





# Complete Coding Genome Sequence of an Influenza A/H3N8 Equine Virus Isolated in Kazakhstan in 2007

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**ABSTRACT** Here, we reported the complete coding sequence of the influenza A/equine/Otar/3/2007 (H3N8) equine virus, first isolated in Kazakhstan in 2007. The hemagglutinin (HA) sequences of the Kazakhstan isolates appeared to be closely related to viruses isolated in early 2000 in Asia. Phylogenetic analysis characterized the Kazakhstan isolates as a member of the Florida sublineage clade 2 by the HA protein sequence.

Equine influenza (EI) is an infectious, acutely contagious disease of the equine family (horses, donkeys, mules, and zebras) (1, 2). The causative agent of EI is an RNA-containing virus belonging to the *Orthomyxoviridae* family and is 80 to 120 nm in diameter (3, 4). Due to the segmentation of the genome, influenza viruses often undergo rearrangement, which leads to reassortment and antigenic variability. The transfer of the virus or its genes into the animal population contributes to the preservation of the causative agent of influenza (5, 6).

It is assumed that the emergence of new pandemic strains occurs because of the reassortment of genes of human and animal influenza viruses. This process is easily reproduced in the laboratory and observed in nature. After every major human influenza epidemic, the corresponding viruses are found in animal populations.

The outbreaks of equine influenza in 2007 and 2012 in Kazakhstan were detected almost at the same time in China and Mongolia (7, 8).

Viral RNAs from nasal swabs were extracted using the QIAmp viral RNA extraction kit (Qiagen) according to the manufacturer's instructions. Sequencing and amplification of eight segments of the virus genome were amplified in the SuperScript one-step reverse transcriptase PCR (RT-PCR) system with Platinum *Taq* DNA polymerase (Invitrogen SRL)

**TABLE 1** Genome characteristics of strain influenza A/equine/Otar/3/2007

Gene/segment	Size (nucleotides)	GC content (%)	Strain with closest relative sequence	Identity at the nucleotide level (%)	GenBank accession no. (NCBI nt)
PB2	2341	42.5	A/equine/Himachal Pradesh/CMVL-YOL2/2008	99.7	<a href="#">MT908915</a>
PB1	2341	42.1	A/equine/Richmond/1/2007	99.9	<a href="#">MT908914</a>
PA	2084	41	A/equine/Richmond/1/2007	99.2	<a href="#">MT965694</a>
HA	1733	39.4	A/equine/Richmond/1/2007	99.8	<a href="#">JF683499</a>
NP	1565	45.4	A/equine/Richmond/1/2007	99.7	<a href="#">MT908913</a>
NA	1406	41	A/equine/Meath/1/2007	99.7	<a href="#">JF683500</a>
M	988	47.5	A/equine/Jammu and Kashmir/CMVL-LEH4/2008	99.8	<a href="#">JF683498</a>
NS	890	40.6	A/equine/Hissar/CMVL-HSR4/2008	99.6	<a href="#">JF683501</a>

