

Outbreak Reports

Three Cases Infected with Avian Influenza A(H5N6) Virus — Chongqing Municipality, China, January–September, 2021

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Summary

What is already known about this topic?

The World Health Organization (WHO) has reported a total of 48 cases by October 15, 2021. The continuous genomic reassortments of H5N6 and other subtype avian influenza viruses (AIVs) pose a long-term threat to public health and the poultry industry.

What is added by this report?

Three new cases of H5N6 that occurred from January to September 2021 in Chongqing Municipality, China were reported in this study. Epidemiological information of the three cases showed raising poultry and visiting live poultry market contributed to these infections, and there was no evidence of human-to-human transmission of H5N6 currently but a potential spatial cluster. An increase of H5N6 cases was recorded in the area.

What are the implications for public health practice?

In case of unexplained pneumonia or severe respiratory infection, the patients' epidemiological history of contact with poultry or live poultry markets (LPMs) may be an important interrogation to help diagnose. Extensive and long-term surveillance of avian influenza viruses in LPMs is essential.

Influenza A (H5N6) has replaced H5N1 as one of the dominant avian influenza viruses (AIV) subtypes in China (1). Since the first case of H5N6 virus infection in 2014, World Health Organization (WHO) has reported a total of 48 cases by October 15, 2021. A sharp increase was recorded in the frequency of human H5N6 infections in the last year (2–3). There were no H5N6 cases in Chongqing Municipality before, however, 3 cases were confirmed within just 9 months. Therefore, it is important to pay closer attention to H5N6 infectivity, especially on H5N6's possibly increasing capability to break through the species barrier to infect humans compared to before. Here, we

reported the first outbreak of human infections with influenza A (H5N6) virus in Chongqing Municipality, China, and presented epidemiological characteristics of this outbreak.

INVESTIGATION AND RESULTS

Human Infections with Influenza A (H5N6) Virus

There were no human infections with influenza A (H5N6) virus reported in Chongqing until January 4, 2021, when a 51-year-old male who had developed acute fever, cough and pneumonia was virologically confirmed in Rongchang District, Chongqing. Another two cases were confirmed in Tongnan District within 9 months (Table 1). These 2 districts are less than 100 kilometers far away from each other and located in the west of Chongqing, China. For the 3 cases, we carried out a detailed epidemiological investigation. A structured questionnaire was employed to collect demographic, epidemiological, and clinical data on each patient through a review of medical records and interviews with patients and their relatives, contacts, and healthcare workers.

Case 1 occurred in a 51-year-old male construction worker (Patient 1) who developed fever, cough, dizziness, weakness, and muscle pain on December 18, 2020, and he bought some cold-related medicine the same day in a private clinic; however, his symptoms became more severe as time moved on. On December 25, 2020, he developed expectoration, shortness of breath, choking cough, and heart tiredness after activity. On December 28, 2020, he was sent to Yongchuan Hospital of Chongqing Medical University and was admitted with fever (39.8 °C) and community acquired pneumonia. On January 1, 2021, the alveolar lavage fluid sample was tested by CapitalBio Medlab using next generation sequencing (NGS), which suggested an infection with avian influenza A (H5N6)

TABLE 1. Demography and epidemiology profiles of the 3 cases in Chongqing Municipality.

Case No.	District	Date of onset	Date of hospitalization	Date of laboratory confirmation	LPM contact	Contacted sick or dead poultry	Ate dead poultry	Outcome
1	Rongchang	2020/12/18	2020/12/25	2021/01/04	Yes	No	No	Recovered
2	Tongnan	2021/06/23	2021/06/30	2021/07/08	Yes	Yes	Yes	Recovered
3	Tongnan	2021/09/16	2021/09/19	2021/09/24	No	Yes	Yes	Death

Abbreviation: LPM=live poultry market.

virus. Thereafter, Chongqing Municipal CDC and China CDC confirmed the laboratory test as positive for H5N6 using real time reverse transcriptase polymerase chain reaction (RT-PCR) (lowest Ct values of H5 and N6 were 28.39 and 28.63, respectively) on January 3, 2021, and January 4, 2021, respectively.

Case 2 occurred in a 66-year-old male farmer (Patient 2) who developed a stuffy nose, sore throat, and cough on June 23, 2021, and he bought some cold-related medicine to relieve these symptoms the next day in a private clinic. However, his symptoms became more severe. On June 30, 2021, he developed shortness of breath and extreme fatigue. On June 30, 2021, he was sent to Tongnan General Hospital and was admitted with pneumonia infection. On July 7, 2021, nasopharyngeal swab was tested for influenza virus and was found to be positive for H5 by Tongnan CDC. On the next day, the respiratory tract samples were sent to Chongqing Municipal CDC and tested as positive for H5N6 using real time RT-PCR (lowest Ct values of H5 and N6 were 29.99 and 29.70, respectively).

