

## CHINA CDC WEEKLY



Vol. 4 No. 1 Jan. 7, 2022

中国疾病预防控制中心周报



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ISSN 2096-7071



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## Commentary

# Innovating for Health in China's Greater Bay Area

Feichau Pang<sup>1</sup>; Puihong Chung<sup>1</sup>; Gabriel M Leung<sup>1,\*</sup>

## The Success of World Metropolitan and Bay Areas

The Tokyo Bay Area, the New York Metropolitan Area, and the San Francisco Bay Area are leading regions for science and technology. They share an economic transformation from industrial or manufacturing bases to predominantly service-based economies with this sector contributing 75% of their gross domestic product (GDP) in 2020. Technology companies in these locations accounted for 60% of the revenue of the top 100 global tech companies in 2018 (1). The success of these locations stems from the convergence of academia, technology giants, government support, and a high density of investors, as well as innovative entrepreneurs (2).

## GBA as Innovation Hub in 14th Five-Year Plan

Since the Qing Dynasty when Guangzhou was the only port open to foreign commerce, Southern China's Pearl River Delta has traded on its international connections. Beginning in the 1980s, Pearl River Delta (PRD) cities transformed into a global manufacturing center, driving urbanization and producing 24.8% of China's exports (3). Today, the region is home to more than 86 million people and has a GDP in excess of 1,600 billion USD (4). In 2019, the Chinese Central Government adopted the Greater Bay Area (GBA) policy, wrapping together 9 cities plus 2 special administrative regions in the PRD, with a vision for the region to become a leader in healthcare technology (healthtech) (5).

The GBA has been officially recognized for its potential to achieve the sci-tech self-reliance and self-strengthening strategy as detailed in the outline of the 14th Five-Year Plan (2021–2025) for National Economic and Social Development and Vision 2035 (6). Under the plan, Guangzhou, Shenzhen, and Hong Kong will connect as one of two technology corridors in the GBA (Figure 1). This corridor boasts 80 universities, mega ports and technology giants, and will

house the world's second-largest biotech fundraising hub.

Elsewhere in China, the Beijing-Tianjin-Hebei region (Jing-Jin-Ji) and the Yangtze River Delta (YRD) are also positioned to become world-class mega city clusters. These regions possess 11,153 tech scalars, according to a 2021 report, second to the USA which has 30,007 tech scalars (7). However, pursuing innovation in life sciences and developing health-tech, which is not simply information technology as applied to the health arena, requires an ecosystem supported by basic science research alongside incubators to nurture new discoveries to translate these for clinical and public health applications. Thus, investing in technology infrastructure is neither sustainable nor efficient if the three regions are in competition for talent and supply chains, particularly if the ultimate goal is global competitiveness.

## Healthtech Clustering

Healthtech dominates technology investment thanks to breakthrough discoveries in sequencing and synthesis and advancements in artificial intelligence (AI) and super-computing data analytics. Clustering of such technologies enables cross-fertilization among technology giants, multinational pharmaceutical companies, start-ups, and researchers to develop solutions that attract venture capital firms to invest. This is exemplified by the Boston area, where 120 biomedical firms sit within a 1.5-kilometer radius. These companies regularly partner with the academic powerhouses of Harvard University and the Massachusetts Institute of Technology to translate innovations (7–8).

In recent years, start-ups have harnessed artificial intelligence, big data, precision medicine, gene editing, and biomanufacturing to build services or products (9–10). In view of the complexity of these innovations, world-class technology hubs are opting to specialize in either biotech, biopharma, medical devices, or digital health. It is therefore crucial for China to create defined roles for each city cluster to avoid duplication and maximize global competitiveness.

