Int J Clin Exp Med 2017;10(11):15588-15598
www.ijcem.com /ISSN:1940-5901/IJCEM0056775

Case Report

Clinical, epidemiological and virological characteristics of the first detected human infection with avian influenza A (H5N6) virus in Anhui Province, China

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Received May 4, 2017; Accepted September 22, 2017; Epub November 15, 2017; Published November 30, 2017

Abstract: After the first detected human infection case of avian influenza A (H5N6) in 2014, 17 cases have been detected to date. The case was the first human case infection with avian influenza A (H5N6) in Anhui Province. This case was onset of influenza-like illness and the throat swab specimens were tested for H5N6 subtype virus infection. The case was with severe bacterial infection, liver and heart damage and the respiratory function deteriorated during the hospitalization. On May 16 died of respiratory failure. The case had a clear history of poultry contact, and the source of infection might be live-poultry market. No human-to-human transmission occurred. The phylogenetic and molecular characteristics analysis showed that the HA gene of AH33162/2016 shared 98% nt identity with A/duck/Guangdong/GD01/2014 (H5N6) (KJ754145) and belonged to clade 2.3.4.4 H5 viruses, while the NA genes shared 98% nt identity with A/chicken/Zhejiang/727022/2014 (H5N6) (KU042822). All of 17 cases in China mainland are sporadic without epidemiological association. Although this novel avian influenza A (H5N6) virus could be low pathogenic in human, its prevalence and genetic evolution should be monitored closely.

Keywords: Avian influenza, epidemiology, clinic, virus

Introduction

Avian influenza is a zoonotic, highly contagious, and worldwide-distributed disease, and it has posed significant risks to public health in recent years. The genome of avian influenza viruses (AIVs) is a segmented, negative-strain RNA that belongs to the Orthomyxoviridae family [1]. On the basis of the external proteins hemagglutinin (HA) and neuraminidase (NA), AIVs can be divided into several subtypes, including various combinations of 18 HAs (H1-H18) and 11 NAs (N1-N11) [2, 3]. Human infection cases of the subtypes H5N1, H5N2, H7N3, H7N7, H7N9, H9N2, H10N7, H10N8, and H5N6 have been reported previously [4-13]. The clinical manifestations of human cases vary with different subtypes of AIVs; common clinical manifestations include fever, cough, conjunctivitis, and other upper respiratory infection symptoms. Some subtypes of viruses can rapidly cause the patients to develop pneumonia, acute respiratory distress syndrome (ARDS), organ failure, and even death.

In May 2014, the National Health and Family Planning Commission (NHFPC) of China announced the world's first detected human case of infection with avian influenza A (H5N6) in Yunnan Province. Till March 15th, 2017, a total of 17 cases of avian influenza A (H5N6) had been reported in mainland China. The 17 cases were distributed in eight provinces: seven cases in Guangdong; three cases in Hunan; two cases in Yunnan; and one case each in Jiangxi, Sichuan, Guangxi, Hubei, and Anhui. Therefore, the occurrence of human infection with avian influenza virus (H5N6) should gain a high level of concern in the field of public health.

At 2:00 am on May 28th, 2016, the National Laboratory for Influenza Surveillance at the

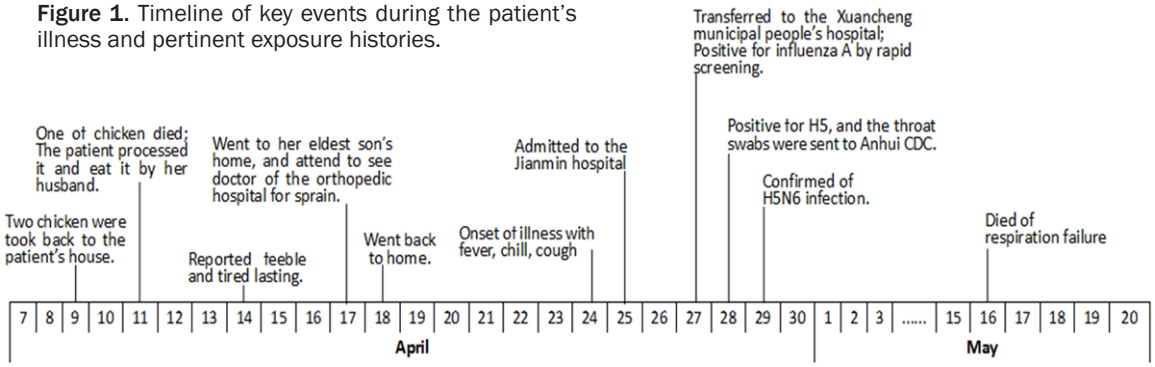


Figure 1. Timeline of key events during the patient's illness and pertinent exposure histories.

