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Characterization of an H5N3 Avian Influenza Virus Isolated from Wild Birds

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Introduction: Avian influenza viruses (AIVs) are classified into two pathotypes on their virulence; low pathogenic avian influenza (LPAI) virus and highly pathogenic avian influenza (HPAI) virus. Among 16 HA subtypes of AIV, only H5 and H7 subtypes have caused HPAI in poultry. However, most H5 or H7 subtype viruses are LPAI. Since the 1997 H5N1 avian influenza outbreak in Hong Kong, AIVs including H5 and H7 HPAI viruses have shown their ability to infect humans directly. An avian H5N3 influenza virus (A/Wild bird feces/Korea/SYG06/2006) was recently isolated from fecal samples of wild birds obtained from the SoYang river in Chuncheon city. In this study, we describe the biological and molecular characterization of the H5N3 avian influenza isolate (WBF/KR/SYG06/06).

Materials and Method: A/wild bird feces/Korea/SYG06/2006(H5N3) isolate was propagated in 10-day-old embryonating chicken eggs inoculated via the allantoic sac. The allantoic fluids from the infected eggs were harvested and used for other tests. Viral RNAs were extracted from the allantoic fluids with viral DNA/RNA extraction kit (Viral Gene-Spin, Intron, Korea). Standard reverse transcription-PCR was performed by use of a One-Step RT-PCR kit (QIAGEN, Valencia, Calif) with segment-specific primer sets. PCR products were separated in an agarose gel by electrophopresis, and amplicons of the appropriate sizes were purified with the QIAquick Gel Extraction Kit (QIAGEN, Valencia, Calif). Sequencing was performed with DNA sequencing service in Macrogen Inc (Macrogen, Korea). Nucleotide and amino acid sequences were compared with other genome sequences of influenza viruses obtained from the GeneBank database by the maximum parsimony method with MEGA4 program. In vitro test to predict pathogenicity potential was performed with Madin-Darby canine kidney (MDCK) cell culture system.

Results: A phylogenetic analysis of the 5 viral genes (HA, NA, M, NS, NP genes) of WBF/KR/SYG06/06 showed that all 5 viral genes belonged to Eurasian lineage. The five gene segments had a close relationship with Chinese or Italian LPAI H5N3 or H5N2 strains from 2000 to 2005. Phylogenetically, WBF/KR/SYG06/06 isolate was clearly different from the HPAI H5N1 strains including human isolates and Italian HPAI H5N2 strains and formed a separate clade. Also, no relationship was found between WBF/KR/SYG06/06 and Korean HPAI H5N1 isolates. The WBF/KR/SYG06/06 isolate had high sequence identity from 98.0 to 99.8% at the nucleotide level and from 97.6 to 100% at the amino acid level with Eurasian lineage isolates.

The HA gene of WBF/KR/SYG06/06 isolate had RETR/GLF motif at the cleavage site, which suggested that the isolate should be LPAI virus. The HA sequences of the isolate had the specific amino acid residues at positions 150, 202, 206, 237, 238 and 240 (position 138, 190, 194, 225, 226 and 228 in H3 numbering), which define the receptor binding site residues typical of AIV. The WBF/KR/SYG06/06 isolate had no amino acid deletion in the stalk region of the neuraminidase protein. In NS1 gene, amino acid changed at positions D92E and V149A, which have been associated with the virulence and resistance antiviral cytokines in pigs and chickens. The H5N3 virus in this study had 92D and 149A but no deletions were observed within the protein. NS2 gene had four C-terminal amino acids, motif ESEV, typical of AIVs.

The WBF/KR/SYG06/06 isolate replicated well and produced cytopathic effects (CPEs) in MDCK cells with the addition of trypsin. However, there were no detectable viruses in MDCK cultures without trypsin, which indicated that this virus was LPAIV.

Conclusions: A phylogenetic analysis of the 5 viral genes (HA, NA, M, NS and NP) showed that the WBF/KR/SYG06/06 isolate belonged to Eurasian lineage. A phylogenetic analysis also showed that the H5N3 isolate was different from HPAI H5N1 strains including human isolates. The H5N3 isolate had avian specific receptor binding site residues in HA gene and avian specific four C-terminal amino acids in NS2 gene. HA gene of this isolate had the typical LPAI motif at the cleavage site and this virus produced no CPEs in MDCK cells without trypsin. From these results, we suggested that an H5N3 avian influenza virus isolated from wild birds in Chuncheon city should be LPAI virus and should have no pathogenic to human.

References

1. **L. Duan, L. Campitelli. X. H. Fan, Y. H. C. Leung, D. Vijaykrishna, J. X. Zhang.** Characterization of Low-Pathogenic H5 Subtype Influenza Viruses from Eurasia: Implications for the Origin of Highly Pathogenic H5N1 Viruses *Journal of Virology* 2007; 00327-07: 7529-7539.