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RESEARCH ARTICLE

The first avian influenza A (H7N9) viral infection in humans in Zhejiang Province, China: a death report

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Abstract **This study reports the first death caused by a novel avian influenza A (H7N9) virus in Zhejiang Province, China. The patient had chronic hepatitis B and history of exposure to poultry. The patient initially complained of diarrhea and influenza-like symptoms on March 7 and 14 respectively. The disease progressed to severe pneumonia, sustained hypoxia, and coagulation abnormalities. The patient died on March 27 because of respiratory failure, multiple organ failure, and disseminated intravascular coagulation without oseltamivir treatment. This H7N9 virus from Zhejiang is highly similar to isolates obtained from Shanghai, Jiangsu, Anhui, etc. Analysis of hemagglutinin, neuramidinase, and matrix genes indicated that the isolates share the same avian origin, have low virulence, and are sensitive to oseltamivir, but are resistant to adamantine. Only the isolate that caused the fatality exhibited substitution of Q226I in the HA gene, which indicates a potentially enhanced human affinity. The secondary transmission rate was 1.6% (2/125). Only two health workers presented with influenza-like symptoms, and they subsequently tested negative for H7N9 RNA. In conclusion, underlying disease, late diagnosis, and untimely antiviral treatment are possible high-risk factors for infections and death caused by the low-pathogenicity avian influenza A (H7N9). Person-to-person transmission of the H7N9 virus was not detected among close contacts, but such transmission should be investigated in the future. Expanding and enhancing surveillance will help in the early discovery and diagnosis of suspected cases, which will reduce the number of severe cases and deaths.**

Keywords avian influenza A (H7N9) virus; epidemiology; contacts; person-to-person transmission

Introduction

An emerging infectious disease that presents with acute high fever, cough, and pink frothy sputum with leukocytopenia was identified in China, from Shanghai, Beijing, Jiangsu, Zhejiang, and Anhui since February 2013 [1,2]. Some of the cases developed severe pneumonia and acute respiratory distress syndrome (ARDS) [3], subsequently requiring intensive care and mechanical ventilation. About 24.43% (32/131) of the patients died of respiratory failure and multiple organ failure (MOF). In March 2013, epidemiologic, clinical, and pathologic investigations were performed that subsequently identified the etiology as a novel re-assortment avian influenza A (H7N9) virus distinct from the previous circulating human influenza A viruses [2,4]. It was the first reported case of a human infected with a low-pathogenicity H7N9 virus that had a fatal outcome.

As of May 16, 2013, 131 laboratory-confirmed cases, including 32 deaths, have been reported in China since the first patient was identified on March 25, 2013 [5]. Zhejiang Province is one of the areas involved, and it is adjacent to Shanghai, Jiangsu, and Anhui. By May 16, 2013, 46 confirmed cases were reported, including 10 deaths, after the first H7N9 case was diagnosed on April 1, 2013 [5]. Sporadic cases were found daily in more areas because of the expanding and enhanced surveillance of influenza/avian influenza, pneumonia, and severe acute respiratory infection since the first H7N9 case was confirmed.

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In the current paper, we describe the discovery and epidemiological, clinical, and virologic characterization of the first fatal case of infection by the novel H7N9 virus in Zhejiang Province. This report is important for assessing and controlling similar cases, and would provide meaningful insights into the early and rapid diagnosis, treatment, prevention, and control of human influenza A (H7N9) viral infections.

Materials and methods

The case and contact definitions were set based on the diagnosis and treatment guidelines (second edition) established by the Chinese Ministry of Health. A confirmed H7N9 case was defined as a suspected case with respiratory specimens positive for H7N9 virus based on isolation of the H7N9 virus or on positive real-time reverse transcription polymerase chain reaction (rRT-PCR) assays for the H7N9 virus. Patients with pneumonia-complicated respiratory failure or other organ failure was defined as severe cases [6].

Contacts were defined as (1) medical staff or relatives of the patient who did not take protective measures upon the diagnosis and treatment of suspected or confirmed cases, or those who took care of the patient; (2) persons who lived with or were in close contact with the suspected or confirmed cases for at least 1 week; (3) the investigators who were also considered close contacts [7].

