HPAI H5N1 vaccine.

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