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Genome Sequences of an H9N2 Avian Influenza Virus Strain Found in Pakistan in 2017

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ABSTRACT In 2017, we isolated an H9N2 avian influenza virus in Pakistan. Genetic analysis showed that the A/chicken/Kasoor/SI36/2017(H9N2) isolate belongs to the G1 lineage. In addition, this isolate possesses mammalian host-specific mutations which could possibly favor interspecies transmission, suggesting that Pakistani H9N2 viruses are still potentially infectious for mammals.

Avian influenza virus (AIV) belongs to the genus *Influenzavirus A* and the family *Orthomyxoviridae*. The H9N2 low-pathogenicity AIV (LPAIV) is endemic in poultry in many countries throughout Asia, the Middle East, and North Africa. Furthermore, some H9N2 LPAIVs have caused sporadic infections in mammalian species, raising public health concerns (1, 2). The first outbreak of the G1 lineage H9N2 LPAIV in Pakistan occurred in 1998 and subsequently evolved through reassortment. The H9N2 LPAIV collected in 2009 to 2015 from chickens in Pakistan contained several mammalian host-specific markers (3). In addition, an H9N2 LPAIV was isolated from a poultry worker during avian influenza virus surveillance in Pakistan in 2015, highlighting the potential for interspecies transmission of H9N2 viruses (4).

Herein, we report the genome sequence of the full-length coding region of an H9N2 strain, A/chicken/Kasoor/SI36/2017(H9N2) (SI36/2017), isolated from a chicken farm in Kasoor, Pakistan, on 7 February 2017.

RNA was extracted using a MagMAX-96 viral RNA isolation kit (Ambion, Inc.). All eight segments were amplified using influenza virus-specific universal primers complementary to the conserved 12 to 13 nucleotides at the end of all segments (5, 6). A sequencing library was prepared using a Nextera XT DNA preparation kit, and paired-end sequencing was performed on a MiSeq sequencer (Illumina) with a MiSeq v2 kit according to the manufacturer's instructions. *De novo* and directed assembly were carried out using the Geneious assembler and mapper in Geneious R8 using default parameters (7). Nucleotide sequences for each gene were aligned using MAFFT (7). The maximum-likelihood (ML) tree was estimated using RAxML (8) using default parameters and a general time-reversible nucleotide substitution model. Bootstrap support values were generated by using 500 rapid bootstrap replicates.