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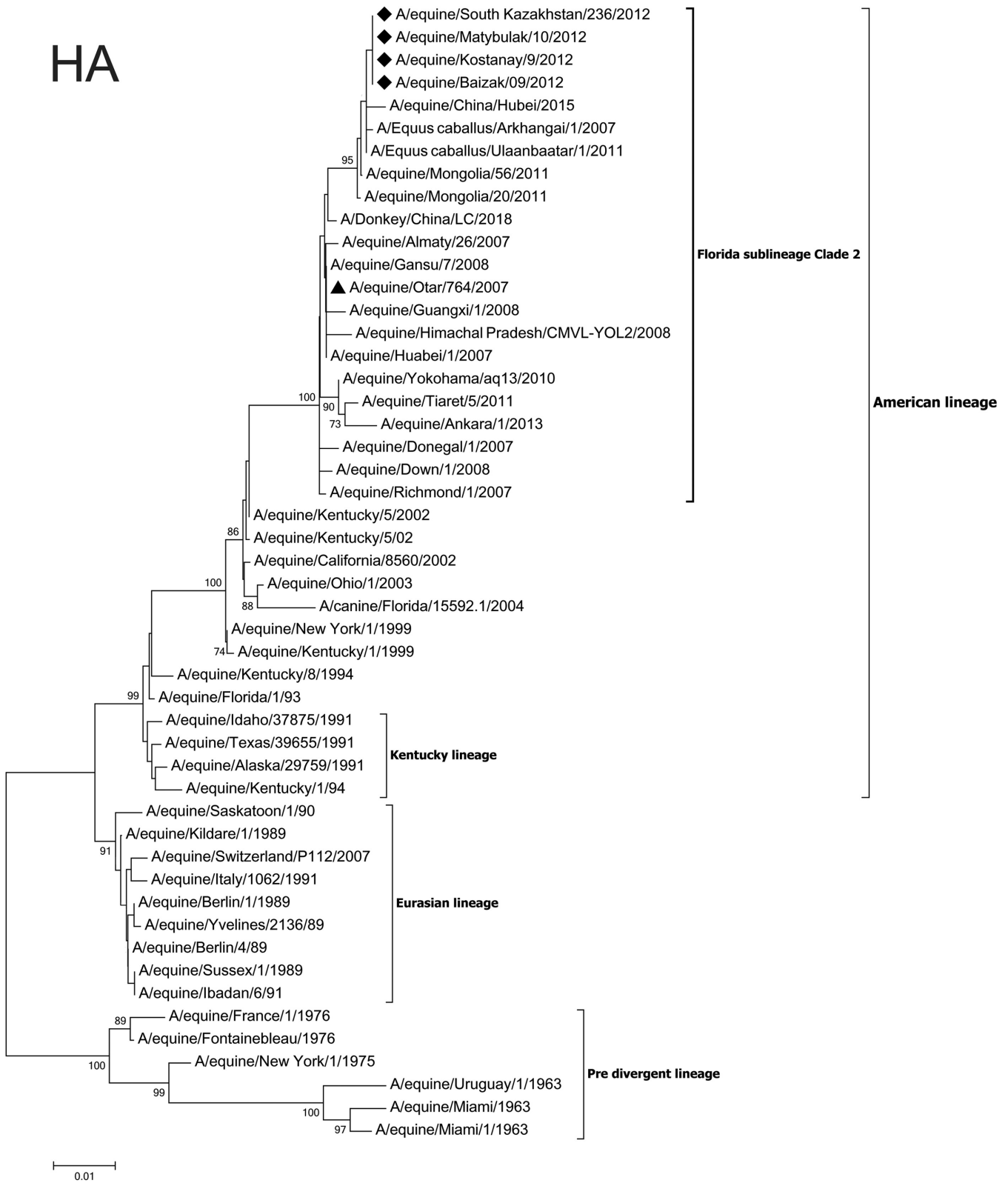
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**FIG 1** Phylogenetic tree for HA gene of the strain A/equine/Otar/3/2007 (H3N8). The evolutionary history of the HA gene was inferred using the neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10,000 replicates) are shown next to the branches (12). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA7 (13, 14). The location of the sequence reported here is indicated with a black triangle. Rhombuses mark Kazakhstan strains of equine influenza virus isolated in 2012 (8).

using the Uni-12 (3-UCGYUUUCGUCC) and Uni-13 (GGAACAAAGAUGA-5) universal influenza primers (9). PCR products were cloned into pJEM plasmids and sequenced using m13 primers. Genome sequencing PCR products were analyzed on a 1.5% agarose gel stained with ethidium bromide and purified using the QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions.

Sequencing was performed with a 16-capillary genetic analyzer AB3130xl automatic sequencer (Hitachi Applied Biosystems) using the BigDye Terminator version 3.1 cycle sequencing kit (ABI, Foster City, CA, USA). Chromatograms were edited and assembled using Sequencer version 5 (Gene Codes Corp.). Alignment of the nucleotide sequence with quality scores above 90% was carried out using the BioEdit version 7.2.5 program (<https://bioedit.software.informer.com/7.2/>).

The data were searched in the GenBank nucleotide (nt) database. The size of each virus segment and identity with the closest strains is shown in Table 1 according to the BLAST software (10).

HA gene alignment analysis of the A/equine/Otar/3/2007 strain showed three nucleotide substitutions relative to A/equine/Richmond/1/2007 strain. When the amino acid sequences of our isolates were aligned with the strain A/equine/Switzerland/P112/2007 six amino acid substitutions (positions 43D-V, 100G-R, 123G-E, 209M-T, 238L-P, 265I-V) were seen.