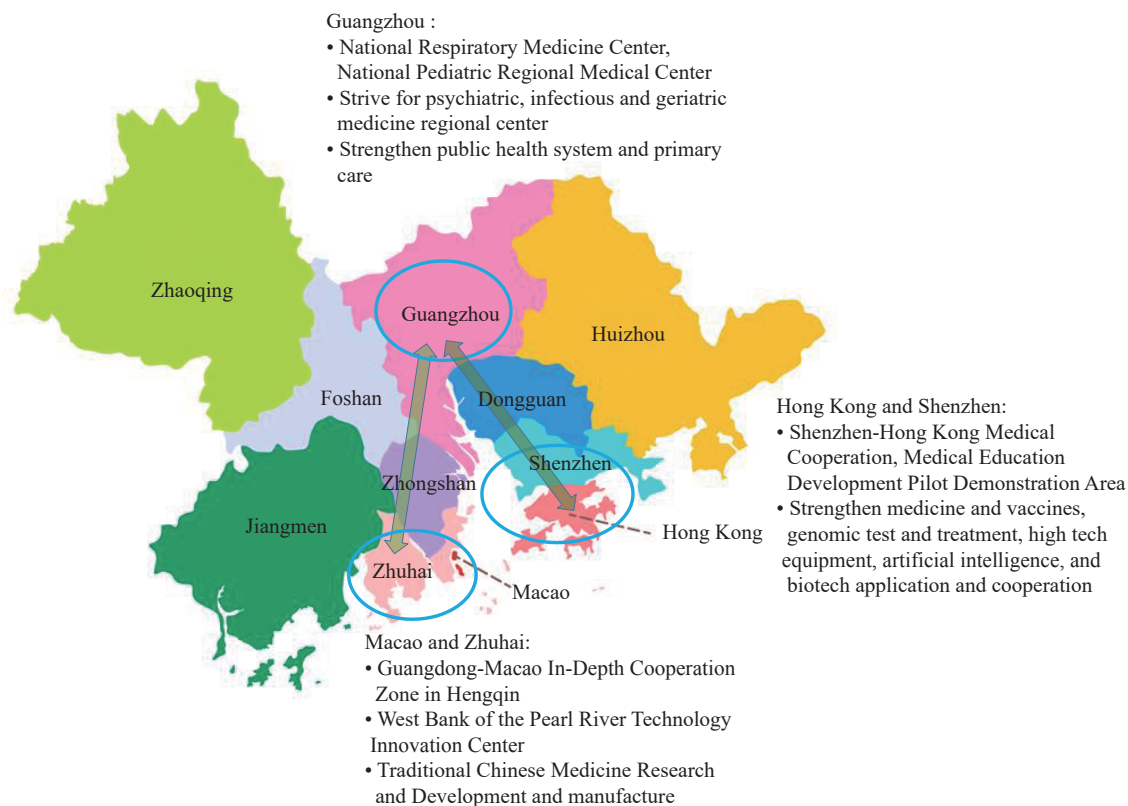


FIGURE 1. Guangzhou-Shenzhen-Hong Kong and Guangzhou-Zhuhai-Macao technology corridor.

## “Coopetition” and Role Delineation Among China’s Mega City Clusters

By comparing the three mega city clusters in Figure 2, it is clear that all 3 clusters have heavily invested across multiple healthtech fields. This uncoordinated approach risks competition for resources and calls for the development of collaborative platforms across academia and services.

This comparison found the YRD region possesses the strongest technology infrastructure for developing biopharma, medical devices, therapeutic diagnostics, and biotech. In 2020, more than 60% of Shanghai’s healthcare investment focused on biopharma, while Anhui Province accounted for over 50% of China’s device funding.

Jing-Jin-Ji is in the leading position for big data analytics and diagnostic tools, with investment in related technologies accounting for 67% of Beijing’s healthcare funding. Biopharma investment secured 26% of that funding, followed by devices at 7% (11).

Global technology giants Tencent, Huawei, and BGI are headquartered in Shenzhen in the GBA, attracting tech talent and investors. Neighboring Guangzhou boasts over 10,000 high-tech start-ups and

is a major regional healthcare center with 70 tertiary hospitals (12–13). Beyond Hong Kong’s role as a global financial center, it boasts two world-class medical schools, a fully-funded public hospital system, and an internationally-recognized specialist training system for over 60 medical sub-specialties. Moreover, the territory’s infectious disease and public health researchers stand at the forefront of COVID-19 research, while 23 local scientists ranked in the top 1% for citations in their fields in 2021. By building on these strengths, the GBA can excel by leveraging its IT capabilities to develop digital health services alongside a focus on biotech research.

## Enhance Competitiveness

The 14th 5-Year Plan synergizes each mega-city cluster, but more centralized planning is required to define each region’s focus and to formulate a cooperative competitive strategy. For the GBA, it can utilize its existing strengths to build data platforms in collaboration with other mega city clusters. Sharing health data to develop big data applications is beneficial for public health systems and potentially lucrative for entrepreneurs. In addition, Hong Kong’s clinical trial data are nationally and internationally

	Jing-Jin-Ji	YRD	GBA
Demographics			
Population (Million)	113	235	86
Average GDP per person (RMB per year)	78,000	110,000	124,000
Technology infrastructure			
Double first-class universities	40	35	5
Number of top-class tertiary hospitals	174	201	100
Proportion of IT Companies in China (%)	32.5	24.8	14.5
Fund raising for healthtech (2018–20) (Billion USD)	3.7	13	2.8
Healthtec companies / services			
Biopharma companies	168	256	85
Biotech R&D services	134	280	63
Therapeutic diagnostic companies	46	192	40
Medical device companies	2,709	7,344	3,747
Digital health IT companies	34	22	8

