

Outbreak Reports

Epidemiological and Genetic Characterization of Three H9N2 Viruses Causing Human Infections — Changsha City, Hunan Province, China, April 2025

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Summary

What is already known about this topic?

A total of 117 H9N2 cases of human infection of Chinese origin had been reported to the World Health Organization (WHO) by May 9, 2025, with 22 of them originating in Hunan Province.

What is added by this report?

This article reported on the investigation of three new H9N2 avian influenza virus (AIV) infections detected in Changsha, Hunan Province, in April 2025. No epidemiological link was found among them. Exposure to live poultry was identified as the primary risk factor for infection. Sequence analysis of the three H9N2 AIVs showed a similarity of 99.71%–99.82% between hemagglutinin (HA), and the homology of the neuraminidase (NA) genes was 98.41%–99.83%. Although the tests showed that the HA had enhanced binding ability to upper respiratory tract cells' receptors, no evidence of sustained human-to-human transmission has been found so far.

What are the implications for public health practice?

This study indicated that H9N2 AIV remains a public health issue in China. We need to strengthen publicity and education efforts to inform people of the potential risk of avian influenza virus, especially to keep children away from poultry and poultry-related facilities, to effectively prevent the occurrence of avian influenza A(H9N2) infection.

ABSTRACT

Introduction: In April 2025, three suspected human cases of avian influenza were identified in Changsha, China. Laboratory testing confirmed three cases of H9N2 AIV infection. This report summarizes the epidemiological findings from cases and contact investigations, along with genetic characterization of

the isolated H9N2 strains.

Methods: Comprehensive epidemiological assessments were conducted for each confirmed case. Virus isolation and culture were performed using throat swab specimens obtained from the cases. Isolated H9N2 strains were sequenced using next-generation sequencing (NGS). HA and NA gene sequences were analyzed for homology; evolutionary trees were constructed; and key antigenic sites were examined to identify genetic features.

Results: All three cases were sporadic. No influenza-like illness was observed among close contacts or live poultry store employees during the 10-day medical monitoring period. Phylogenetic analysis indicated that the HA gene of all three H9N2 strains belonged to the A/Duck/Hong Kong/Y280/97 (Y280-like) clade within the Eurasian lineage. HA gene sequence homology was 99.7%–99.8%, and NA gene homology was 98.4%–99.8%. The HA protein cleavage site was identified as PSRSSR↓GLF. Several HA protein site mutations were detected — H191N, A198T/V, Q226L, and Q234L — that had been previously associated with increased binding to receptors. HA-232H, 234L, and 236G support a binding preference for the human-type sialic acid- α -2,6-galactose receptors.

Conclusion: All three H9N2 avian influenza cases were mild and involved reported exposure to poultry or related environments. Genetic analysis revealed high homology of HA and NA among the isolated viruses. No epidemiological links were identified between cases, and no evidence was found of sustained human-to-human transmission. Continued avian influenza surveillance and public health education are warranted.

The H9N2 avian influenza virus (AIV) is the most prevalent avian influenza virus circulating among

poultry in China (1). While it primarily leads to economic losses in the poultry industry, it has also repeatedly crossed the species barrier to infect humans, raising public health concerns. Since 2015, China has consistently reported human H9N2 infections to the WHO, with 117 cumulative cases by May 9, 2025 (2). Among these, 19 cases were reported in Hunan Province by the end of 2024 (3), followed by three additional cases in April 2025. Therefore, it is crucial to closely monitor variations in H9N2 infectivity — particularly its potential for cross-species transmission to humans. This study presents the epidemiological profiles of three human H9N2 cases detected through influenza-like illness (ILI) surveillance from Changsha, China, along with the molecular characteristics of the corresponding H9N2 viruses.

INVESTIGATION AND RESULTS

Human Infections with H9N2 AIVs

In April 2025, three new human H9N2 cases were reported in Changsha City, Hunan Province. All three cases were children and were confirmed as cases of H9N2 infection by Hunan Provincial CDC and Changsha CDC.