Study design

We conducted a retrospective descriptive study of the clinical, laboratory, and radiographic characteristics of a confirmed case of avian influenza A (H7N9) viral infection during the study period (April 5, 2013 to April 10, 2013). All available medical records were reviewed by 10 clinicians, using a standardized data collection tool. Epidemiologists and local public health doctors interviewed family members and medical staff using a standard questionnaire. Pharyngeal swabs from the patient and from 46 of the 125 contacts were submitted to Zhejiang CDC and China CDC for testing for H7N9 viral RNA via rRT-PCR assays.

Rapid viral RNA extraction and detection via rRT-PCR

Avian influenza A (H7N9) virus RNA extraction was performed following the instructions of the kit (Qiagen Company), and 200 μl of template was dissolved in double-distilled water, aliquoted into four samples (50 μl each), and stored at –80 °C. rRT-PCR was used to detect the HA, NA, and M genes of seasonal influenza viruses (H1, H3, and B), H5N1, H7N9, severe acute respiratory syndrome coronavirus (SARS-CoV), and novel coronavirus. Their specific primer and probe sets were provided by the China CDC.

Viral culture and isolation

A pharyngeal swab specimen positive for H7N9 viral RNA was further cultured in MDCK cells maintained in Hank's balanced solution at 37 °C for 7 days. The cell cultures were observed for 2 weeks. The subtypes were identified using H7N9 HA and NA type-specific primers when the cells exhibited the characteristic cytopathic effect (CPE). The subtypes were determined via a hemagglutination inhibition test (HAI) assays using type-specific reference antibodies.

Sequence analysis

The nucleotide sequences of the amplified products were directly determined via dideoxy sequencing using an ABI Prism BigDye Terminator cycle sequencing kit. The HA, M, and NA segments were analyzed and compared using Mega 5.05 and DNASTAR. Phylogenetic trees were constructed via the neighbor-joining method to estimate the viral gene relationships with selected influenza A virus strains obtained from the GISAID database (http://platform.gisaid.org/epi3/frontend#575253) and GenBank (http://www.ncbi.nlm.nih.gov/nuccore/?term = H7N9) [2].

Results

Epidemiologic features

On March 7, 2013, a 39-year-old male cook with chronic hepatitis B who works in Suzhou, Jiangsu Province and lives in Hangzhou, Zhejiang Province, China, reported cough and diarrhea. He consulted an outpatient clinic in Hangzhou on March 9 and he was admitted to the Second Hospital of Jiande in Hangzhou on March 18, 2013 for the first time. On admission, he had fever (39.5 °C), cough, and expectoration of bloody sputum, and his chest radiograph showed shadows in the right lower lobes. Blood tests showed a white cell count of 2.33×10^9/L and his C-reactive protein (CRP) was 43.7 mg/L. On March 20, 2013 he was transferred to the First People's Hospital in Xiaoshan in Hangzhou to hospitalization for the second time, because the cefodizime + levofloxacin treatment was ineffective. His second chest radiograph showed diffuse bilateral consolidation with right pleural effusion. He was diagnosed with "acute pneumonia." His condition continued to deteriorate and he developed multiple organ failure (MOF) on March 24 despite oxygen therapy, broad-spectrum antibiotics (ceftriaxone + tazobactam), and corticosteroid (Urbason) treatment. Consequently, he was intubated and ventilated on the same day. On March 27, he died of ARDS, disseminated intravascular coagulation (DIC), and MOF. The swab samples collected on March 24, 2013 and the RNA tested positive for influenza A but excluding seasonal influenza A/H1, H3, H5N1, 2009 pandemic H1N1

and influenza B, SARS-CoV, and HCoV-Erasmus Medical Center under rRT-PCR assays. He was confirmed as an avian influenza A (H7N9) virus case by analyzing eight segments, performed in China CDC on April 1, 2013. The delay in seeking medical care resulted in the patient presenting with severe acute lower respiratory symptoms with several complications upon hospital admission. Thus, he was diagnosed with avian influenza A (H7N9) 5 days after he died and he did not receive antiviral treatment during the clinical course of the disease.

