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GENOME ANNOUNCEMENT

Complete Genome Sequence of an Avian Leukosis Virus Isolate Associated with Hemangioma and Myeloid Leukosis in Egg-Type and Meat-Type Chickens

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Subgroup J avian leukosis virus (ALV-J) was first isolated from meat-type chickens that developed myeloid leukosis (ML). In recent years, field cases of hemangioma (HE) or HE and ML, rather than ML alone, have been reported in commercial layer flocks exposed to ALV-J with a high incidence in China. Here we report the complete genomic sequence of an ALV-J isolate that caused both HE and ML in egg-type and meat-type chickens in China. These findings will provide additional insights into the molecular characteristics in genomes, host range, and pathogenicity of ALV-J.

The J subgroup of avian leukosis virus (ALV-J) is the latest addition to the list of avian leukosis virus subgroups identified in chickens. Since ALV-J was first isolated in 1989 from white meat-type chickens with myelocytomatosis in Great Britain, ALV-J has primarily induced myeloid leukosis (ML) in meat-type chickens and caused enormous economic losses for the poultry industry worldwide (1, 2, 4, 7, 8). In recent years, field cases of infection and tumors caused by ALV-J in commercial layers and breeders have been emerging in China, which resulted in bleeding claws and surrounding skin, yellowish-white tumors on the visceral surface, and drastically reduced egg production (1, 2, 4, 9). A new isolate, designated GD1109 ALV-J, was isolated from a commercial layer flock that suffered from a high incidence of spontaneous hemangioma (HE) (>30%) and a low incidence of ML (<5%) in Guangdong province, China, in 2011. GD1109 ALV-J was propagated in a chicken embryo fibroblast cell line (DF-1). The whole genome of GD1109 was amplified by PCR, cloned into the pMD19-T vector (TaKaRa Bio Inc., Japan), sequenced three times, and assembled using DNAStar version 7.0. Multiple-sequence alignment was performed with Clustal X (BioEdit version 7). The transcriptional regulatory elements in noncoding regions of the genome were analyzed with SoftBerry (Softberry, Inc., Mount Kisco, NY).

Comparative analyses showed that the *pol* and *gag* genes of the GD1109 isolate genome shared homology ranging from 96.4% to 99.5% and 94.0% to 98.9%, respectively, with the reported ALV-J sequences in GenBank, suggesting high conservation of the *pol* and *gag* genes of ALV-J. The homology of the *env* gene was 98.2% between the GD1109 isolate and the HE strain JL093-1 but less than 94.9% with reported ML strains. In addition, the GD1109 isolate's genome harbored three unique nucleotide substitutions (117R, 189G, and 218T) distributed within the central region of the gp85 subunit region in comparison to most layer isolates, which might be responsible for the variability of the GD1109 isolate in host range and pathogenicity (3). A 19-nucleotide insertion (CGGTTGCTCTGCGTGATTC, bases 582 to 600) in the leader sequence of GD1109 was identified and found to be in good agreement with previous reports (5, 6). A single nucleotide deletion in the E element of the GD1109 genome was identified in comparison to HPRS-103, which resulted in a distinct binding site for c-Ets-1. Animal trials using specific-pathogen-free (SPF) White Leghorn and native yellow meat-type chickens challenged with GD1109 resulted in 36% and 67% HE, respectively, and 10% ML for both types of chickens. A novel single nucleotide substitution (T to C) was also identified at position 82 of the U3 region of the long terminal repeat (LTR) in the GD1109 genome. This rare but significant mutation resulted in the loss of the first of two highly conservative inverse Y boxes (ATTGG), suggesting some degree of dispensability for viral fitness but not for tumor type (5, 6).

Nucleotide sequence accession number. The complete genome sequence of the GD1109 isolate was submitted to GenBank, and the assigned accession number is JX254901.

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All experimental chickens in the challenge trials were managed during and euthanized at the end of the experiments according to the South China Agricultural University's Guidelines for Animal Care and Use (revised April 2000) and the *Guide for the Care and Use of Laboratory Animals* (5a).

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