Case 1 was a seven-year-old female primary school student (patient 1), residing in Kaifu District. She developed a fever and cough on April 1 and sought care at the Pediatrics Department of Changsha First Hospital. She presented with a paroxysmal productive cough and was prescribed medication, remaining homebound from April 1 to 5 without exposure risks. On April 6, she returned for a follow-up.

Case 2 was a 16-month-old girl from Wangcheng District, who developed cough and low-grade fever (38.5 °C) on April 3. She was admitted to Changsha First Hospital on April 4 at 11:00 a.m. with a diagnosis of bronchopneumonia and was discharged on April 8 after recovery.

Case 3 was a five-year-old boy living in Kaifu District. He experienced fever onset on April 19 and sought medical care on April 20. Initial testing was

positive for influenza A (H9N2), and his symptoms were resolved by April 25 (Table 1).

Epidemiological Investigation

Epidemiological investigations showed that none of the three cases had had pre-onset contact history with people experiencing fever or respiratory symptoms, and the three children had had no contact with each other. However, cases 1 and 2 had been exposed to live poultry markets, and case 3 had had contact with diseased and dead poultry.

Opposite the entrance of the community where Case 1 lives were six live poultry stores in Maojiaqiao Market, which was only 40 meters away. Case 1 passes by these live poultry stores every day on her way to school. On April 8, four environmental smear samples collected from her home tested negative for influenza A virus nucleic acid. Meanwhile, 13 environmental samples (7 smear samples and 6 water samples) from the live poultry stores all tested positive for influenza A virus. All three close contacts of Case 1 tested negative for influenza virus nucleic acid.

Case 2 had visited a live poultry store near her home within seven days before symptom onset. On April 16, four environmental samples collected from this store tested positive for H9N2 nucleic acid. All seven close contacts of Case 2 tested negative.

Case 3 bought a duckling as a pet on April 8, and the duckling died on April 15. Nucleic acid testing of four close contacts sampled on April 21 was negative for influenza virus nucleic acid.

H9N2 AIVs RNA Extraction and Sequencing

The H9N2 viruses were sequenced by Hunan Provincial CDC and Changsha CDC, with the following strain designations: Case 1: A/Hunan-Kaifu/1294/2025(H9N2), Case 2: A/Hunan-Kaifu/8210/2025(H9N2), and Case 3: A/Hunan-Kaifu/CS992/2025(H9N2). H9N2 AIVs RNA were extracted from the three patients using the Viral RNA Mini Kit (Qiagen, Germany). Reverse transcription

TABLE 1. Demography and epidemiology investigation of three H9N2 AIV cases in Changsha, April 2025.

Case No.	Sex	Age (Years)	District	Date of onset	Date of hospitalization	Date of laboratory confirmation	Case type	LPM contact	Contacted sick or dead poultry
1	Female	7	Kaifu	2025.04.01	2025.04.01	2025.04.04	Outpatient	Yes	No
2	Female	1	Wangcheng	2025.04.03	2025.04.04	2025.04.11	Inpatient	Yes	No
3	Male	5	Kaifu	2025.04.19	2025.04.20	2025.04.24	Outpatient	Yes	Yes

Abbreviation: LPM=live poultry market; AIV=avian influenza virus.

and amplification were performed with the SuperScript® III One-Step RT-PCR System with Platinum Taq High Fidelity (Invitrogen, Carlsbad, USA). Libraries were constructed using the Nextera® XT Library Prep Kit (Illumina, USA). Sequencing was conducted on the MiSeq platform with the MiSeq V2 Kit (Illumina, USA). All library prep reagents, sequencing kits, and the sequencer were supplied by Illumina (USA). Raw FastQ data were assembled using CLC Genomics Workbench (Qiagen, Germany). The sequencing depth and coverage of HA and NA nucleic acids in H9N2 AIVs are presented in [Supplementary Table S1](#) (available at <https://weekly.chinacdc.cn/>).

Bioinformatics Analysis

Global Initiative on Sharing All Influenza Data (GISAID) EpiFlu Database was used for sequence alignment of H9N2 hemagglutinin (HA) and neuraminidase (NA) genes. Reference sequences were downloaded for comparison. MAFFT (version 7.526, Advanced Industrial Science and Technology, Tokyo, Japan) performed multiple sequence alignment (4). Maximum-likelihood phylogenetic trees were constructed with 1,000 bootstrap replicates for statistical validation.

