# First human case of avian influenza A (H10N3) in Southwest China

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# Abstract

In recent years, the avian influenza virus has emerged as a significant threat to both human and public health. Despite this, only two cases of human infection with the H10N3 strain have been documented. Here, we present the initial instance of human infection with avian influenza virus H10N3 in Yunnan Province, Southwest China. The patient, a previously healthy 51-year-old male, presented with recurrent fever peaking at 39°C, accompanied by symptoms such as cough, expectoration, chest tightness, and shortness of breath. Diagnosis revealed severe pneumonia, type I respiratory failure, and infection with avian influenza virus H10N3. Additionally, the patient experienced complications from Candida albicans and Staphylococcus epidermidis infections. Following treatment with appropriate antiviral drugs and antibiotics, the patient's condition improved. Molecular analysis of the viral strain identified four mutations potentially hazardous to human health. This underscores the importance of continuous and vigilant monitoring of the dynamics surrounding the H10N3 subtype of avian influenza virus.


# Introduction

Influenza A virus belongs to the family Orthomycoviridae. Influenza A viruses can infect a variety of birds, humans, and animals such as pigs, horses, seals and whales[1]. It is an important pathogen that poses risks to both human and animal health. Currently, only two subtypes of influenza A viruses, H1N1 and H3N2, are known to circulate in humans[1]. However, humans can also be infected by other subtypes, collectively referred to as avian influenza viruses (AIV).

In recent years, human infection with AIV has been frequently reported around the world, particularly involving subtypes H5, H7, and other[2–4]. Among these, human infections with H10 avian influenza virus have been reported globally, including subtypes H10N7, H10N8, and H10N3 [5,6]. The H10N3 subtype of avian influenza virus has been circulating among waterfowl and poultry in East and South Asia for decades, with rare instances of human infection[7]. The first recorded human cases of Avian-Origin Influenza A (H10N3) virus occurred in Jiangsu, China, in April 2021[8], followed by a second case reported in Zhejiang in June 2022[9]. Importantly, no instances of human-to-human transmission were detected in either case.

# Results

A previously healthy 51-year-old male experienced recurrent fever for a week, reaching a maximum temperature of 39°C, along with symptoms of cough, expectoration, chest tightness, and shortness of breath. Despite seeking medical attention at the local community health service center, his symptoms did not significantly improve. Consequently, he was transferred to the Department of Respiratory and Critical Care Medicine at a hospital in Yunnan Province, Southwest China, on March 6, 2024. The patient had a history of raising various birds, including chickens, ducks, geese, pigeons, peacocks, and ostriches. Notably, more than 20 chickens and geese died in the week preceding the onset of his illness, and he had

a history of slaughtering these birds. There was no reported contact with individuals exhibiting respiratory symptoms within the preceding month.

Upon admission (7 days after the onset of illness), the patient presented with a temperature of 39℃, a pulse rate of 110 beats per minute, a respiratory rate of 28 breaths per minute, oxygen saturation of 78%, and blood pressure measuring 105/70 mmHg. Laboratory tests revealed a low white blood cell count, elevated neutrophil percentage, decreased platelet count, and elevated levels of infectious markers. Additionally, the nucleic acid test for influenza A virus was positive (Table 1). Chest computed tomography revealed multiple patchy and increased density shadows in both lungs, characterized by unclear boundaries and uneven density (Fig. 1). The initial diagnosis upon admission included severe pneumonia, type I respiratory failure, and influenza attributed to influenza A virus.

The patient was administered oseltamivir (150mg, twice daily) and methylprednisolone (80mg, once daily) for treatment. Subsequent sputum culture results revealed infection with Candida albicans and Staphylococcus epidermidis, prompting the administration of appropriate antibiotics. Following this, Samples were sent for mNGS detection on March 8th, processed on March 12th, with the detection of positive influenza A virus on March 13th. Confirmation of the H10N3 subtype was achieved through sequence analysis and alignment on March 14th, and samples were subsequently sent to the CDC (Centers for Disease Control and Prevention). Confirmation of the H10N3 subtype through PCR was obtained on March 15th. Following this, the CDC conducted nanopore sequencing (Nanopore, GridION X5) on the samples and obtained the whole genome information of the samples (GenBank accession number SUB14344866, PP555666-555673). The patient's fever subsided on March 17th (18 days after illness onset), and on March 19th (20 days after illness onset), the nucleic acid test for influenza A virus returned negative results for the first time. Subsequent test results on March 21st (22 days after illness onset) indicated normalization of the patient's white blood cell count, along with a decrease or return to normal levels of infection markers. However, the patient exhibited prolonged prothrombin time. Chest computed tomography scans showed a reduction in lesions compared to previous scans (Fig. 1).