In early March 2013, he worked as a cook in Suzhou, Jiangsu Province and bought meat and poultry every morning from the local live poultry market 2 miles away, and then prepared lunch and dinner for about 30 company staff. The patient had no travel history before onset on March 7. According to the epidemiologic investigation, the patient had 125 close contacts, including 13 relatives, 45 colleagues, 57 doctors and nurses, and 10 patients exposed within the same ward. Among these contacts, one doctor and one nurse developed cough one day after the patient was diagnosed and treated. Both of the symptomatic contacts were negative for H7N9 avian influenza RNA under rRT-PCR assay. The symptoms resolved spontaneously within 2 days. A total of 98 serum and 46 throat swabs samples from close contacts were tested using rRT-PCR on April 2, and none tested positive for H7N9.

The demographic and epidemiologic characteristics of the patient are summarized in Table 1.

Table 1 Demographic, epidemiologic, and virology, clinical characteristics of the first confirmed influenza A (H7N9) case in Zhejiang, China

Characteristics		First confirmed influenza A (H7N9) fatality in Zhejiang
Demographic information	Age (year)	39
	Sex	Male
	Occupation	Cook
	Underlying conditions	Chronic hepatitis B
Epidemiologic information	Area of origin	Jiangsu
	Exposure in chicken market in past 7 days	Yes
	Date of specimen collection	March 24, 2013
	Date of laboratory confirmation of virus	April 1, 2013
	Number of contacts	125
	Contacts with symptoms	2 (1 nurse and 1 doctor)
	Number of contact samples	46 swabs and 98 serum samples (all negative)
Clinical information	Date of illness onset	March 7, 2013
	Date of admission	March 18, 2013 for the first time and March 20, 2013 for the second time
	Admission to ICU	March 25, 2013
	Viral isolation	A/Zhejiang/1/2013 (H7N9)
	Complications	
	Septic shock	No
	Respiratory failure	Yes
	ARDS	Yes
	Acute renal damage	Yes
	Encephalopathy	No
	MOF	Yes
	DIC	Yes
	Secondary infections	No
	Oxygen therapy	NIPPV + mechanical ventilation
	Extracorporeal membrane oxygenation	No
	Continuous renal-replacement therapy	Yes
	Antibiotic therapy	Piperacillin sodium and tazobactam sodium moxifloxacin hydrochloride, imipenem, and cilastatin sodium
	Antiviral agent (oseltamivir)	No
	Glucocorticoid therapy	Yes
	Intravenous immune globulin therapy	Yes
	Length of stay in hospital	7 days
	Date of death	March 27, 2013

Clinical diagnosis and treatment

The initial symptoms reported on March 7 and March 14 were diarrhea and cough respectively, which worsened, and was accompanied by fever (39.5°C) and hemoptysis on March 17. Then patient developed shortness of breath, weakness, poor appetite, cyanosis, coma, and anuria because of progression to severe pneumonia with pleural effusion, and MOF. He had died of ARDS and MOF.

Blood cell account

The laboratory tests show that the patient's white blood cell count decreased to 1.6×10^9 /L during the early stages, which slowly increased to normal levels after treatment, and reached abnormally increased levels in the later stages. The neutrophil count exhibited a similar trend as the white cell count. The lymphocyte and mononuclear cell counts were consistently lower than normal, but the red cell count remained stable at normal levels. See Fig. 1.

Coagulation index

D-dimer remained abnormally high and the platelet counts remain low. Activated partial thromboplastin time (APTT), prothrombin time, and thrombin time were initially normal levels, but became prolonged in the later stage. See Fig. 2.