Phylogenetic analysis revealed that the HA and NA genes of all three viruses clustered within the A/Duck/Hong Kong/Y280/97(H9N2) (Y280-like) evolutionary lineage of the Eurasian branch ([Figure 1](#)). Nucleotide similarities among the three strains were 99.71%–99.82% for HA and 98.41%–99.83% for NA. Compared with the reference strain A/Hunan-Louxing/11086/2022(H9N2) (LX11086) isolated in Loudi, Hunan Province, in 2022, HA gene homology was 96.8%–96.9%, and NA gene homology was 95.0%–95.2%.

The basic local alignment search tool (BLAST) in the GISAID EpiFlu database indicated that the HA genes of the three H9N2 AIVs shared high homology (98.41%–98.51%) with A/chicken/Vietnam/NCVD-23CB2V7S5-38/2023(H9N2), a strain isolated from Vietnamese chickens. The NA genes were highly homologous (97.42%–97.57%) to A/environment/Xiamen/01/2021(H9N2) ([Table 2](#)).

Protein sequence analysis using LX11086 as a reference revealed that the HA cleavage site sequences of HA1 and HA2 of all viruses were PSRSSR↓GLF (positions 333aa–341aa), which did not have a continuous acidic amino acid (lysine, arginine, or histidine) sequence, consistent with low-pathogenicity

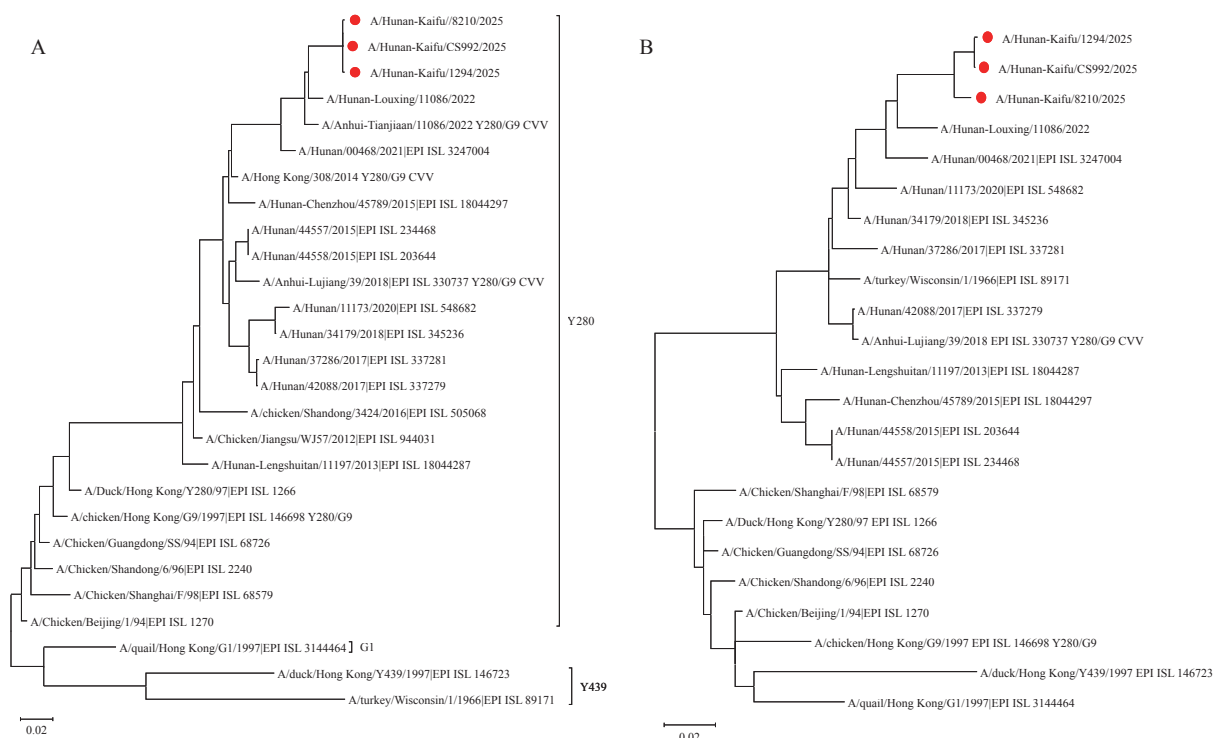


FIGURE 1. Maximum likelihood phylogenetic relationships of H9N2 viruses. (A) HA genes; (B) NA genes.