Xuancheng Center for Disease Control and Prevention (CDC) detected AIV in a throat swab specimen that had been collected by a sentinel hospital of the China Influenza Surveillance System. The sample was positive for H5 but negative for N1. The specimen was then tested for H5N6 subtype by the Anhui CDC, with a positive result; the result was confirmed by the National Influenza Center of the China CDC. This patient was the first human case of infection with avian influenza A (H5N6) in Anhui Province and the tenth such case in China. This article will report the clinical, epidemiological, and virological results of this H5N6 infected case, as well as the H5N6 virus gene sequence characteristics.

Material and methods

Clinical and epidemiological data collection

A standardized questionnaire designed by the China CDC was used to collect the following clinical and epidemiological data: demographic characteristics; past medical history; exposure to poultry, birds, or other animals; exposure to live-poultry markets; clinical symptoms; laboratory testing results; antiviral treatment; clinical testing, treatment, and outcomes.

Close contacts were defined as individuals who had provided care to, had been living with, or had potentially or directly been exposed to respiratory secretions or body fluids of the patient during the 14 days before the onset of illness in the patient.

Viral analysis

Each throat swab specimens of the patient were collected, injected into nine-day-old specific-pathogen-free (SPF) chicken eggs, and propagated in the eggs for 72 hours at 37°C. According to the manufacturer's instructions, the QIAamp Viral RNA Mini Kit (Qiagen, Germany) was used to extract the viral RNA from the allantoic fluid of the inoculated eggs. This virus was named A/Anhui/33162/2016 (H5N6). Specific real-time RT-PCR or conventional RT-PCR assays can be used for detecting seasonal influenza virus (H1, H3, or B) and avian influenza viruses (H5, H7, H9, H10, and N1-N9 subtypes) [14].

The full genome of the virus was amplified using the Qiagen OneStep RT-PCR Kit for sequencing (Germany). PCR products were purified from agarose gel using the QIAquick Gel Extraction Kit (Qiagen, Germany). All sequencing processes were performed by the National Influenza Center of the China CDC. Phylogenetic trees were constructed using Molecular Evolutionary Genetics Analysis (MEGA 6), utilizing the neighbor-joining method to calculate distance. Bootstrap values were estimated for 1,000 replicates.

Ethics considerations

Data collection from this patient was part of a routine surveillance and outbreak investigation and was therefore exempt from oversight by the institutional review board (IRB). The IRB of Anhui CDC approved the assessment of close contacts; written consent was obtained from each close contact.

Results

Exposure survey

The patient was a 65-year-old female farmer, living with her husband in an independent house located beside the mountain in the

Wanjia village of Xuancheng city. She had suffered from diabetes and hypertension for about four years. She raised local chickens in the courtyard and planted tea tree on the mountain before the onset of illness. The patient had two sons and two daughters, all living in Xuancheng city and coming back home occasionally. On April 9th, 2016, the patient's second son came home to visit and brought two black-bone chickens that had been bought from the Nanmen live poultry market in Xuancheng city. The two black-bone chickens were kept separate from the local chickens, and the patient took care of them every day, including feeding and cleaning feces without individual protection. On April 11th one of the black-bone chickens died, and then the patient cleaned and cooked it by herself. Her husband ate the chicken, but she didn't. On April 17th she went to her eldest son's home and to the Ningguo Osteopathic Hospital for an ankle sprain in the afternoon. On April 18th she came back home to the village (**Figure 1**). From April 17th to 18th, she didn't eat poultry or eggs and didn't have any live-poultry or live-poultry market exposure. She had no other travelling history in April and denied contact with any other similar cases of fever.

Clinical history

On April 14th the patient experienced fatigue and felt tired, but she didn't take her temperature or receive any treatment. At 4:00 pm on April 24th she began to develop influenza-like symptoms, such as fever, chills, cough, expectoration, and muscular soreness. On April 25th she went to Jianmin Hospital of Ningguo city and was admitted with a diagnosis of fever of unknown origin and diabetes mellitus type 2. The computed tomography (CT) chest scan showed the right lung with a little moist rale. She was treated with cefoxitin sodium and amikacin sulfate, but the symptoms did not improve. On April 27th the CT chest scan showed high density in both lungs and bilateral pleural effusion; the doctor advised that she should be transferred for better treatment. At 10:00 am that same day, the patient was transferred to Ningguo People's Hospital and was admitted to the Department of Respiratory Medicine with the following symptoms and signs: temperature of 38.7°C, pulse of 90 beats per minute, respiration of 20 breaths per minute, blood pressure of 144/66 mmHg, and both lungs with moist rale. A rapid test for the influenza A antigen indicated a positive result. Ceftriaxone, azithromycin, and oseltamivir were used for anti-infective and antiviral therapy. Ningguo People's Hospital immediately organized a consultation about the case, and the panel concluded that this patient had viral pneumonia with the possibility of an avian influenza infection and that the patient should be given further pathogen detection. The result of blood routine test and biochemical indices are summarized in **Table 1** and **Table 2**. At the same time, the patient should be transferred to the Intensive Care Unit (ICU) for treatment. The throat swab specimen of the patient was then collected and sent to the National Laboratory for Influenza Surveillance at the Xuancheng CDC. There, the sample tested positive for H5 but negative for N1 on April 28th, and the specimen was sent to the Anhui CDC. The clinical outcomes of this patient included a temperature of 37.0°C, ecphysesis, decreasing SpO_2, and acute respiratory distress syndrome (ARDS), the result of blood gas analysis result are summarized in **Table 3**. A tracheal cannula treatment was given for improvement of oxygenation. On April 29th at the Anhui CDC, the patient's throat swab specimen tested positive for H5N6 subtype viral infection, and the result was confirmed by the China CDC on April 30th. On May 2nd, the CT chest scan showed increasing double lung markings, multiple patchy shadows with high density, and a lesion with unclear margins and uneven density, showing partial lesion absorption compared to CT results from April 27th. The chest CT conducted on May 6th showed that lesions had developed slightly in comparison to the previous scans. On May 16th, 2016, the patient died of respiratory failure.

