



Influenza (Flu)

Technical Update: Summary Analysis of Genetic Sequences of Highly Pathogenic Avian Influenza A(H5N1) Viruses in Texas

This is a technical summary of an analysis of the genomic sequences of viruses associated with an outbreak of highly pathogenic avian influenza (HPAI) A(H5N1) viruses in Texas. This analysis supports the conclusion that the overall risk to the general public associated with the ongoing HPAI A(H5N1) outbreak has not changed and remains low at this time. The genome of the virus identified from the patient in Texas is publicly posted in [GISAID](#) and has been submitted to [GenBank](#).

April 2, 2024 – CDC has sequenced the influenza virus genome identified in a specimen collected from the patient in Texas who was confirmed to be infected with highly pathogenic avian influenza A(H5N1) [“HPAI A(H5N1)”] virus and compared these with HPAI A(H5N1) sequences from cattle, wild birds and poultry. The virus sequences are HA clade 2.3.4.4b HPAI A(H5N1) with each individual gene segment closely related to viruses detected in dairy cattle available from USDA testing in Texas. While minor changes were identified in the virus sequence from the patient specimen compared to the viral sequences from cattle, both cattle and human sequences maintain primarily avian genetic characteristics and for the most part lack changes that would make them better adapted to infect mammals. The genome for the human isolate had one change (PB2 E627K) that is known to be associated with viral adaptation to mammalian hosts, and which has been detected before in people and other mammals infected with HPAI A(H5N1) virus and other avian influenza subtypes (e.g., H7N9), but with no evidence of onward spread among people. Viruses can undergo changes in a host as they replicate after infection. Further, there are no markers known to be associated with influenza antiviral resistance found in the virus sequences from the patient’s specimen and the virus is very closely related to two existing HPAI A(H5N1) candidate vaccine viruses that are already available to manufacturers, and which could be used to make vaccine if needed. Overall, the genetic analysis of HPAI A(H5N1) viruses in Texas supports CDC’s conclusion that the human health risk currently remains low. More details are available in this technical summary below:

Technical Summary:

Viral RNA extractions obtained from nasopharyngeal swab and conjunctival swab specimens collected from the patient were used as template to perform next generation sequencing using Illumina and Oxford Nanopore Technologies (ONT) platforms. Codon complete consensus sequence was successfully generated only from the conjunctival specimen and was assembled using CDC’s Iterative Refinement Meta Assembler (IRMA). Illumina and ONT yielded identical sequences at the consensus sequence level. In concordance with the rRT-PCR cycle threshold (Ct) values obtained from each specimen (i.e., approximately 18 for the conjunctival specimen and 33 for the nasopharyngeal sample), viral RNA from the nasopharyngeal sample failed to generate PCR amplicons suitable for sequencing. Notably, the patient reported only conjunctivitis with no respiratory or other symptoms, which likely resulted in lower viral RNA concentrations detected in the nasopharyngeal sample and is suggestive of a lack of respiratory infection in the patient.

The virus sequence was confirmed to be HA clade 2.3.4.4b HPAI A(H5N1) with each individual gene segment determined to be closely related to viruses detected in dairy cattle in Texas. The genotype was classified as B3.13 (1) and corresponds to the same genotype described by USDA for the virus detected in Texas cattle (2). This genotype contains PA, HA, NA and M gene segments from Eurasian wild bird lineages and PB2, PB1, NP and NS gene segments from American wild bird lineages. Other viruses with this genotype have been sporadically detected in wild birds, poultry and one skunk since November 2023 in the U.S. CDC’s real-time RT-PCR diagnostic test used for the detection of A(H5) virus in human samples has not been impacted by genetic changes in B3.13 genotype viruses.