Phylogenetic analysis of the HA protein of our A/equine/Otar/3/2007 strain clustered the virus among the American lineages and in particular the Florida sublineage clade II. Phylogenetic trees constructed with the NJ method using the HA sequence are shown in Fig. 1 (11).

**Data availability.** Complete genome sequence of strain A/Equine/Otar/3/2007 (H3N8) was published (deposited) in GenBank under the following numbers: [MT908915](https://doi.org/10.1093/mbe/mtz089), [MT908914](https://doi.org/10.1093/mbe/mtz089), [MT965694](https://doi.org/10.1093/mbe/mtz089), [JF683499](https://doi.org/10.1093/mbe/mtz089), [MT908913](https://doi.org/10.1093/mbe/mtz089), [JF683500](https://doi.org/10.1093/mbe/mtz089), [JF683498](https://doi.org/10.1093/mbe/mtz089), and [JF683501](https://doi.org/10.1093/mbe/mtz089).

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## REFERENCES

- Cullinane A, Newton JR. 2013. Equine influenza - a global perspective. *Vet Microbiol* 167:205–214. <https://doi.org/10.1016/j.vetmic.2013.03.029>.
- Karamendin K, Kydyrmanov A, Sayatov M, Strochkov V, Sandybayev N, Sultankulova K. 2016. Retrospective analysis of the equine influenza virus A/equine/Kirgizia/26/1974 (H7N7) isolated in Central Asia. *Pathogens* 5:55. <https://doi.org/10.3390/pathogens5030055>.
- Cullinane A, Elton D, Mumford J. 2010. Equine influenza-surveillance and control. *Influenza Other Respir Viruses* 4:339–344. <https://doi.org/10.1111/j.1750-2659.2010.00176.x>.
- Singh RK, Dhama K, Karthik K, Khandia R, Munjal A, Khurana SK, Chakraborty S, Malik YS, Virmani N, Singh R, Tripathi BN, Munir M, van der Kolk JH. 2018. A comprehensive review on equine influenza virus: etiology, epidemiology, pathobiology, advances in developing diagnostics, vaccines, and control strategies. *Front Microbiol* 9:1941. <https://doi.org/10.3389/fmicb.2018.01941>.
- Rash A, Morton R, Woodward A, Maes O, McCauley J, Bryant N, Elton D. 2017. Evolution and divergence of H3N8 equine influenza viruses circulating in the United Kingdom from 2013 to 2015. *Pathogens* 6:6. <https://doi.org/10.3390/pathogens6010006>.
- Woodward AL, Rash AS, Blinman D, Bowman S, Chambers TM, Daly JM, Damiani A, Joseph S, Lewis N, McCauley JW, Medcalf L, Mumford J, Newton JR, Tiwari A, Bryant NA, Elton DM. 2014. Development of a surveillance scheme for equine influenza in the UK and characterisation of viruses isolated in Europe, Dubai and the USA from 2010–2012. *Vet Microbiol* 169:113–127. <https://doi.org/10.1016/j.vetmic.2013.11.039>.
- Yondon M, Zayat B, Nelson MI, Heil GL, Anderson BD, Lin X, Halpin RA, McKenzie PP, White SK, Wentworth DE, Gray GC. 2014. Equine influenza A(H3N8) virus isolated from Bactrian Camel, Mongolia. *Emerg Infect Dis* 20:2144–2147. <https://doi.org/10.3201/eid2012.140435>.
- Burashev Y, Strochkov V, Sultankulova K, Orynbayev M, Sansyzybay A, Sandybayev N, Nurabayev S, Savitskaya I, Rock DL, Tulman ER. 2018. Complete genome sequencing of two equine influenza A(H3N8) virus strains isolated in Kazakhstan. *Genome Announc* 6:e00574-18. <https://doi.org/10.1128/genomeA.00574-18>.
- Hoffmann E, Stech J, Guan Y, Webster RG, Perez DR. 2001. Universal primer set for the full-length amplification of all influenza A viruses. *Arch Virol* 146:2275–2289. <https://doi.org/10.1007/s007050170002>.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>.
- Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791. <https://doi.org/10.1111/j.1558-5646.1985.tb00420.x>.
- Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16:111–120. <https://doi.org/10.1007/BF01731581>.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874. <https://doi.org/10.1093/molbev/msw054>.