Through online analysis using BLASTN software on the GISAID website, it was determined that all eight gene segments of the H10N3 virus strain in our case originated from Eurasian avian influenza viruses. The phylogenetic tree indicated that the H10N3 strain from this patient belonged to the same group as the first patient in Jiangsu and the H10N3 strains found in poultry across various provinces in China (Fig. 2). Specifically, the H10N3 strain from this patient showed a closer genetic relationship to a chicken (GISAID#EPIISL15737164) from Jiangsu Province. Molecular characterization revealed a mutation at the 226th amino acid residue in the receptor binding site of the HA protein, where the amino acid changed from Q to L. This mutation makes the virus more adept at binding to human α-2,6-sialic acid receptors, significantly increasing the likelihood of human infection[10]. The mutation D701N in the PB2 protein has been shown to enhance the replication activity of avian influenza RNA polymerase within the human body. This mutation also increases the adaptability and pathogenicity of the virus to the human host, potentially serving as a crucial factor in avian influenza viruses crossing the host species barrier[11]. The presence of the S409N mutation in the PA protein suggests the potential for infectivity in humans and

may contribute to increased pathogenicity of this particular virus strain[12]. The S31N mutation in the M2 protein has been associated with resistance to adamantanes, a class of antiviral drugs[13].

## Discussion

The patient in this case not only exhibited infection with the H10N3 subtype of influenza A virus but also presented with a mixed infection involving bacteria and fungi, making the condition complex. It's worth noting that severe pneumonia patients infected with avian influenza often experience concurrent or secondary bacterial and fungal infections. Therefore, it is recommended to conduct repeated sputum culture, respiratory tract aspirate culture, or metagenomic Next Generation Sequencing (mNGS) detection in clinical settings to identify the types of bacteria or fungi present, as well as their susceptibility or resistance patterns. This approach enables clinicians to make informed decisions regarding antibiotic selection and guide appropriate clinical treatment strategies.

The clinical manifestations of avian influenza virus infection vary depending on the virus subtypes involved. For instance, infection with H5N1 and H7N9 subtypes can lead to severe pneumonia and related complications in patients. Conversely, certain subtypes such as H7 and H9 may only induce conjunctivitis or mild respiratory symptoms. It's important for healthcare providers to be aware of these differences in clinical presentation when diagnosing and managing cases of avian influenza virus infection[1]. As of now, only two cases of human infection with the H10N3 subtype have been reported. The symptoms observed in the patient infected with H10N3 in this case closely resemble those documented in the two previously known cases of H10N3 infection. Notably, all cases resulted in severe pneumonia in the affected patients[8,9]. In light of our findings, the identification of HA-Q226L, PB2-D701N, PA-S409N, and M2-S31N mutations in the protein of the Yunnan H10N3 virus strain underscores the potential for increased harm posed by H10N3 in humans. Therefore, it is imperative to closely monitor the dynamics of this subtype.

The case of human infection with H10N3 avian influenza virus highlighted in this study involved close contact with live birds, particularly through the handling and slaughtering of dead birds. This contact ultimately led to the patient contracting avian influenza and experiencing severe illness. This underscores the importance of paying special attention to instances of unexpected bird deaths and promptly reporting such cases. Moreover, it emphasizes the necessity of establishing a comprehensive avian influenza surveillance system, not only within Yunnan but also globally, to continuously and vigilantly monitor the H10N3 virus strain and its potential impact on human health.