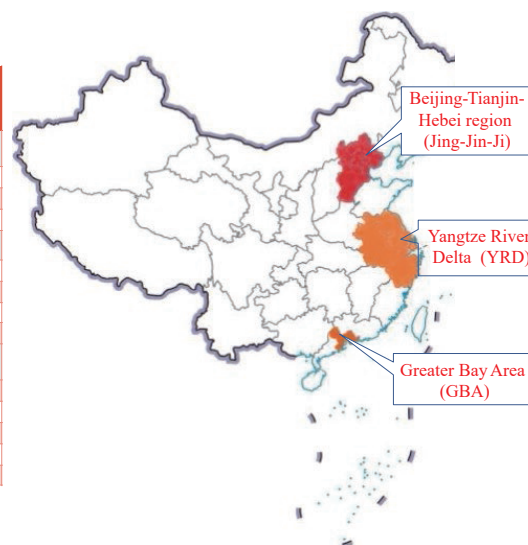


FIGURE 2. Comparison of Three Mega-city Clusters in Healthtech Development (4,7,11–19).

accredited, positioning the territory as a guide and resource for the two other city clusters to enhance competitiveness. Against this cooperative backdrop, the GBA can narrow its healthtech focus and partner with national and international counterparts to compete against global technology hubs.

doi: 10.46234/ccdcw2021.274

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Submitted: December 15, 2021; Accepted: December 20, 2021

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## Outbreak Reports

# Epidemiological Investigation of an Inhalational Anthrax Patient Traveling for Medical Treatment in Beijing Municipality, China, August 2021

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## Summary

### What is already known about this topic?

Inhalational anthrax, also known as pulmonary anthrax, is an infectious disease caused by *Bacillus anthracis*. The patients are usually infected by inhaling aerosolized *B. anthracis* spores from dead animals or animal products. Compared to cutaneous anthrax, inhalational anthrax is rare and deadly and few cases in China were reported.

### What is added by this report?

This report covers all information of clinical features, laboratory testing, and epidemiological characteristics as well as exposure history of a recent primary inhalational anthrax patient who was seeking medical treatment in Beijing Municipality in August 2021. New laboratory techniques, including second-generation sequencing, polymerase chain reaction, and rapid test for serum antibody, played an important role in the process.

### What are the implications for public health practice?

The information provided in this report, including the correct sample type, epidemiological investigation details, and application of the new diagnostic criteria of anthrax, could assist public health professionals in dealing with anthrax epidemics.

At 16:18 on August 8, 2021, a hospital in Beijing Municipality reported a suspected case of inhalational anthrax. Six hours later, Beijing CDC reported that pleural effusion sample of the case tested positive for nucleic acids of *Bacillus anthracis* using fluorescent real-time polymerase chain reaction (PCR). On August 13, 1 strain of *B. anthracis* was isolated from dead cattle in the patient's village. On August 19 and 20, 2 samples of pleural effusion collected from the patient with 24-hour interval were negative for *B. anthracis* both by

real-time PCR and bacterial culture. This met the requirements for discharging anthrax cases from hospital isolation. A total of 127 close contacts of the case in Beijing were quarantined at designated sites or at home for 12 days from the last contact with the patient.

## INVESTIGATION AND RESULTS

The patient was a 46-year-old female from Weichang Manchu and Mongolian Autonomous County, Chengde City, Hebei Province. The patient had sudden chest pain on July 30, 2021 at home with continuous tingling in the right chest, radiating to the right shoulder. The pain worsened when respiring deeply, accompanied by chest tightness, shortness of breath, and asthenia. However, there was no fever, cough, sputum, or hemoptysis. She went to the local county-level and then prefecture-level hospitals on July 31 and was suspected of cardiovascular diseases, such as myocardial infarction and aortic dissection. Fever appeared at 14:30 on August 1. After screening negative for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the fever clinic, the computed tomography (CT) examination at 21:00 showed lesions in the right pulmonary hilar and mediastinum with suspected space-occupying lesions, bilateral pleural effusion, atelectasis in both lungs, and pericardial effusion. The results of two CT examinations by the hospitals in Beijing were similar.

The patient came to Hospital A in Beijing by an ambulance at 02:00 on August 3, accompanied by her families. After screening negative for the SARS-CoV-2 in the fever clinic, the patient was given symptomatic anti-inflammatory, analgesic, and antipyretic treatments in the emergency department of Hospital A. Symptoms of pain were relieved. The results of the CT examination showed bilateral pleural effusion,



atelectasis in both lungs, and space-occupying lesions in the mediastinum.