Of the 150,158 sequencing reads acquired, 142,555 reads (94.9%) were mapped to A/chicken/Pakistan/10A/2015(H9N2) virus (GenBank accession no. KU042907 to KU042914). Next-generation sequencing (NGS) covered 100% of the coding regions of the polymerase basic 2 (PB2) (2,283 bp), PB1 (2,334 bp), polymerase acidic (PA) (2,151 bp), hemagglutinin (HA) (1,683 bp), nucleoprotein (NP) (1,497 bp), neuraminidase (NA) (1,410 bp), matrix (M) (982 bp), and nonstructural (NS) (838 bp) segments to average depths of coverage of 1,913.1, 278.5, 1,607.7, 1,491.0, 1,936.9, 1,232.6, 3,839.6,
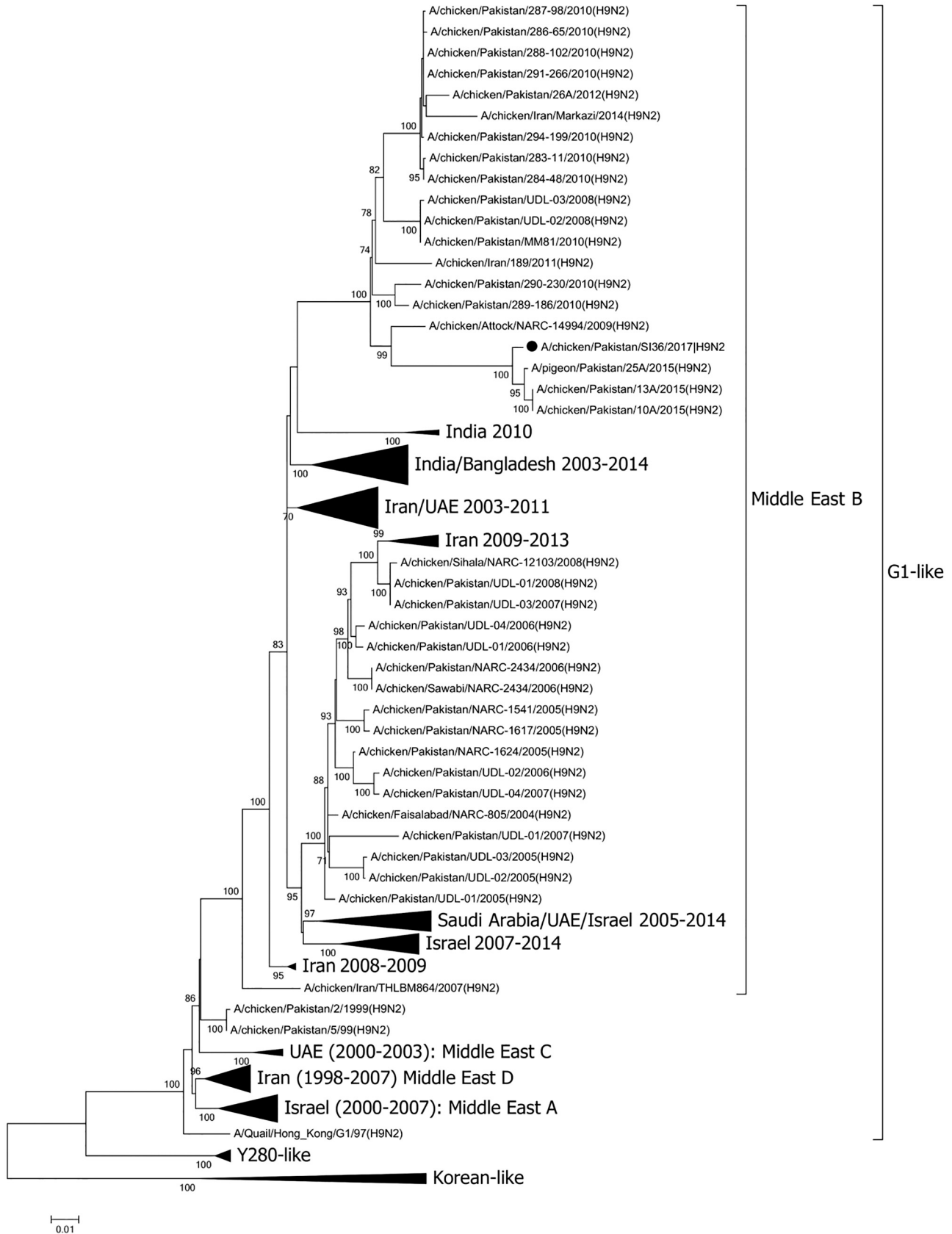
FIG 1 Maximum likelihood phylogeny of the hemagglutinin gene. At each branch, the number indicates a bootstrap value (>70%). The black circle indicates the A/chicken/Pakistan/SI36/2017(H9N2) virus. Scale bar, nucleotide substitutions per site.

TABLE 1 Nucleotide identities between the H9N2 virus identified in Pakistan in 2017 and nearest homologs in GenBank as of 13 April 2018

Gene[a]	Virus	Identity (%)
PB2	A/chicken/Attock/NARC-14994/2009(H9N2)	98.8
PB1	A/chicken/Pakistan/10A/2015(H9N2)	99.6
PA	A/chicken/Pakistan/10A/2015(H9N2)	99.6
HA	A/pigeon/Pakistan/25A/2015(H9N2)	99.6
NP	A/chicken/Pakistan/10A/2015(H9N2)	99.5
NA	A/chicken/Pakistan/10A/2015(H9N2)	99.5
MP	A/chicken/Pakistan/10A/2015(H9N2)	99.8
NS	A/chicken/Pakistan/10A/2015(H9N2)	99.6

[a] HA, hemagglutinin; MP, matrix; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural; PA, polymerase acidic; PB1 and 2, polymerase basic 1 and 2.

and 2,267.4, respectively. The genomic G+C content of the coding regions ranged from 42.9 to 47.0%. The SI36/2017 was found to be LPAIV based on the amino acid sequence, PAKSSR/G, at the HA proteolytic cleavage site. The H9N2 LPAIV isolates identified in Pakistan from 2006 to 2015 were reassortants between the G1 lineage and the H7N3 high-pathogenicity AIV (HPAIV) that circulated in Pakistan and belong to group B (3, 9, 10). Our ML phylogeny of the HA gene shows that SI36/2017 belongs to group B of the G1 lineage (Fig. 1) (10). Other segments of SI36/2017 also clustered with H9N2 viruses from Pakistan identified in 2008 to 2015 (data not shown) (3). Homology BLAST searches showed high sequence identity with H9N2 isolates identified in Pakistan (Table 1). These data suggest that the SI36/2017 virus likely evolved through genetic drift in the absence of further reassortment. Consistent with previous findings that H9N2 viruses identified from Pakistan in 2012 to 2015 possessed mammalian host-specific markers (3), the SI36/2017 virus possessed identical mutations which could possibly favor interspecies transmission (Table 1), highlighting the need for enhanced surveillance.

Data availability. The genome sequences of the SI36/2017 virus have been deposited in GenBank under the accession no. MK603213 to MK603220.

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