## Methods

# Data collection

On March 6, 2024 the patient went to Kunming Third People's Hospital for treatment due to continuous fever for many days, and was diagnosed with severe pneumonia, type I respiratory failure and infection

by avian influenza virus. After the diagnosis of avian influenza virus infection, the patient investigated by using questionnaires, including demographic information, poultry contact history, basic diseases, etc.

# Genomic analysis and genome assembly

Multiple amplification products were obtained by using influenza A virus genotyping gene targeted amplification kit (BaiyiTech, Hangzhou). The amplified products were purified using ampure XP beads nucleic acid magnetic bead Purification Kit (Beckman, USA) and the library was constructed. The library was constructed by ligation method with the kit sqk-nbd114.24 (Nanopore, UK). After the library was constructed, it was added to the flo-min 114 sequencing chip (Nanopore, UK), and high-throughput sequencing was performed on the gridion X5 third-generation sequencer. All experimental steps were carried out in strict accordance with the relevant kit instructions and nanopore third-generation high-throughput sequencing requirements.

# Phylogenetic analysis

The nucleotide sequences obtained were analyzed in the Genbank and GISAID databases using the BLASTn tool to initially determine the virus subtypes. Similar HA nucleotide sequences were downloaded for phylogenetic analysis. The nucleotide and amino acid sequences were aligned using MAFFT (v7.310), and the phylogenetic tree was constructed based on the neighbor-joining method using MEGA-X.

# Declarations


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### Author contributions

The author contributions are as follows. J.Y.D., J.Z., J.W.X., Y.Y.L. and G.M.L. conceived, designed and supervised the study. J.W.X. treated the patient. P.Z., Y.D.D. and Q.J.L. gathered data and interviewed patients. M.H., X.H.X., Q.Q.J. and Y.Y.L did the laboratory tests, P.Z., Y.D.D. and Q.J.L. performed the data analyses and explored the mutation site. J.Y.D., J.Z., J.W.X., Y.Y.L. and G.M.L. wrote the drafts of the manuscript and interpreted the findings. All authors read the manuscript, provided feedback, and approved the final version.

### Ethics declaration

The patient and his family members signed consent forms approving the investigation, sample collection and its publication. The procedures were in accordance with the Helsinki declaration of 1975, as revised

in 1983. According to local regulations in China, institutional review board approval is not required for case reports, but only the written consent of the patient. The Third People's Hospital of Kunming City ethics committee reviewed this work and determined that institutional review board approval was not required.

### Competing interests

The authors declare that there is no conflict of interest.

### Data availability statement

The sequence data generated in this study have been deposited in the NCBI GenBank database under accession number SUB14344866, PP555666- PP555673.

