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GENOME ANNOUNCEMENT

# Complete Genome Sequence of a Mammalian Species-Infectious and -Pathogenic H6N5 Avian Influenza Virus without Evidence of Adaptation

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**An H6N5 avian influenza virus (AIV) strain, designated A/aquatic bird/Korea/CN5/2009 (H6N5), was isolated from fecal swabs of aquatic birds in 2009, and surprisingly, it showed infectivity and pathogenicity in mammalian species without evidence of adaptation. In this study, we report the first complete genome sequence containing 3′ and 5′ noncoding regions (NCRs) of a mammalian species-infectious and pathogenic H6N5 AIV, which will help provide important insights into the molecular basis of pathogenesis, transmission, and evolution of AIV.**

Avian influenza virus (AIV) is a segmented, negative-sense, single-stranded RNA virus belonging to the family *Orthomyxoviridae*, genus *Influenzavirus A*. Aquatic birds are the natural reservoirs of influenza A viruses (3, 16). The A/aquatic bird/Korea/CN5/2009 (H6N5) [A/AB/Kor/CN5/09 (H6N5)] strain was isolated from fecal swabs of aquatic birds in 2009. Surprisingly, it showed infectivity and pathogenicity in mammalian species without evidence of adaptation (6). To date, primers targeting the noncoding regions (NCRs) have generally been used to obtain complete genomes of influenza A viruses. Thus, the exact sequences of NCRs have scarcely been determined. Moreover, a complete genome sequence containing 3′ and 5′ NCRs of a mammalian species-infectious and pathogenic H6N5 AIV with no evidence of adaptation has not been reported despite multifunctions of NCRs in the replication of influenza A viruses (4, 5, 14, 15, 18). For those reasons, it is necessary to analyze the complete genome sequence containing 3′ and 5′ NCRs of A/AB/Kor/CN5/09 (H6N5) and understand its molecular characteristics.

Viral RNA was isolated from allantoic fluids of embryonated eggs infected with the A/AB/Kor/CN5/09 (H6N5) ($10^{7.75}$ 50% egg infective doses [$EID_{50}$]/ml) by using an RNeasy minikit (Qiagen) and circularized with T4 RNA ligase as described previously (2, 13). The PCR products produced by RNA ligation-mediated reverse transcriptase PCR (RT-PCR) were purified, cloned (9), and sequenced to determine its exact complete genome sequence containing 3′ and 5′ NCRs on an automated DNA sequencer (ABI system 3700; Applied Biosystems Inc.) by utilizing universal primers (7) with simple modifications and newly designed segment-specific primers.

The complete genome of A/AB/Kor/CN5/09 (H6N5) is 13,607 nucleotides (nt) long; segments 1 (Seg-1) to 8 (Seg-8) are 2,341, 2,341, 2,233, 1,765, 1,565, 1,467, 1,027, and 890 nt, respectively. They encode 12 viral proteins with amino acid lengths as follows: PB2, 759; PB1, 757; N40 (an N-terminally truncated and functionally distinct variant of PB1) (17), 718; PB1-F2, 90; PA, 716; HA, 566; NP, 498; NA, 472; M1, 252; M2, 97; NS1, 230; and NS2 (nuclear export protein [NEP]), 121.

The sizes of NCRs of viral RNA of A/AB/Kor/CN5/09 (H6N5) were variable (17 [Seg-4] to 45 [Seg-5] and 20 [Seg-7] to 58 [Seg-3] nt at the 3′ and 5′ NCRs, respectively) in the different genome segments, but the terminal 12 (3′UCGYUUUCGUCC-) and 13 (-GGAACAAAGAUGA5′) nt of the 3′ and 5′ ends, respectively, were highly conserved among all genome segments, which is consistent with results of previous studies (1, 8, 11). Furthermore, a uridine-rich region (5 to 6 U's), which serves as the polyadenylation site (10, 12), was observed from positions 15 through 16 at the 5′ end of each segment.

This is the first report of the complete genome sequence containing 3′ and 5′ NCRs of H6N5 AIV showing infectivity and pathogenicity in mammalian species without evidence of adaptation. We hope that these data will help elucidate the molecular basis of pathogenesis, transmission, and evolution of AIV as well as other influenza A viruses.

**Nucleotide sequence accession numbers.** The complete genome sequence of the A/AB/Kor/CN5/09 (H6N5) has been deposited in GenBank under accession numbers JX465637 to JX465644 for Seg-1 to Seg-8.

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