Environment survey

Thirteen specimens from the live poultry market where the two black-bone chickens were bought and 11 specimens from the patient's house were collected. Xuancheng CDC testing showed that two of the market specimens were positive for both H5N6 and H9N2 subtypes, while seven of the other market specimens were positive for only the H9N2 subtype. The specimens from the patient's house were all negative, including the local chickens raised by the patient.

Close contact monitoring

According to the definition of close contacts, five of the patient's family members, 36 medi-

Table 1. The main blood routine test result of the patient

Items	Reference Value	Unity	April				May												
			27th	28th	29th	30th	1th	2th	3th	4th	5th	6th	7th	8th	9th	10th	11th	13th	15th
WBC counts	4-10	10^9/L	4.80	4.40	5.60	5.40	7.80	11.40	13.70	14.60	22	24.10	20.30	19.40	11.30	10.60	12.60	13.80	22.60
RBC counts	3.5-5	10^{12}/L	3.65	3.57	3.18	3.03	3.21	3.17	3.23	2.93	3.48	3.07	3.26	3.01	2.49	3.24	3.08	3.47	3.53
Hemoglobin	110-150	g/L	100	95	84	81	86	81	83	80	89	80	86	89	66	91	81	95	99
Hematocrit	35-45	%	32.20	30.30	26.80	26.10	27.50	27.40	27.20	25.70	29.30	28	29.20	26.70	22.70	30.50	27.70	34.30	37.8
PLT counts	100-300	10^9/L	136	136	158	169	235	199	162	161	189	154	118	102	79	67	68	84	106
NEU counts	2-7.7	10^9/L	4.50	4.10	5.20	4.90	6.60	10.20	12.70	14	20.80	23.10	19.30	19.10	10.60	9.60	11.40	12.80	20.60
Lymphocyte counts	0.8-4	10^9/L	0.30	0.20	0.30	0.30	0.30	0.50	0.40	0.40	0.50	0.60	0.70	0.20	0.40	0.60	0.70	0.60	1.30
Monocytes counts	0.12-0.8	10^9/L	0	0.10	0.10	0.20	0.70	0.70	0.60	0.10	0.70	0.40	0.20	0	0.10	0.10	0.20	0.10	0.30
Eosinophils counts	0.05-0.5	10^9/L	0	0	0	0.01	0.02	0	0.01	0.03	0	0.02	0.08	0.04	0.12	0.32	0.26	0.32	0.43
Basophils counts	0-0.1	10^9/L	0	0	0.02	0.02	0.09	0.06	0.04	0.01	0.03	0.02	0.02	0.02	0.01	0.02	0.04	0.03	0.05
Neutrophil ratio	50-70	%	93	92.70	92.90	90.70	85.70	89.10	92.50	96	94.80	95.60	95	98.30	94	90.20	90.50	92.70	91.10
Lymphocyte ratio	20-40	%	6.10	4.80	4.50	5.20	4.10	4.30	2.90	3	2	2.40	3.50	1.20	3.90	5.70	5.70	4	5.70
Monocytes ratio	3-10	%	0.70	2.40	2.30	3.60	8.90	6.10	4.20	0.70	3	1.80	1	0.20	0.90	0.90	1.50	0.80	-
Eosinophils ratio	0-5	%	0.10	0	0	0.10	0.20	0	0.10	0.20	0	0.10	0.40	0.20	1.10	3	2	2.30	-
Basophils ratio	0-1	%	0.10	0.10	0.30	0.40	1.10	0.50	0.30	0.10	0.20	0.10	0.10	0.10	0.10	0.20	0.30	0.20	-
Platelet distribution width	15-17	%	16.50	16.30	16.40	16.50	16.30	16.20	16.20	16.20	16.40	16.50	16.50	17	17.40	17.60	16.40	17.50	-
Mean platelet volume	6-14	fL	8.90	10.90	10.90	9.90	8.90	9	9.20	8.10	9.80	7.90	8.10	8.50	8.80	8	9.80	9.70	-