At 14:00 on August 4, the patient was transferred to Hospital B by a Beijing Emergency Medical Center ambulance. The medical record for admission of the patient showed that she had no erythema, papules, or vesicles on the skin of unknown cause and no fever, nausea, or vomiting. Moist rale was found by lung auscultation and the CT showed lesions in the right pulmonary hilar and mediastinum, with suspected space-occupying lesions, bilateral pleural effusion, atelectasis in both lungs, and pericardial effusion. The CT results were similar to previously reported cases (1–2). Other clinical findings were liver injury and hypoalbuminemia. A total of 200 mL of hemorrhagic pleural fluid was collected via the closed thoracic drainage method. The patient was treated with imipenem-cilastatin sodium injection and moxifloxacin hydrochloride sodium chloride injection. The seventh group of lymph nodes was positioned by endobronchial ultrasound (EBUS) and punctured, and some purulent secretions were observed in the puncture fluid. Metagenomic sequencing detected sequences of *B. anthracis* in the puncture fluid.

Various samples of the patient were collected on August 8, 2021. The sample of pleural effusion was positive for nucleic acid of *B. anthracis* by fluorescent real-time PCR. Serum antibody against *B. anthracis* was positive by colloidal gold test (Table 1). As a confirmed case, the patient was transferred to the designated infectious disease hospital for quarantine and treatment on the same day. On August 19 and 20, two samples of pleural effusion within a 24 h-interval collected from the case were negative for *B. anthracis* by real-time PCR and bacterial culture.

The patient's home is in the Bashang Grassland, which was a semi-pastoral area, and the patient often had close contact with cattle and sheep. However, the course of the anthrax often progresses rapidly for cattle and sheep and no chronic or carrier states exist. The probability of infection through touching

asymptomatic cattle or sheep was estimated to be very low. The patient was also engaged in restaurant operations, so she often visited the meat wholesale market to purchase beef and mutton every two or three days before the disease onset. However, contact with meat or blood of dead animals is more likely to cause cutaneous anthrax instead of inhalational anthrax. The patient had no sign of cutaneous anthrax, no erythema, papule, or verruca, as shown in the medical records of Hospital B, so the probability of infection from exposure to meat from the market was also very low.

The two cattle raised by the patient's brother-in-law died of an unknown illness in the morning of July 26 and were slaughtered at once in the backyard of the patient's house. At the same time, the patient's brother-in-law was told by the doctor that he might have cutaneous anthrax; he called from hospital to stop the slaughtering of the two cattle. The meat, furs, and other parts of the two cattle were disposed outside the village. The slaughter site was immediately disinfected with 1,000 mL of cresol soap solution, the ground was washed with a high-pressure water gun, and the sewage entered the drainage ditch through a drain. In the afternoon, and the next day, the other two cattle died and were buried directly without slaughter. The spore-containing droplets produced by high-pressure water gun washing could be the possible cause of the infection of the patient because she had to pass the slaughter site to go to the toilet.

The patient's brother-in-law was a 54-year-old male. He felt itchy on the back of his right hand on July 23 and found a rice grain-sized rash that gradually reddened and became swollen with pain. The site of skin turned black after the ulceration without fever. In the morning of July 26, he went to the county hospital. The doctor suspected cutaneous anthrax but results of stained smear examination for the damaged skin was positive for cocci and no bacilli were detected at the time. The doctor still prescribed levofloxacin infusion therapy for him.

The patient's brother-in-law received levofloxacin

TABLE 1. Test results of the patients' samples on August 8, 2021.

Sample type	Colloidal gold test		Real-time PCR target genes			Bacterial culture
	Antigen	Antibody	<i>pagA</i>	<i>cap</i>	<i>rpoB</i>	
Oropharyngeal swab	Neg	Not tested	Neg	Neg	Neg	Neg
Nasopharyngeal swab	Neg	Not tested	Neg	Neg	Neg	Neg
Pleural effusion	Neg	Not tested	Pos	Pos	Pos	Neg
Blood	Neg	Pos	Neg	Neg	Neg	Neg

Abbreviations: PCR=polymerase chain reaction; Neg=negative; Pos=positive.

infusion therapy for 10 days. He went again to the hospital in town due to pain in his right hand. The hospital found his skin damage site on the back of his right hand to be black with eschar and reported the case. On August 9, the Chengde CDC reported real-time PCR negative results for nucleic acid of *B. anthracis* of his skin smear sample, but positive for serum antibody against *B. anthracis* by colloidal gold test. The possible source of infection for the patient's brother-in-law could be the *B. anthracis* spores in the soil. The village is located within a historically anthrax-endemic region. The heavy rainfall this year may have caused the spores in the soil to be revealed on the surface and contaminated the grass. The time of infection could be the same as the cattle or when the patient disposed of the sick cattle.

On August 9, 3 samples of furs and 3 samples of beef were collected from the buried dead cattle. The beef samples were positive for nucleic acid of *B. anthracis* and one strain of *B. anthracis* was isolated by bacterial culture from one sample of the beef.

Inhalational anthrax is usually caused by inhaled spores of *B. anthracis* from animal fur, wool, textile mill, or by bioterrorist attack (3). The current case was also related to infected animals because the case was preliminarily attributed to being caused by droplets produced by the high-pressure water gun in the process of washing the grounds where the cattle were slaughtered. In addition, the patient was in poor health and vulnerable to infection. She likely passed the site of slaughtering and became infected.