Case 3 occurred in a 72-year-old male farmer (Patient 3) who developed dizzy, fever, and chill on September 16, 2021, and he bought some cold-related medicine to relieve these symptoms the next day in a private clinic. However, his symptoms became more severe. On September 19, 2021, he developed pneumonia and was admitted with pneumonia infection by Tongliang Traditional Chinese Medicine Hospital. On September 22, 2021, he was transferred to Tongliang General Hospital. On September 23, 2021, he was sampled by nasopharyngeal swab, tested for influenza virus, and found to be positive for H5 by Tongliang CDC. On the same day, the respiratory tract samples were sent to Chongqing municipal CDC and tested as positive for H5N6 using real time RT-PCR (lowest Ct values of H5 and N6 were 25.37 and 25.70, respectively).

All the 3 patients were in critical condition after hospital admission, and were treated with anti-viral, mechanical ventilation, and other intense supportive therapies in intensive care unit (ICU). Case 1 and Case

2 resulted in recovery and were discharged on Days 49 and 61 after illness onset, respectively, while Case 3 resulted in death on Day 8 after illness onset.

Infection Source Investigation

The epidemiological investigation of the infection source found that all three patients had no contact with known febrile persons or anyone who had respiratory symptoms, nor had they travelled out of Chongqing, and none of the patients had been in contact with each other. However, they all had been exposed to poultry and poultry-related premises 10 days before their disease onset.

Patient 1 lived in an urban area. He brought back a live duck from his father's house on December 5, 2020 and slaughtered it and stored it in the refrigerator until he and his family ate it on December 20, 2020. In addition, he visited a nearby live poultry market where there were live chickens and ducks for sale every 2 to 3 days before the disease onset.

Patient 2 lived in a rural area. He and his wife bought 12 baby chickens and 15 baby ducks from the local live poultry market in March 2021 and raised them in their backyard; 8 chickens successively became ill in June. His wife slaughtered these sick chickens and stored them in the refrigerator. He had cooked and eaten these chickens within 10 days before disease onset. In addition, he visited the local live poultry market within 10 days before disease onset.

Patient 3 also lived in a rural area. He and his wife raised 15 chickens, 4 geese, and 2 ducks in their backyard. Their geese and chickens became ill from early September, and some of them died. He had contacted the sick or dead poultry every day within 10 days before disease onset. In addition, there were chickens, ducks and geese who died in his neighborhood of the village in September.

There were H5N6 or H5 positive environment samples on the premises where the 3 patients lived or visited 10 days before their disease onset (Table 2). For Patient 1, the positive percentage of H5N6 was 6.7% (3/45) in the LPMs he visited. For Patient 2, the positive percentage of H5N6 was 11.1% (2/18) and

33.3% (1/3) in his family and the LPM he visited, respectively. For Patient 3, the positive percentage of H5N6 was 36.3% (4/11) in his family.

Human-to-Human Transmission Investigation

To determine whether there was human-to-human transmission, a 10-day medical follow-up was performed for the identified close contacts and strengthened surveillance on influenza-like illness and pneumonia of unknown origin. A total of 59 close contacts were identified, and none of them developed respiratory symptoms during the medical follow-up. None of the nasopharyngeal swabs collected at the start and end of their medical observation were positive for influenza A (H5N6) virus by real time RT-PCR. No more human infections with H5N6 virus were identified during the strengthened surveillance on influenza-like illness and pneumonia of unknown origin. These results indicated that no human-to-human transmission occurred in the epidemic.

Bioinformatics Analysis

The H5N6 viruses were sequenced by Chinese National Influenza Center (CNIC) and named A/Chongqing/00013/2021(H5N6) for Case 1 and A/Chongqing/02/2021(H5N6) for Case 2. The RNA was extracted with RNeasy Mini Kit (Qiagen, Hilden, Germany) and reversed by SuperScript[®] III One-Step RT-PCR System with Platinum Taq High Fidelity (Thermo Fisher, Carlsbad, USA). The library was performed with Illumina Nextera[®] XT Library Prep Kit (Illumina, San Diego, USA) and sequenced on Miseq. The raw data was filtered and assembled by CLC Genomics Workbench 11.0 (Qiagen, Hilden, Germany). The two H5N6 sequences were aligned by MAFFT v7.037b (<https://mafft.cbrc.jp/alignment/software/>) with other H5 sequences which were downloaded from GISAID (www.gisaid.org) and NCBI (www.ncbi.nlm.nih.gov). The phylogenetic analyses were conducted in MEGA X (version 10, Pennsylvania State University, USA, <https://www.mega-software.net/>) by using Neighbor-Joining Method, and the bootstrap value was 1,000. The HA gene of A/Chongqing/00013/2021(H5N6) belongs to clade 2.3.4.4h, while A/Chongqing/02/2021(H5N6) belongs to clade 2.3.4.4b (Figure 1).