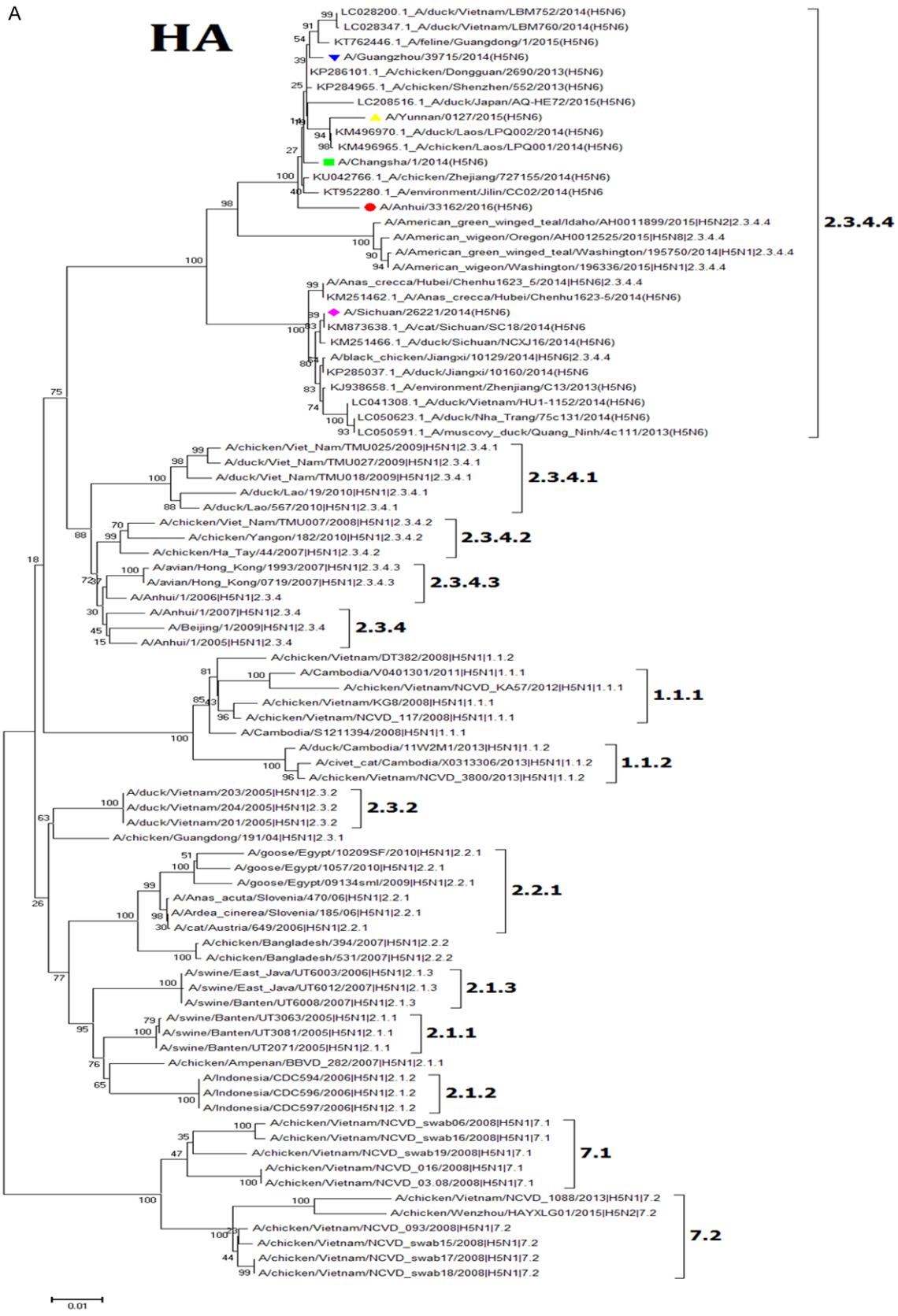
Table 2. Summary of the result of related blood biochemical indices

