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 reassembly 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 reassembly of genes from 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).

| 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                                | MT908915                        |
| PB1          | 2341               | 42.1           | A/equine/Richmond/1/2007              | 99.9                                | MT908914                        |
| PA           | 2084               | 41             | A/equine/Richmond/1/2007              | 99.2                                | MT965694                        |
| HA           | 1733               | 39.4           | A/equine/Richmond/1/2007              | 99.8                                | JF683499                        |
| NP           | 1565               | 45.4           | A/equine/Richmond/1/2007              | 99.7                                | MT908913                        |
| NA           | 1406               | 41             | A/equine/Meath/1/2007                 | 99.7                                | JF683500                        |
| M            | 988                | 47.5           | A/equine/Jammu and Kashmir/CMVL-LEH4/2008 | 99.8                                | JF683498                        |
| NS           | 890                | 40.6           | A/equine/Hissar/CMVL-HSR4/2008        | 99.6                                | JF683501                        |
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, MT908914, MT965694, JF683499, MT908913, JF683500, JF683498, and JF683501.

ACKNOWLEDGMENTS

The work was conducted under the support of the Science Committee, RK Ministry of Education and Science within the Scientific and Technical Program "Biological safety of the Republic of Kazakhstan: threat assessment, scientific and technical basis for their prevention and elimination" for 2021 to 2023.

We declare no conflict of interest.

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