The nucleotide sequences similarity of A/Chongqing/00013/2021(H5N6) and A/Chongqing/02/2021(H5N6) were analyzed with the online Basic Local

Alignment Search Tool (BLAST) (Table 2). The HA and NA segments of A/Chongqing/02/2021(H5N6) was found to have more than 99% similarity with A/Sichuan/06689/2021(A/H5N6), respectively, and A/Chongqing/00013/2021(H5N6)'s 8 segments were found to have more than 99% similarity with A/Anhui/2021-00011/2020(A/H5N6), respectively. This result is consistent with the results of phylogenetic analysis (Figure 1).

DISCUSSION

Severe infection with avian influenza A (H5N6) virus in humans was identified first in 2014 in China (4). Since then, a total of 48 laboratory-confirmed cases of human infection with influenza A (H5N6) virus including 25 deaths have been sporadically reported to the WHO in the Western Pacific Region by October 15, 2021. H5N6 likely has a high case fatality rate of around 52.1%, similar to H5N1 at 51.6%, but much higher than that of seasonal influenza virus at 0.1% (5–6). For Chongqing, no human infections with influenza A (H5N6) virus were reported before 2020. In neighboring Sichuan province, at least 5 H5N6 cases were recorded during the same period (7). All this evidence indicates an increase in the frequency of human H5N6 infections and implies a likelihood of more cases, while the incidence of seasonal influenza and other respiratory diseases declined because of the non-pharmaceutical interventions (NPIs) and behavioral changes to mitigate COVID-19 (8–9).

A study performed from November 2014 to April 2016 had found that H5N6 has replaced H5N1 as the dominant AIV subtype in southern China, especially in ducks (1). Therefore, enough attention should be paid to the unusual increase of H5N6 incidence in the last year. To determine whether H5N6 is more capable of breaking through species barrier to infect humans than before, epidemiological transmission patterns and genetic evolution of H5N6 virus should be closely monitored.

In this study, the epidemiological investigation showed that all 3 H5N6 cases had no contact with confirmed H5N6 cases, known febrile people or anyone who had respiratory symptoms, or traveled out of Chongqing. However, the epidemiological investigations indicated that contact with sick or dead poultry and visiting LPMs contributed to their infections, which was consistent with previous studies (4,7,10–11). The fact that H5N6 cases had history of