## PUBLIC HEALTH RESPONSE

The current event of inhalational anthrax was reported as a level IV public health emergency. The patient was quarantined and treated in a single room at the designated infectious hospital. For contact tracing, in accordance with the *Anthrax Diagnosis, Treatment, and Management Plan (2005)*, "family members of the patient, caregivers, and medical staffs who had direct contact with the patient, those who had contact with the excrements of the patient, and individuals who stayed in the same room or stayed with the patient for at least 30 minutes within 5 meters of the patient" should be defined as close contacts. There were 9 family members, 45 medical staff members from various hospitals, 5 medical staff members in the ambulance, 37 hospitalized patients, 30 caregivers of patients, and 1 person collecting clinical samples identified as close contacts. Among them, 9 family

members not only had close contact with the patient but also had a history of common environmental exposure in the patient's village. All were quarantined for 12 days from the last contact with the patient. They were required to measure body temperature and report health status everyday. No secondary case was found. The possible contaminated areas in Hospital A and B, as well as the ambulance for transferring the patient were disinfected.

## DISCUSSION

There are still misunderstandings about anthrax. There was no report on person-to-person transmission of inhalational anthrax under natural conditions (4). The medical staff members in local hospitals in Hebei Province and Beijing Municipality had primary personal protection, including working clothes and surgical masks. No infection was observed. Person-to-person transmission has only been reported with cutaneous anthrax, where discharges from skin lesions might be infectious (5). In this report, the fluorescent real-time PCR results of the throat swab and nasopharyngeal swab were negative, while the result of pleural effusion was positive. These findings were consistent with the pathological findings that the anthrax lesions existed mainly in the mediastinum rather than the lungs (2,6). However, currently existing regulations still require quarantine of close contacts of inhalational anthrax cases. Therefore, it is necessary to further update the technical guidelines based on integrating evidence from previous cases and documentation.

Under natural conditions, primary inhalational anthrax is rare and fatal (7). The droplets aroused by this high-pressure water gun should contain a relatively small number of bacteria. Healthy people washing the ground may not be infected but the patient was not in good health and likely vulnerable. However, people with a history of common exposure were at the same risk, so we suggest that these people need to be quarantined.

The patient's first symptoms were atypical, with no fever in the early stage; the chest pain and tightness was suspected at first to be caused by cardiovascular disease. This perhaps was related to being exposed to particles containing lower levels of *B. anthracis*. The patient's medical history in multiple hospitals showed that no epidemiological exposure history was asked by the doctors, which should be one of the reasons for the misdiagnosis. The physicians should be informed to



pay more attention to epidemiological history.

The doctors in the local hospitals of Hebei Province showed good awareness of cutaneous anthrax. The doctor in the county-level hospital immediately suspected anthrax and prescribed correct antibiotics for the patient's brother-in-law. Inhalational anthrax was hard to distinguish clinically from other illnesses and was once diagnosed with cardiovascular disease. New techniques, like second generation sequencing would effectively assist the diagnosis. Training of medical staff members with knowledge of anthrax should be carried out.

Beijing has high-quality medical resources; patients from other regions tend to come to Beijing to seek medical treatment. Cases of infectious diseases, such as the 2 cases of pneumonic plague in 2019 may be imported to the city (8). This inhalational anthrax patient entered Beijing by ambulance, so there were no other contacts outside of hospitals. This reminds us that for cases that do not rule out infectious diseases, remote transfer should be further standardized, and the transfer and receiving process should be optimized to ensure that the cases can be treated in time, while at the same time conducive to the prevention and control of infectious diseases.

doi: 10.46234/ccdcw2021.275

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Submitted: August 28, 2021; Accepted: November 19, 2021

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## Outbreak Reports

## A H9N2 Human Case and Surveillance of Avian Influenza Viruses in Live Poultry Markets — Huizhou City, Guangdong Province, China, 2021

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### Summary

#### What is already known about this topic?

An increasing number of human infected avian influenza A (H9N2) cases have been reported. In 2021, 11 human infections with influenza A virus subtype H9N2 (A/H9N2) have been reported in China.

#### What is added by this report?

A new case of H9N2 that occurred in April 2021 in Huizhou City, Guangdong Province, China, was reported in this study. Epidemiological and laboratory information of the case and routine influenza surveillance data of avian influenza A were presented in this report.

#### What are the implications for public health practice?

The emergence of a human infected with Avian Influenza Virus H9N2 demonstrates that there is an urgent need to strengthen the surveillance of influenza-like illness and live poultry market.