Note: Red circle means The H9N2 AIVs researched in this study.

Abbreviation: HA=hemagglutinin; NA=neuraminidase; AIV=avian influenza virus.

TABLE 2. Similarity of HA and NA segments of three H9N2 AIVs analyzed by online BLAST.

Case NO.	Virus	Date of collection	Abbreviation	Segment	Strain with the highest similarity in GISAID	GISAID ID	Similarity (%)
1	A/Hunan-Kaifu/1294/2025(H9N2)	2025.04.02	KF1294	HA	A/chicken/Vietnam/NCVD-23CB2V7S5-38/2023	EPI4316643	98.51
				NA	A/environment/Xiamen/01/2021	EPI2161506	97.42
2	A/Hunan-Kaifu/8210/2025(H9N2)	2025.04.07	KF8210	HA	A/chicken/Vietnam/NCVD-23CB2V7S5-38/2023	EPI4316643	98.46
				NA	A/environment/Xiamen/01/2021	EPI2161506	97.57
3	A/Hunan-Kaifu/CS992/2025(H9N2)	2025.04.21	CS992	HA	A/chicken/Vietnam/NCVD-23CB2V7S5-38/2023	EPI4316643	98.51
				NA	A/environment/Xiamen/01/2021	EPI2161506	97.56

Abbreviation: BLAST=basic local alignment search tool; GISAID=Global Initiative on Sharing All Influenza Data; HA=hemagglutinin; NA=neuraminidase; AIV=avian influenza virus.

avian influenza virus (LPAIV) H9 strains. Analysis of receptor-binding sites showed that the HA proteins had mutations at amino acid positions H191N, A198V, Q226L, and Q234L, which potentially enhanced the binding ability of the virus to the receptor (5–6). The amino acids of the HA protein were N232H, Q234L, and 235M, binding to the human receptor sialic acid- α 2,6-galactose, and G236 is a typical feature of avian viruses. No resistance-associated mutations were detected in the NA protein (Table 3).

PUBLIC HEALTH RESPONSE

The Kaifu CDC and Wangcheng CDC have conducted a 10-day medical observation on the close contacts of the three cases and the employees of the related live poultry stores. None of them developed influenza-like symptoms such as fever and cough. The disease control departments have also strengthened the monitoring of avian influenza in live poultry markets and assisted hospitals with training for the prevention and control of avian influenza in ILI cases.

The Changsha CDC conducted emergency monitoring of the sources of chickens and ducks in the live poultry stores of Case 1 and Case 2, as well as the Shuiduhe Market and Huangxing Town Market, on April 27, 2025. A total of 62 environmental samples were collected, and the nucleic acid test results showed that 33 samples were positive for H9N2 AIV, and 2 samples were positive for H5 AIV.

DISCUSSION

Avian influenza viruses are categorized into highly pathogenic avian influenza virus (HPAIV) and LPAIV based on their pathogenicity. HPAIV consists of subtypes H5 (e.g., H5N1, H5N6) and H7 (e.g., H7N9), while H9N2 is the predominant LPAIV.

Most human infections with H9N2 result in mild and self-limiting symptoms, so the public and clinicians paid less attention compared to H5 or H7 infections. However, H9N2 AIV demonstrates a notable capacity for cross-species transmission. Serological evidence indicated a higher seropositivity rate among poultry workers for H9N2 than for H5N1 or H7N9 (7). Moreover, genetic analyses reveal that H9N2 frequently contributes internal gene segments to emerging reassortant viruses, such as H3N8, H10N8, H5N6, and H7N9. Co-circulation of LPAIVs with other subtypes provided opportunities for the novel AIVs to cross the species barrier.