Items	Reference Value	Unity	April				May											
			27th	28th	29th	30th	1th	2th	3th	5th	6th	7th	8th	9th	10th	11th	13th	15th
Total bilirubin	0-20	umol/L	-	11.50	-	5.20	-	5.80	11.90	15.10	-	-	-	15.80	-	20.30	12.50	-
Direct bilirubin	0-11	umol/L	-	3.80	-	2.40	-	1.40	4.30	5.10	-	-	-	5.90	-	8.50	5.70	-
Indirect bilirubin	0-10	umol/L	-	7.70	-	2.80	-	4.40	7.60	10	-	-	-	9.90	-	11.80	6.80	-
Glutamic-pyruvic transaminase	0-40	U/L	-	32.70	72.4	42.70	-	58.40	56.80	356.50	-	-	-	102.20	-	66.70	36.60	-
Glutamic oxalacetic transaminase	0-40	U/L	-	62.90	-	78.60	-	70.20	87.60	230.20	-	-	-	40.20	-	43.30	26.10	-
Lactate dehydrogenase	109-245	U/L	-	596.10	606.3	606.50	-	512.50	694.20	742.10	-	-	-	730	-	689.70	646.80	-
Total protein	65-80	g/L	-	61.80	-	65.50	-	74.40	72.70	66.30	-	-	-	60.90	-	64.80	63.30	-
Albumin	35-55	g/L	-	31.40	-	27	-	29.50	30.50	31.70	-	-	-	34	-	35.50	31.50	-
Globulin	20-35	g/L	-	30.40	-	38.50	-	44.90	42.20	34.60	-	-	-	26.90	-	29.30	31.80	-
Creatinine	40-84	umol/L	-	80.30	-	86.60	-	66.70	49.80	62.60	92.70	-	27.00	-	-	70.60	65.10	110.60
Urea	2.8-8.2	mmol/L	7.50	7.60	-	10.50	8.50	9.90	9.80	17.10	21.50	-	13.90	-	-	18.10	16.20	23.50
Creatine kinase	25-200	U/L	-	329.85	314.98	924.55	-	458.05	347.62	295.53	-	-	-	88.17	-	-	134.64	-
Creatine kinase isoenzyme	0-28	U/L	-	28.50	18.10	24.90	-	24.20	30.60	24.50	-	-	11.20	32.20	-	-	20.00	-
Alpha-hydroxybutyric acid	70-180	U/L	-	389.10	390.90	402.80	-	366.40	479.50	459.80	-	-	-	485.10	-	-	432.10	-
C-reaction protein	0-8.2	mg/L	150.70	140.90	134.30	103.20	53.12	59.50	44.20	83.60	-	-	133.80	129.60	83.06	94.20	151.10	144.71
Procalcitonin	0.5-2.0	ng/ml	0.18	-	-	1.03	-	0.378	0.195	0.430	-	0.2	-	-	-	1.10	1.67	-
Prothrombin time	9-13	S	-	-	-	11.70	-	12.30	12.60	12.10	-	13.80	16.40	-	-	-	14.10	-
Activated partial thromboplastin time	23-40	S	-	-	-	33.30	-	29.40	29.80	19.10	-	22.86	22.32	-	-	-	29.60	-
pro-BNP	0-125	pg/ml	-	-	-	708.50	-	7443	5973	2893	-	-	2200	-	-	-		-

Table 3. Summary of the results of the blood gas analysis

Items	Reference Value	Unity	April						May														
			27th		28th		29th	30th	1th	3th	4th	5th	6th	7th	8th		9th	11th	12th	13th	14th	15th	
			10:16	22:32	9:10	22:58	9:03	9:07	8:53	8:25	9:05	8:46	8:40	8:52	8:41	9:54	8:30	8:48	8:55	9:40	9;03	9:01	11:08
pH	7.35-7.45		7.47	7.420	7.53	7.37	7.38	7.36	7.37	7.53	7.48	7.56	7.37	7.55	7.51	7.63	7.43	7.42	7.53	7.39	7.35	6.90	7.11
PCO_2	32.0-48.0	mmHg	30	28.60	26.10	32.10	34.70	35.20	37.70	31.10	35.90	32	47.30	32.70	44.70	35.50	46.40	53.40	44.10	59.90	57.40	103.70	71.60
PO_2	83.0-108.0	mmHg	42.70	53	48	59	56	45	43	41	48	62	66	109	27	40	54	68	42	60	48	53	38
Lac	1.0-1.4	mmol/L	-	-	1.21	1.59	2.57	1.70	1.13	4.20	2.60	3.24	2.05	3.62	1.80	1.99	2.12	3.65	2.01	2.11	-	9.34	7.56
BE-ecf	-3-+3	mmol/L	-1.40	-1	-1	-6	-5	-5	-3	4	3	6	2	6	13	16	7	10	14	11	6	-13	-7
HCO_3	18-24	mmol/L	21.50	21.50	21.70	18.70	20.40	20	21.80	26.20	26.50	28.70	27.10	28.50	35.90	37.10	30.80	34.80	36.90	36.20	32	20.10	22.60
TCO_2	18-24	mmol/L	-	19	23	20	21	21	23	27	28	30	29	29	37	38	32	36	38	38	34	23	25
sO_2			-	88%	89%	90%	86%	80%	77%	83%	86%	94%	92%	99%	56%	85%	88%	93%	83%	89%	81%	59%	53%

pH: Potential of Hydrogen; PCO2: Partial pressure of arterial carbon dioxygen; PO2: Partial pressure of arterial oxygen; Lac: Lactic acid; BE-ecf: Base excess extracellular fluid; HCO3: Calculated bicarbonate concentration; TCO2: Total carbon dioxide Content; SO2: Arterial oxygen saturation.