Since the first human infection of low pathogenicity avian influenza A (H9N2) virus was detected in Hong Kong, China in 1999 (1), this strain has been reported to intermittently infect humans. In April 2021, a case of H9N2 was reported in Huizhou City, Guangdong Province, China. The patient was sampled by throat swabs, which were sent to Huizhou CDC for quantitative reverse transcription-polymerase chain reaction (RT-PCR) testing. An investigation group carried out epidemiological and environmental investigations to find other avian influenza cases.

## INVESTIGATION AND RESULTS

Emergency sampling of environment was conducted; both the viral RNA from the throat swab of the patient and environment were extracted using the QIAamp<sup>®</sup> Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions, and

RT-PCR assays with specific primers and probe sets were used for detecting the avian influenza A virus(H9N2) M gene. The virus gene segments were obtained through metagenomic sequencing. Genome sequences were aligned with the reference genomes downloaded from GenBank using the basic local alignment search tool (BLAST). The avian influenza virus activity in live poultry markets in Huizhou was analyzed using IBM SPSS Statistics for Windows (version 22.0. Armonk, NY: IBM Corp, USA).

The case occurred in a 30-year-old female living in Huicheng District of Huizhou City. On April 12, 2021, she experienced symptoms of sore throat but no fever. After medical treatment, her symptoms were alleviated. On April 20, 2021, she had symptoms of fever with an auxiliary temperature of 38.5 °C and was admitted to the Huizhou Central People's Hospital for treatment. Her respiratory symptoms were paroxysmal cough, mainly dry cough, and wheezing. Other symptoms included chills, headache, and general malaise. The blood routine examination confirmed that the absolute value of neutrophils increased and that of lymphocytes decreased. The patient refused X-ray check as she thought it was unnecessary due to her perceived mild symptoms. On April 21, the patient's throat swabs were collected and sent to the Huizhou CDC as a sample for routine influenza surveillance. On April 23, she was found to be positive for H9N2.

The patient had a history of travel to Dali Bai Minority Region, Yunnan Province from April 14 to 18 but denied having any direct contact history with live birds or other poultry. Photographs indicated that the patient did not wear a mask as required in a park. Before she went to Yunnan Province, she left her car in a surface parking lot, which was surrounded by many trees and birds. Her son was in a kindergarten near a live poultry market and was picked up by his grandmother. Their shoes may have been contaminated by sewage, poultry manure, and feathers when they passed through the live poultry market.

Through RT-PCR assays, the cycle threshold value (Ct value) of the patient's throat swab was 32, which indicated the viral load of the sample was fairly low. As a result, the 8 sequences of H9N2 were unobtainable and only 9 gene reads were obtained (2 HA gene reads, 2 NA gene reads, 4 PB2 gene reads, 1 M gene reads), with the length being 50 bp for each read. The BLAST results showed that these gene reads had high homology with chicken H9N2 strains isolated from Guangdong Province, Zhejiang Province, and Shandong Province. The molecular characterization of the gene reads was analyzed, and the amino acid residues in proteins of these gene reads were the same or similar to reference strains A/chicken/China/GD2021/2021(H9N2) and A/Zhongshan/201501/2015(H9N2), but different to reference strain A/chicken/Hong Kong/G9/1997(H9N2) as shown in the Table 1. A total of 40 environment samples of the live poultry market near the patient's son's kindergarten were collected. The nucleic acid positive rate of avian influenza A virus was detected, the H9

subtype positive rate was 50% (20/40), but H5 and H7 subtype were not detected.

A total of 386 environment samples were collected in poultry markets in Huizhou. The nucleic acid positivity rate of avian influenza A was detected in 176 of these samples from December 2020 to August 2021. The nucleic acid positive rate of subtype H9 fluctuated between 30.00% and 65.38% among different months, without significant difference ( $\chi^2=56.00$ ,  $P>0.05$ ) (Figure 1). The highest positivity rate of avian influenza A virus was in August 2021, and the lowest was in April 2021.

## DISCUSSION

So far in 2021, 11 human infections with A/H9N2 have been reported (2–3). Among them, Guangdong Province reported four cases, Yunfu City reported two cases, Meizhou City and Huizhou City each reported one case (4–5). According to the routine influenza surveillance of Huizhou City, avian influenza A virus

TABLE 1. The molecular characteristics of the H9N2 gene reads from Huizhou City.

Virus	HA		NA		PB2				
	334*	501	432	434	299	327	334	336	381
A/chicken/Hong Kong/G9/1997(H9N2)	A	K	K	T	K	G	K	F	M
A/chicken/China/GD2021/2021(H9N2)	S	Q	H	P	R	G	S	S	L
A/Zhongshan/201501/2015(H9N2)	S	Q	Q	P	R	G	S	S	L
The H9N2 gene reads from Huizhou City	F	Q	H	P	R	C	S	S	L

Abbreviations: N=Asparagine; T=Threonine; L=Leucine; M=Methionine; G=Glycine; S=Serine; R=Arginine; K=Lysine; P=Proline; F=Phenylalanine.