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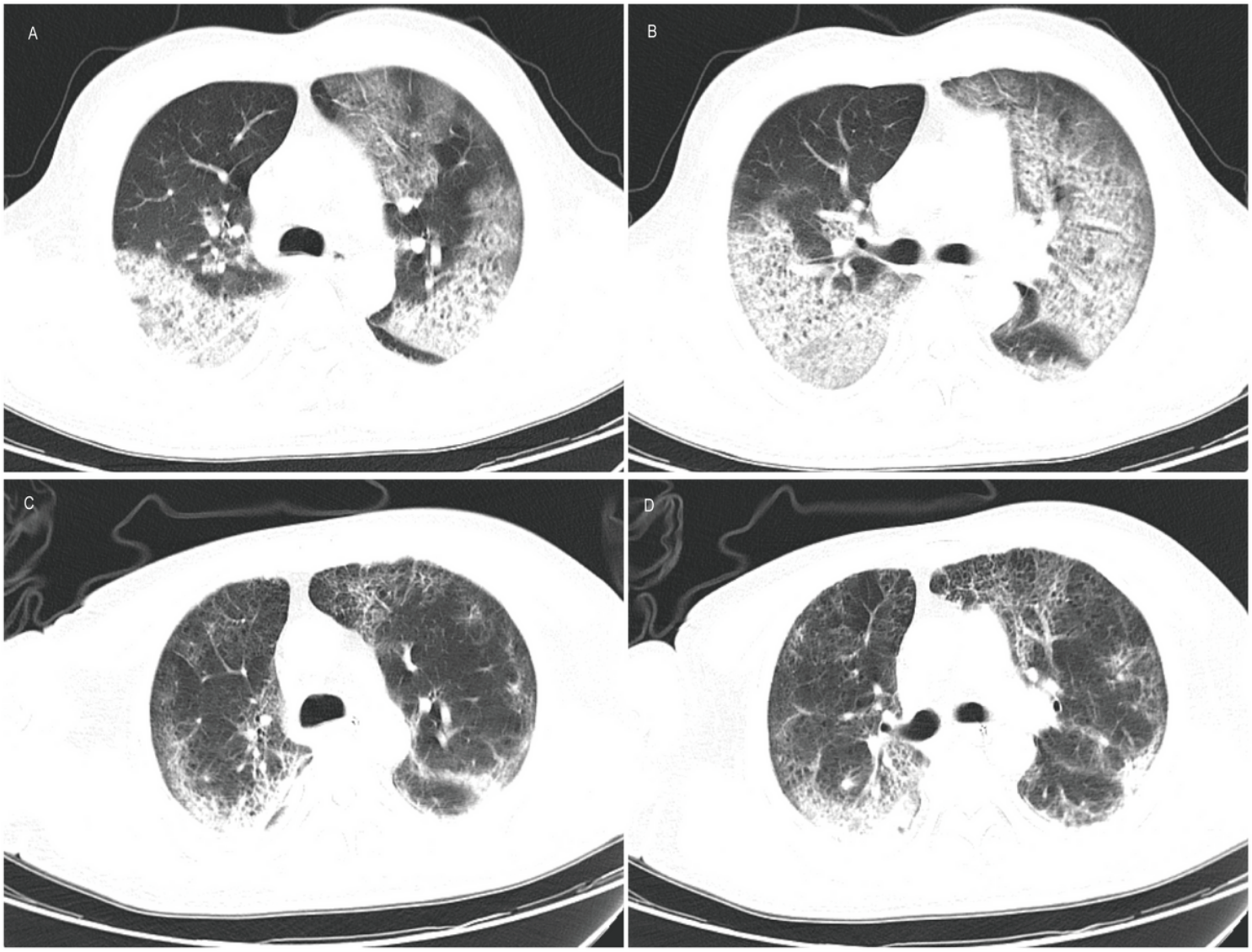
# Table