Three children infected with H9N2 AIV were identified in Changsha in April 2025, and no epidemiological links were found between these mild and sporadic cases. Genetic analysis showed that the H9N2 viruses had enhanced binding ability to upper respiratory tract receptors, particularly the α 2,6-sialic acid receptors.

During the infection cycle of the influenza virus, the binding of the HA protein to the sialic acid receptor on the cell surface is a prerequisite for AIV to infect the host. The surface receptor of human upper respiratory tract epidermal cells is the α 2,6-sialic acid receptor, which is species-specific to the α 2,3-sialic acid receptor of avian cells. The mutations of Q234L in the AIV HA protein enabled an AIV-prioritized binding receptor shift from α 2,3-sialic acid to α 2,6-sialic acid. Liu et al. found that between 2005 and 2011, the H9N2 subtype of AIV acquired a stronger binding ability to the α 2,6-sialic acid receptor (8).

As the main antigenic proteins of the influenza virus, the HA protein and NA protein are subject to recognition by the host immune system and induced immune responses, thus facing greater immune selection pressure. Variations in the HA sequence may have a significant impact on the virulence (high pathogenicity and low pathogenicity), transmissibility,

TABLE 3. Molecular features of the genes of H9N2 viruses isolated from humans.

Virus	LX11086	KF1294	KF8210	CS992
Cleavage peptides	333–341	PSRSSRGLF	PSRSSRGLF	PSRSSRGLF
	109	Y	Y	Y
	161	W	W	W
	163	N	N	N
	191	N	N	N
Receptor binding sites of HA	198	V	V	V
	202	L	L	L
	203	Y	Y	Y
	226	L	L	L
	232–236	NGLMG	HGLMG	HGLMG
	281	L	L	L
Antigenic sites of HA	285	S	S	S
	296	G	G	G
	334	S	S	S
Sites of NA	62–64	del	del	del
	119	E	E	E
	292	R	R	R

Abbreviation: HA=hemagglutinin; NA=neuraminidase.

and host specificity (infection of humans, poultry, or other animals) of the virus. Mutations at the key receptor binding sites of the HA protein can change the ability of the virus to infect different tissues and organs. If AIV HA acquires a stronger ability to bind to the receptors of lower respiratory tract cells, AIV will be more likely to invade the lungs, and its pathogenicity and infection consequences will be significantly enhanced. The key sites of the NA sequence are related to the sensitivity of the virus to influenza virus drugs. Mutations such as E119D/I/V and R292K, which increased the drug resistance of the influenza virus, have not been found in the three strains of the H9N2 virus, indicating that clinical drugs (Oseltamivir, Zanamivir) are still effective in the treatment of the H9N2 influenza virus. All NA proteins of the three virus strains had three amino acid residues TEI (positions 62–64) deletion in the NA stems, which was consistent with the AIV sequence of the Y280 lineage (9). The NA proteins were more efficient at hydrolyzing sialic acid, promoting viral replication and transmission, and therefore better suited to transmission from wild birds to poultry (10).

The H9N2 avian influenza outbreaks pose significant challenges to public health systems. It is imperative to strengthen environmental surveillance in live poultry markets, improve early recognition of AIVs

in clinical settings — particularly in cases of unexplained pneumonia — and enhance interdisciplinary collaboration among agricultural, public health, and market regulatory agencies. Popularizing knowledge about AIV is equally imperative. The public should minimize contact with chicken, ducks, and other birds in areas known to be affected by AIVs, including markets and stores where live poultry may be sold or slaughtered (11).

Conflicts of interest: No conflicts of interest.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Sequencing depth and coverage of HA and NA of three H9N2 AIVs.

Virus	Segment	Sequencing depth	Sequencing coverage (%)
KF1294	HA	47,422	100.00
	NA	33,462	100.00
KF8210	HA	4,118	100.00
	NA	274	100.00
CS992	HA	31,243	100.00
	NA	15,614	100.00

Abbreviation: HA=hemagglutinin; NA=neuraminidase; AIV=avian influenza virus.