The hemagglutinin (HA) gene codes for one of the two surface glycoproteins and is central to species specificity because it is responsible for virus attachment and fusion with host cells. Like the viruses detected in cattle, the analysis of the HA gene from the human specimen shows that it is closely related to HPAI A(H5) viruses in HA clade 2.3.4.4b recently detected in wild birds and poultry and lacks amino acid changes that improve recognition of mammalian receptors or fusion of the viral membrane with the host endosomal membranes. The HA is also the primary target of neutralizing antibodies elicited by infection or vaccination, and the HA of virus from the human specimen is very closely related to the A/Astrakhan/3212/2020-like pre-pandemic candidate vaccine virus (CVV) (IDCDC-RG71A) and the A/American wigeon/South Carolina/22-000345-001/2021-like CVV (IDCDC-RG78A), both of which are available to vaccine manufacturers (3). There are only four amino acid changes (L104M, L115Q, T195I, V210A) between the HA1 of the virus from the Texas case and A/Astrakhan/3212/2020-like CVV and only two changes (L115Q, T195I) compared to the A/American wigeon/South Carolina/22-000345-001/2021-like CVV. The changes are not in major antigenic epitopes strongly suggesting that antibodies elicited by A/Astrakhan/3212/2020-like and A/American wigeon/South Carolina/22-000345-001/2021-like vaccines would be expected to have good cross-reactivity – and therefore protection – against this virus.

The neuraminidase (NA) gene encodes the other surface protein of the virus. The major role of the NA is to release new progeny virions from an infected cell by enzymatically cleaving sialic acid receptors, which aids virus spread to uninfected cells within an infected host. The enzymatic activity of NA is inhibited by one class of antiviral drugs that are FDA-approved for treatment of influenza (i.e., NA inhibitors). Analysis of the N1 NA gene from the Texas human specimen showed that it did not have any known or suspected markers of reduced susceptibility to this class of antivirals, which includes oseltamivir. Furthermore, the NA has a full-length stalk which is consistent with viruses that naturally circulate in wild birds. In previous HPAI A(H5N1) virus outbreaks and zoonoses the NA stalk region often had deletions (e.g., a 20 amino acid deletion at positions 49–68 relative to A/goose/Guangdong/1/1996) that enhances replication and/or pathogenesis in terrestrial poultry and mice (4-6).

Analysis of the other gene segments (PB2, PB1, PA, NP, M, NS) was also conducted. No known or suspected markers of reduced susceptibility to antiviral compounds that target the PA (i.e., baloxavir marboxil) or M2 (i.e., amantadine, rimantadine) were found.

In addition to the HA and NA, the RNA transcription and replication complex (PB2, PB1, PA, NP) also have species-specific determinants that impact efficient replication in humans and other mammals, particularly polymerase basic protein 2 (PB2). The PB1, PA and NP lacked markers of mammalian adaptation. The PB2 of the human specimen had a change of PB2 E627K compared to the PB2 genes of viruses available from USDA detections in Texas dairy cattle and typically found in A(H5N1) viruses circulating in wild birds. This mutation is, however, commonly found in humans and other mammals that are infected with HPAI A(H5N1) viruses and is understood to be associated with mammalian adaptation because it improves RNA polymerase activity and replication efficiency in mammalian cells; based on experimental studies in mice, guinea pigs and ferrets, it has the potential to impact pathogenesis or transmission in infected mammals (7-8). Despite previous identification of PB2 E627K in human cases of HPAI A(H5N1) virus, there is no evidence of onward transmission among humans after infection with viruses containing this mutation. It is important to note that this substitution has not been seen in available PB2 genes from viruses circulating in wild birds and poultry or in the recently described cattle viruses detected in Texas, suggesting the mutation may have been acquired in the patient during the development of conjunctivitis. Viruses can undergo changes in a host as they replicate after infection, and it is not uncommon or surprising for HPAI A(H5N1) viruses to undergo this and other polymerase gene changes in infected patients (9). Additional data from A(H5N1) virus-infected animals from the premises where the person was likely exposed is needed to support this hypothesis.

The protein products from the M (M1 and M2) and NS (N1 and N2) genes lacked markers associated with mammalian adaptation. Collectively, epidemiologic, and viral genomic analyses indicate that this case represents a single zoonotic event and while the HA lacked changes likely to enhance transmission to mammals, it did acquire substitutions in PB2 likely to enhance replication in mammals, which illustrates that we have to remain vigilant and continue to characterize zoonotic viruses.

Overall, the genomic analysis of the virus from this human case does not change CDC's risk assessment related to the HPAI A(H5) clade 2.3.4.4b viruses. The overall risk to human health associated with the ongoing HPAI A(H5) outbreaks in poultry and detections in wild birds and cattle remains low.

Note: HPAI A(H5) viruses, predominantly HPAI A(H5N1) clade 2.3.4.4b viruses, have been circulating globally in wild birds in the U.S. since late 2021. These viruses have caused outbreaks in commercial and backyard poultry, with spillover resulting in sporadic infections in mammals.

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