\*The mutation sites found in the detected 9 gene reads.

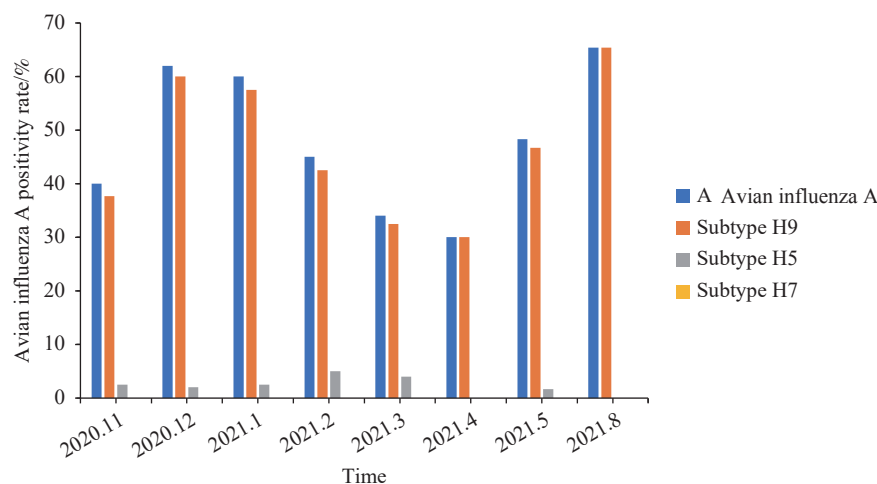


FIGURE 1. The monthly distribution of avian influenza A virus positive rate in Huizhou City.

Note: The subtype H7 AIV was undetected among the monitoring period; The subtype H5 AIV was undetected in April 2021 and August 2021.

had a high positivity rate in poultry markets with subtype H9 being the most common subtype of avian influenza virus in poultry. H9 had a high positivity rate, while other subtypes were relatively rare.

Molecular characteristics analysis suggested that the risk of human infection was not higher than that of previous H9N2 avian influenza viruses. Combined with the epidemiological investigation, clinical symptoms of the patient, and the surveillance of avian influenza in the live poultry market, it is believed that the cause of the disease was mainly associated with exposure to the market that was contaminated by H9N2 avian influenza viruses. Therefore, she was likely infected by inhaling aerosols containing viruses (6).

New cases of human infection with avian influenza have been constantly occurring in China (7). There have also been several outbreaks of human infection with H5N6 and H7N9 avian influenza in Huizhou City (8). It is important to prevent avian influenza outbreaks by understanding the risks of avian influenza virus infection using detailed epidemiological investigation of patients and emergency environmental monitoring. Enhanced environmental monitoring of live poultry market is helpful to fully understand the contamination situation of avian influenza virus in the whole province. Therefore, closing the live poultry market and enhancing publicity and education in a timely manner are essential due to the high positivity rate of H9N2 in environmental samples. This case highlights a weakness of influenza surveillance in humans. Since the reported H9N2 case just had mild clinical symptoms and was found through influenza-like illness (ILI) surveillance, it is recommended that routine ILI surveillance be improved by increasing the sample number and frequency of sampling. It is also important to carry out surveillance in the population who work in the live poultry markets and poultry farms.

The findings in this report were subject to some limitations. First, the viral load of the sample was fairly low, so 8 gene sequences of H9N2 could not be obtained. Second, although emergency sampling of

environment was conducted, bird populations in the live poultry market were not stable, so infected poultry may have been missed. Third, environment samples in Yunnan Province could not be obtained, so the relationship between travel history and H9N2 infection could not be excluded.

**Conflicts of interest:** No conflicts of interest declared.

doi: 10.46234/ccdcw2021.273

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Submitted: October 10, 2021; Accepted: November 30, 2021

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## 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<sup>®</sup> III One-Step RT-PCR System with Platinum Taq High Fidelity (Thermo Fisher, Carlsbad, USA). The library was performed with Illumina Nextera<sup>®</sup> 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](http://www.gisaid.org)) and NCBI ([www.ncbi.nlm.nih.gov](http://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