Table 1
Laboratory Test Results

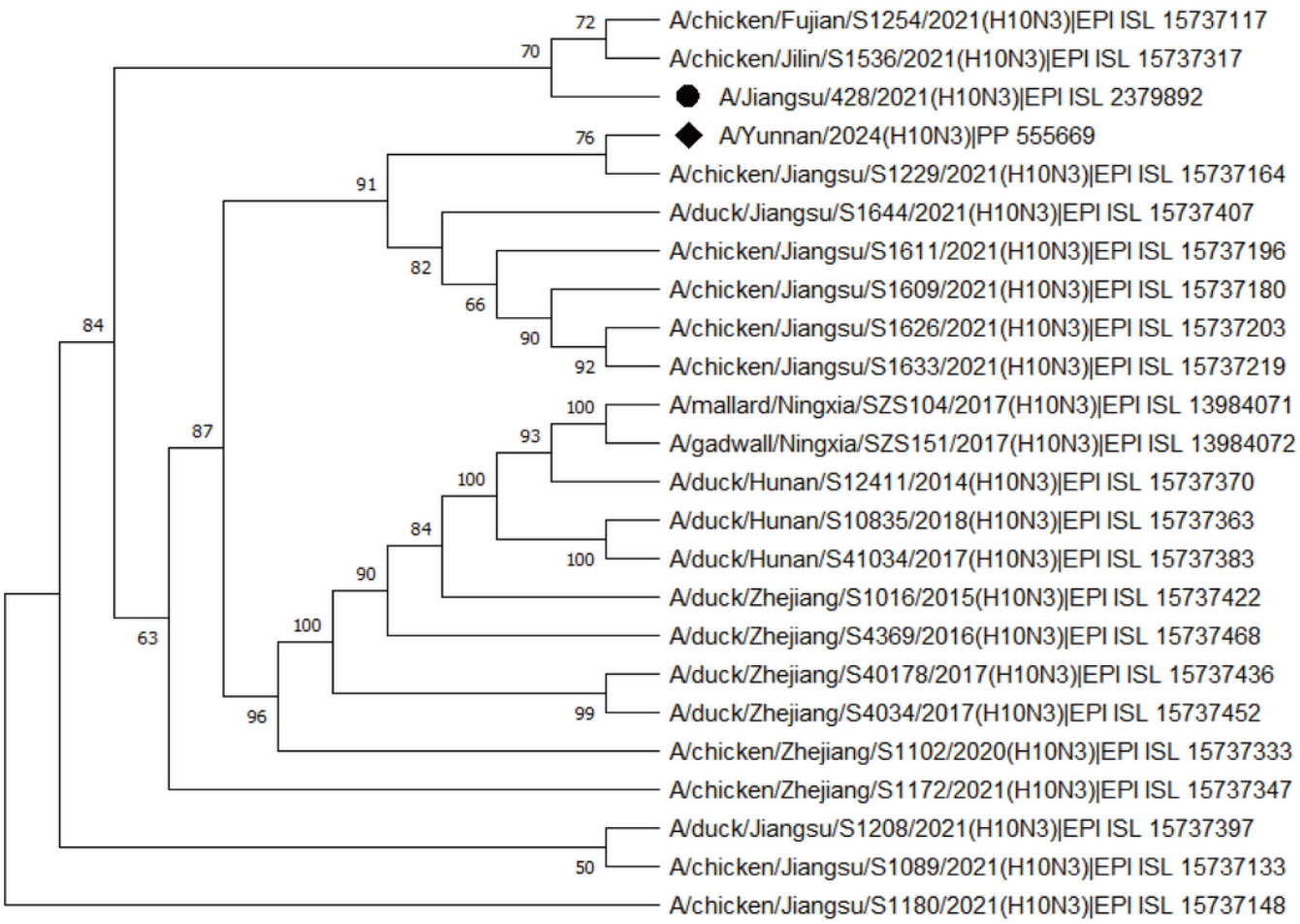
| | Day 7 | Day 22 | Normal range |
|---|---|---|---|
| White Blood Cell ($\times 10^9$cells/L) | 2.12 | 8.58 | 3.50–9.50 |
| Neutrophil ($\times 10^9$cells/L) | 1.80 | 7.19 | 1.80–6.30 |
| Neutrophil percentage (%) | 84.90 | 83.90 | 40.00–75.00 |
| Lymphocyte ($\times 10^9$cells/L) | 0.26 | 0.74 | 1.10–3.20 |
| Lymphocyte percentage (%) | 12.30 | 8.60 | 20.00–50.00 |
| Blood platelet ($\times 10^9$cells/L) | 79 | 223 | 125–350 |
| Prothrombin time (s) | 15.9 | 16.6 | 14.0–16.0 |
| Hypersensitive C-reactive protein (mg/L) | 249.41 | 21.93 | 0.00–6.00 |
| Lnterleukin-6 (pg/mL) | 78.99 | 8.26 | 0.00–7.00 |
| Procalcitonin (ng/mL) | 14.040 | 0.248 | < 0.500 |
| $pO_2$ (mmHg) | 32.00 | 68.40 | 80.00-100.00 |
| $pCO_2$ (mmHg) | 32.00 | 52.10 | 35.00–45.00 |
| Nucleic acid testing for influenza A virus | Positive | Negative | Negative |

# Figures




## Figure 1

**Computed tomography of lung.** (**A** and **B**). Results on March 6, 2024 showed that multiple patchy and patchy increased density shadows were seen in both lungs, with unclear boundary and uneven density. (**C** and **D**). Results on March 23, 2024 showed a reduction in lesions compared to previous scans.




## Figure 2

**Phylogenetic tree of 24 H10N3 strains from China.** The Phylogenetic tree was downloaded from the GISAID database (https://gisaid.org) using the neighbor-joining method in MEGA X. The diamond indicates the H10N3 strain in this study, and the octagon indicates the H10N3 strain from the first case in Jiangsu.