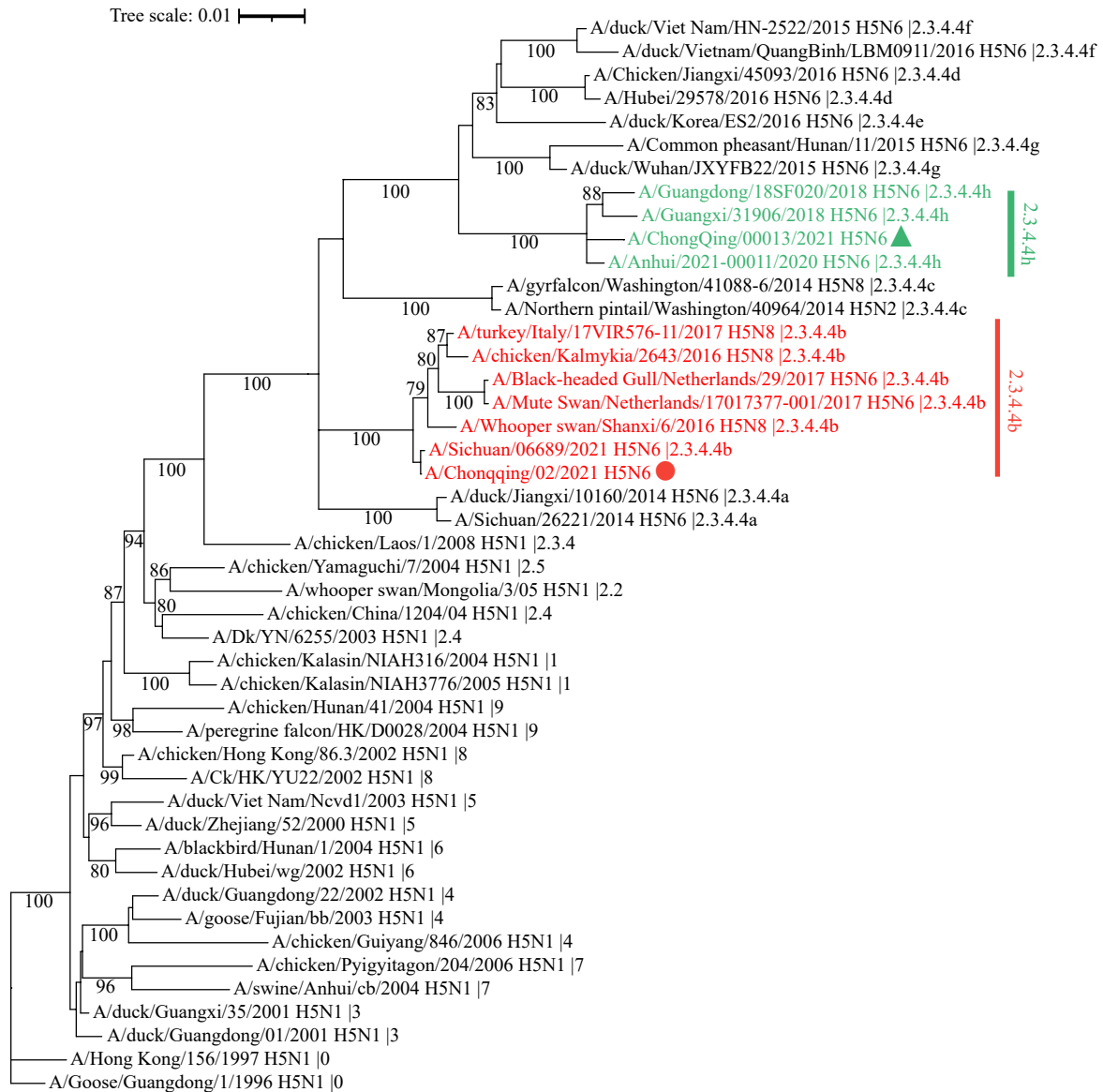


FIGURE 1. Neighbor-joining phylogenetic relationships of H5 viruses' hemagglutinin (HA) genes.
 Note: Green Triangle: The 2.3.4.4h virus isolated in this study. Red circle: The 2.3.4.4b virus isolated in this study.

TABLE 2. Similarity of 8 segments of the viruses analyzed by online BLAST.

Segments	A/Chongqing/00013/2021(H5N6), Identity/length (%)	A/Chongqing/02/2021(H5N6), Identity/Length (%)	Collection location
PB2	A/Anhui/2021-00011/2020 (A/H5N6) 2,327/2,341 (99%)	-	Anhui, China
PB1	A/Anhui/2021-00011/2020 (A/H5N6) 2,308/2,329 (99%)	-	Anhui, China
PA	A/Anhui/2021-00011/2020 (A/H5N6) 2,202/2,219 (99%)	-	Anhui, China
HA	A/Anhui/2021-00011/2020 (A/H5N6) 1,745/1,759 (99%)	A/Sichuan/06689/2021 (A/H5N6)1,767/1,771 (99%)	Anhui/Sichuan, China
NP	A/Anhui/2021-00011/2020 (A/H5N6) 1,558/1,565 (99%)	-	Anhui, China
NA	A/Anhui/2021-00011/2020 (A/H5N6) 1,426/1,432 (99%)	A/Sichuan/06689/2021 (A/H5N6)1,426/1,427 (99%)	Anhui/Sichuan, China
MP	A/Anhui/2021-00011/2020 (A/H5N6) 1,024/1,027 (99%)	-	Anhui, China
NS	A/Anhui/2021-00011/2020 (A/H5N6) 869/875 (99%)	-	Anhui, China

Note: -, no sequence was obtained.
 Abbreviation: BLAST=basic local alignment search tool.

contact with sick or dead poultry is different from a previous finding that all H7N9 human patients in Chongqing were infected after exposure to live chickens in 2017 (12). Not as young as H5N1 cases (13–14), all the H5N6 patients in Chongqing were older and their symptoms progressed rapidly and the health condition worsened rapidly from disease onset, which was consistent with previous studies (7,14). Therefore, to diagnose human infection with avian influenza as early as possible, doctors should ask patients with unexplained pneumonia or severe respiratory infection of their epidemiological history.

Currently, the overall risk of human infection with avian influenza is controllable, sporadic cases of human infection with avian influenza, including new subtypes may continue to occur in the future. With continuous evolution of AIVs (1,15–17), H5N6, H7N9, H5N1, or other subtypes of AIV would pose a long-term threat to public health and the poultry industry (18), especially considering the potential features gained for human-to-human transmissibility.

Although the H5N6 viruses isolated in Chongqing had the poultry origin, they were not identical and clustered in two clades of the phylogenetic tree, and the online BLAST results indicated that these viruses were similar to viruses collected in different regions, which suggested that these H5N6 viruses might be of different sources. In China, the live poultry trade enhances the transmission and dissemination of AIVs among poultry (16,19) and the chances of human contact with AIV-carrying poultry from LPMs (1). LPMs play an important role in the evolution and dissemination of AIV (1,17,19). Therefore, extensive and long-term surveillance of avian influenza viruses in LPMs is essential (1,20).

Conflicts of interest: No conflicts of interest.

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