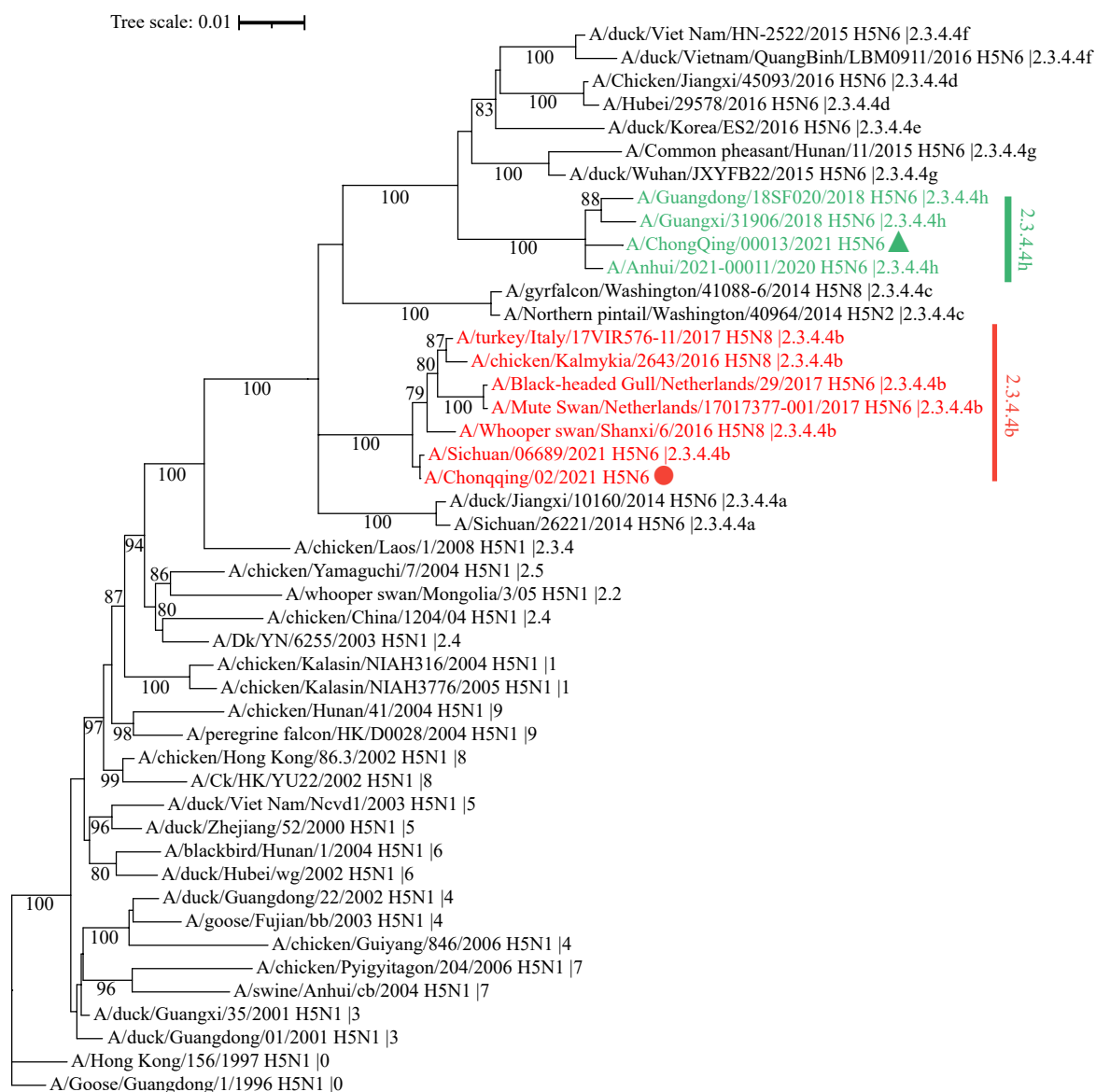


FIGURE 1. Neighbor-joining phylogenetic relationships of H5 viruses' hemagglutinin (HA) genes.

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.

**Acknowledgements:** Staff members at Tongnan, Rongchang, Yongchuan, and Tongliang CDC and the authors, originating, and submitting laboratories of the influenza A(H5N6) virus sequences from GISAID's EpiFlu Database and NIH's Genbank used in the phylogenetic analysis.

**Funding:** The National Subsidy Program of prevention of human infection with avian influenza A virus and grants (2017MSXM003) from Chongqing Municipal Commission of Health and Family Planning, and Chongqing Municipal Science and Technology Commission and Health Commission Joint Medical Research Project: 2020FYYX080, 2020FYYX120.

doi: 10.46234/ccdcw2021.278

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Submitted: October 21, 2021; Accepted: December 08, 2021

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The inauguration of *China CDC Weekly* is in part supported by Project for Enhancing International Impact of China STM Journals Category D (PIIJ2-D-04-(2018)) of China Association for Science and Technology (CAST).



*Vol. 4 No. 1 Jan. 7, 2022*

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**Responsible Authority**

National Health Commission of the People's Republic of China

**Sponsor**

Chinese Center for Disease Control and Prevention

**Editing and Publishing**

China CDC Weekly Editorial Office

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Tel: 86-10-63150501, 63150701

Email: [weekly@chinacdc.cn](mailto:weekly@chinacdc.cn)

**CSSN**

ISSN 2096-7071

CN 10-1629/R1