A
HA
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LC028347.1_A/duck/Vietnam/LBM760/2014(H5N6)
KT762446.1_A/feline/Guangdong/1/2015(H5N6)
A/Guangzhou/39715/2014(H5N6)
KP286101.1_A/chicken/Dongguan/2690/2013(H5N6)
KP284965.1_A/chicken/Shenzhen/552/2013(H5N6)
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A/Yunnan/0127/2015(H5N6)
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KM496965.1_A/chicken/Laos/LPQ001/2014(H5N6)
A/Changsha/1/2014(H5N6)
KU042766.1_A/chicken/Zhejiang/727155/2014(H5N6)
KT952280.1_A/environment/Jilin/CC02/2014(H5N6
A/Anhui/33162/2016(H5N6)
A/American_green_winged_teal/Idaho/AH0011899/2015|H5N2|2.3.4.4
A/American_wigeon/Oregon/AH0012525/2015|H5N8|2.3.4.4
A/American_green_winged_teal/Washington/195750/2014|H5N1|2.3.4.4
A/American_wigeon/Washington/196336/2015|H5N1|2.3.4.4
2.3.4.4
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LC050591.1_A/muscovy_duck/Quang_Ninh/4c111/2013(H5N6)
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2.3.4.1
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A/avian/Hong_Kong/0719/2007|H5N1|2.3.4.3
A/Anhui/1/2006|H5N1|2.3.4
2.3.4.3
A/Anhui/1/2007|H5N1|2.3.4
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A/Anhui/1/2005|H5N1|2.3.4
2.3.4
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A/Cambodia/V0401301/2011|H5N1|1.1.1
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A/chicken/Vietnam/KG8/2008|H5N1|1.1.1
A/chicken/Vietnam/NCVD_117/2008|H5N1|1.1.1
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A/cat/Austria/649/2006|H5N1|2.2.1
2.2.1
A/chicken/Bangladesh/394/2007|H5N1|2.2.2
A/chicken/Bangladesh/531/2007|H5N1|2.2.2
A/swine/East_Java/UT6003/2006|H5N1|2.1.3
A/swine/East_Java/UT6012/2007|H5N1|2.1.3
A/swine/Banten/UT6008/2007|H5N1|2.1.3
2.1.3
A/swine/Banten/UT3063/2005|H5N1|2.1.1
A/swine/Banten/UT3081/2005|H5N1|2.1.1
A/swine/Banten/UT2071/2005|H5N1|2.1.1
2.1.1
A/chicken/Ampenan/BBVD_282/2007|H5N1|2.1.1
A/Indonesia/CDC594/2006|H5N1|2.1.2
A/Indonesia/CDC596/2006|H5N1|2.1.2
A/Indonesia/CDC597/2006|H5N1|2.1.2
2.1.2
A/chicken/Vietnam/NCVD_swab06/2008|H5N1|7.1
A/chicken/Vietnam/NCVD_swab16/2008|H5N1|7.1
A/chicken/Vietnam/NCVD_swab19/2008|H5N1|7.1
A/chicken/Vietnam/NCVD_016/2008|H5N1|7.1
A/chicken/Vietnam/NCVD_03.08/2008|H5N1|7.1
7.1
A/chicken/Vietnam/NCVD_1088/2013|H5N1|7.2
A/chicken/Wenzhou/HAYXLG01/2015|H5N2|7.2
A/chicken/Vietnam/NCVD_093/2008|H5N1|7.2
A/chicken/Vietnam/NCVD_swab15/2008|H5N1|7.2
A/chicken/Vietnam/NCVD_swab17/2008|H5N1|7.2
A/chicken/Vietnam/NCVD_swab18/2008|H5N1|7.2
7.2
0.01

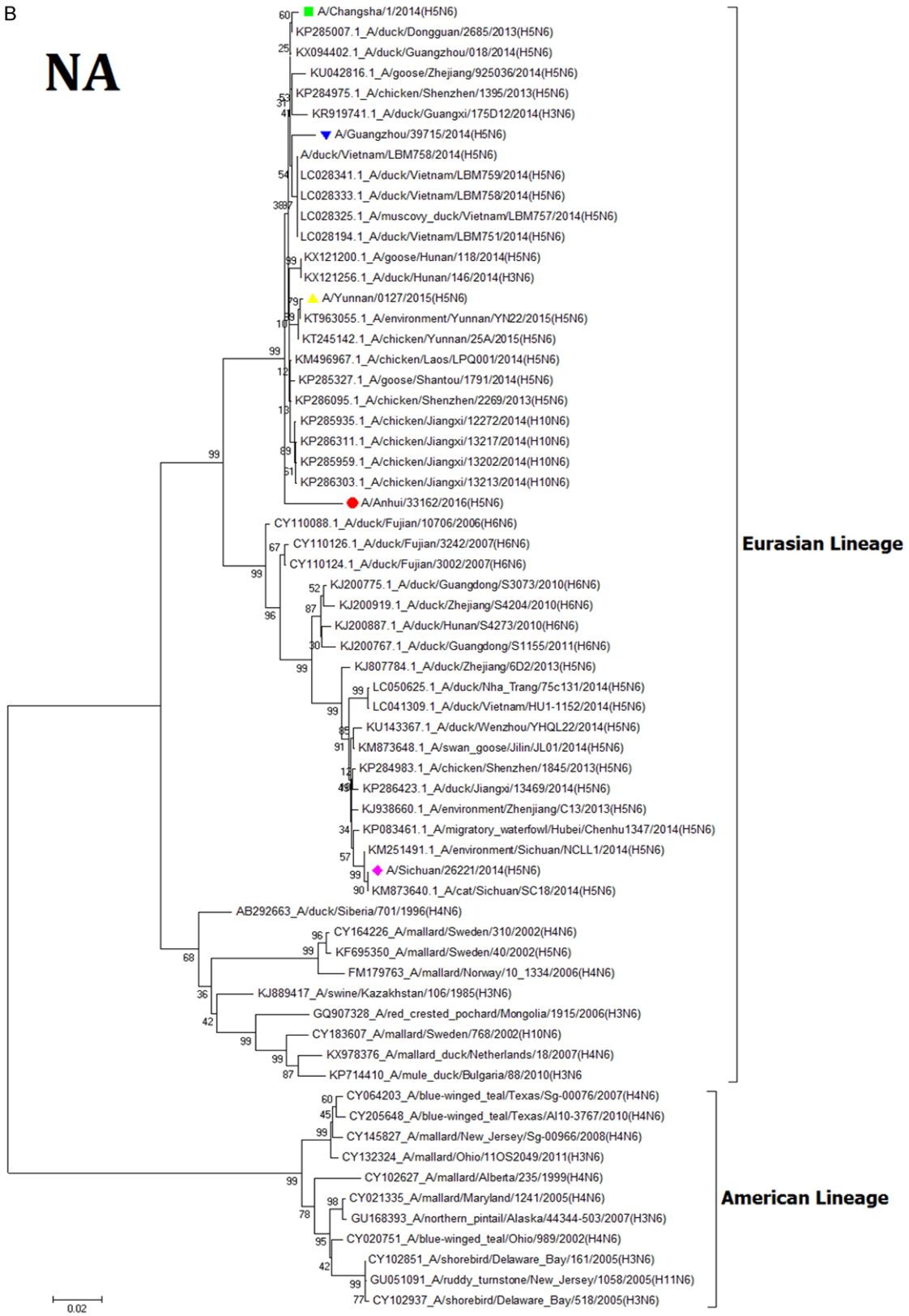

Figure 2. A. Phylogenetic tree of the hemagglutinin (HA) genes of influenza A (H5N6) virus isolated from a patient in Xuancheng, China in 2016. B. Phylogenetic tree of the neuraminidase (NA) genes of the novel influenza A (H5N6) virus isolated from a patient in Xuancheng, China in 2016.

cal staff at the hospitals the patient visited, and two other patients in the same ward were defined as close contacts. Following a two-week medical observation, no signs of influenza-like illness were recorded, and real-time RT-PCR testing showed that 35 throat swab specimens were negative for influenza virus.

Phylogenetic and molecular characteristics analysis

The samples of the patient were positive for H5N6 subtype. Multiple-sequence alignment showed that the HA gene of AH33162/2016 shared 98% nt identity with A/duck/Guangdong/GD01/2014 (H5N6) (KJ754145), and NA genes shared 98% nt identity with A/chicken/Zhejiang/727022/2014 (H5N6) (KU042822). **Figure 2** shows the phylogenetic tree of the HA and NA genes of this influenza A (H5N6) virus. The HA gene of AH33162/2016 belonged to clade 2.3.4.4 H5 viruses. The Sichuan 26221/2014 virus mentioned above is also within clade 2.3.4.4 but clusters in a distinct sub-lineage. Notably, the HA genes of the H5N1, H5N2, and H5N8 viruses that were recently detected in five northwestern U.S. states (California, Idaho, Oregon, Utah, and Washington) also belong to this clade (**Figure 2A**), indicating that viruses from this clade are becoming globally widespread. Demonstrating other widespread characteristics, Anhui 33162/2016 virus clustered with a sub-lineage that includes H5N6 isolates from Zhejiang, Guangdong, and Jilin Provinces, China, and from Laos, Vietnam, and Japan. The NA gene of Anhui 33162/2016 belonged to Eurasian lineage viruses, which are closely related to a group of H6N6 viruses circulating in Southern China. Similarly, the HA and NA genes of Anhui 33162/2016, Changsha 1/2014, Yunnan 0127/2015, and Guangzhou 39715/2014 viruses clustered with one sub-lineage, separate from that of the Sichuan 26221/2014 virus strain (**Figure 2B**).

The HA gene segments of the Anhui 331-62/2016 virus strain contained multiple instances of the amino acid arginine "PLRERRRK/GLF" at the cleavage site between HA1 and HA2. The amino acid substitutions Q226L and G228S (H3 numbering) in the HA gene, which are known to enhance binding to mammalian receptors, were not found. An 11-aa deletion at the NA stalk and a deletion of 5 aa residues from positions 80-84 in the nonstructural 1 protein were found in the Anhui 33162/2016 virus strain. The polymerase basic 2 protein E627K mutation was present in the strain. No neuraminidase inhibitor (oseltamivir) resistance markers were present in the NA genes, while the strain contained S31N mutation in M2 proteins, indicating amantadine resistance.

Discussion

Avian influenza viruses circulate in wild bird and poultry flocks, and they occasionally cause human infection [15-17]. In recent years, AIVs of subtypes H5N6, H7N9, and H10N8 have been circulating in China's poultry flocks, and human infection cases have been reported occasionally [18-20]. On May 6th, 2014, in Sichuan Province, China, the world's first case of human infection with H5N6 was reported [21]. Through March 15th, 2017, 17 cases in total have been reported in Guangdong, Hunan, Yunnan, Jiangxi, Sichuan, Guangxi, Hubei, and Anhui Provinces. There are likely a lot more undetected H5N6 cases without severe symptoms, especially in children [22]. It is believed that the virus contains an HA from clade 2.3.4.4 H5 viruses, an NA from H6N6, and 6 internal genes from a clade 2.3.2.1 H5 virus [23-25]. Of the HA, NA, or 6 internal genes, at least one strain of them had derived its 6 internal genes from AIV H9N2 [18]. Influenza A (H5N6) was first isolated from mallards in North America in 1975; influenza viruses continued to evolve and reassort to generate novel and highly pathogenic viruses [26]. The highly pathogenic reassorted H5 AIV subtypes include the H5N2, H5N5, H5N6, and H5N8 viruses, which have spread throughout poultry and wild bird flocks in Europe, North America, and Asia [22-28]. Since 2013, the H5N6 subtype virus has been found in ducks and chickens in mainland China, indicating that the virus is circulating in this area [29, 30]. Therefore epidemiological, virological, and ecological research including host animal monitoring should be strengthened.

The clinical symptoms of the case of human H5N6 infection presented here were similar to other subtypes' infections, such as H7N9 and H10N8. The clinical presentation included fever, cough, sore throat, pneumonia, and acute respiratory distress syndrome (ARDS). Although the patient felt fatigue from an early stage, that

symptom was not consistent with AIV infection. The real time of illness onset was April 24th, when the patient presented with fever, cough, and chills, which were in accordance with the symptoms of influenza-like illness and AIV infection. Before May 2nd, white blood cell counts were below the standard value, which also meets the criterion of AIV infection. The level of c-reaction protein was 5.39 times higher than the normal value, which indicated the existence of serious infection. Although the patient received anti-infective treatment, the infection wasn't controlled very effectively. The patient presented with liver and heart damage. The biochemical indices lab result demonstrated that the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), NT-proBNP, and lactate dehydrogenase (LDH) were increased significantly. The clinical findings in this study are also supported by previous clinical characterization of other human cases of H5N6 [19, 31]. The CT scan showed severe infection in both lungs. Blood gas analysis showed the patient had severe lung dysfunction, and lung function continued to deteriorate for the duration of treatment.

Based on this study's phylogenetic analysis, it appears that a number of influenza A (H5) clade 2.3.4.4 viruses may have emerged in China and subsequently spread both eastward and westward. The genetic characteristics of this H5N6 subtype virus are highly pathogenic for poultry, with increased virulence and replication capacity. The PB2-E627K mutation indicates that the virus has adapted to mammals [32-35]. Molecular characteristics of oseltamivir resistance were not found; however, characteristics of amantadine resistance were present. As this virus has obtained at least partial capacity for mammalian adaptation, the H5N6 virus should be prioritized for continuous virological monitoring.

This case was identified by the National Laboratory for Influenza Surveillance system, suggesting that the system is sensitive and efficient in discovering cases of new avian influenza virus infection. The epidemiological survey found that from the first day of illness onset, the time interval to the day of seeing a doctor was one day; to the day of hospitalization was one day; to the day of using oseltamivir treatment was three days; and to the day of laboratory confirmation was five days. There were four major causes of this patient's infection and fatality. First, the patient slaughtered poultry without any respiratory protection and did not seek medical treatment in a timely manner after illness onset. Second, the hospital lacked awareness of and sensitivity to AIV infection, diagnosing and treating the patient as having fever of unidentified origin and pneumonia after she was admitted to Jianmin Hospital. Third, oseltamivir was given to the patient after illness onset, exceeding the most effective time of drug use. Fourth, the patient's pre-existing conditions might have aggravated the AIV infection.

The epidemiological investigation of this case proved that there was a clear exposure history to poultry, with H5N6 acids detected in the environment where the patient's two black-bone chickens were bought. Therefore, the infective source might be the live poultry market. The incubation period of this patient is estimated at 13-15 days, which exceeds the average incubation period of AIV infection. Assessment of 43 close contacts excluded the possibility of influenza and AIVs infection during medical observation, and there was no evidence of human-to-human transmission. All 17 cases of H5N6 infection that have been reported in mainland China are sporadic cases without epidemiological association, but the possibility of human-to-human transmission should not be ignored. Enhanced active monitoring of sites frequented by aquatic wild birds and waterfowl is also recommended.

Acknowledgements

This study was supported by the fund for the project of the Development and Demonstrating Application of Early Warning and Joint Prevention and Control of Priority Emerging Communicable Diseases in Yangtze River Delta Area of Anhui International Scientific Collaboration Program (1503062008, to Jiabing Wu).

Disclosure of conflict of interest

None.

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