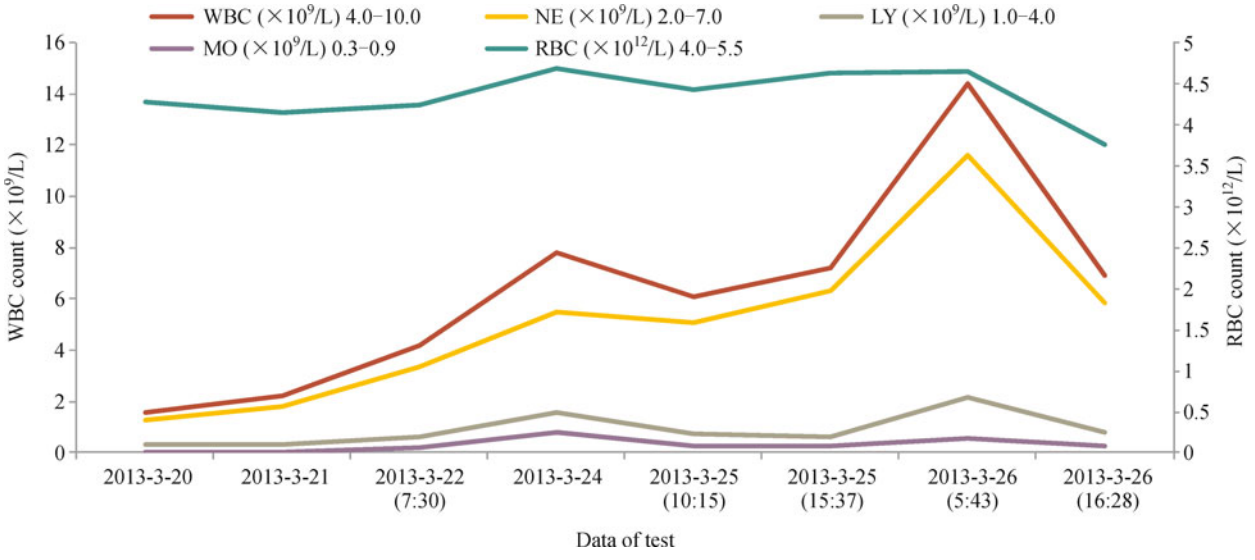

Fig. 1 Blood cell counts of the first avian influenza A (H7N9) fatality. WBC, white blood cell; LY, lymphocyte; NE, neutrophil; RBC, red blood corpuscle; MO, mononuclear cell. Reference ranges for each test are listed.

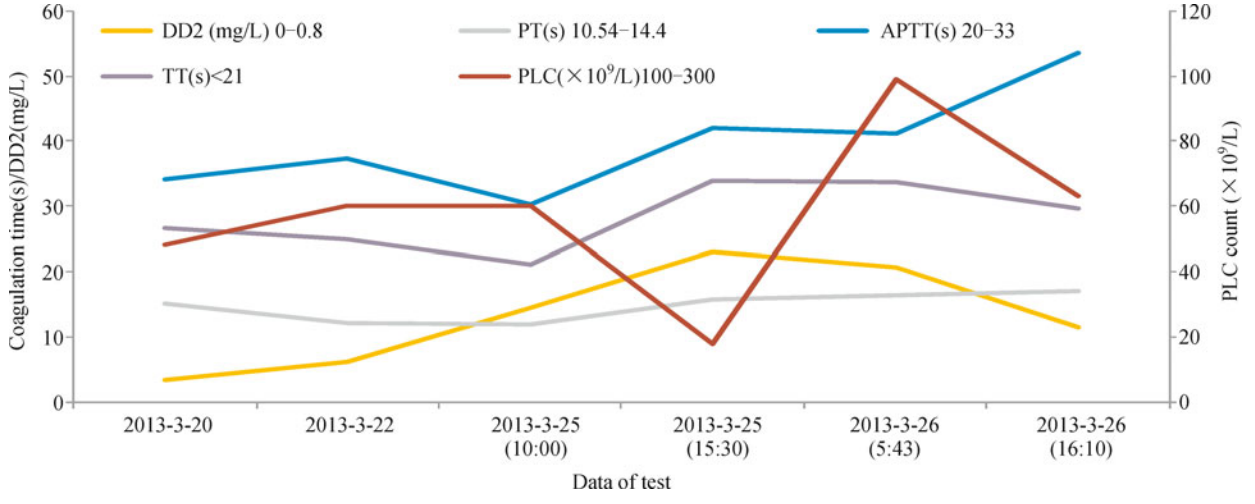

Fig. 2 Coagulation index of the first confirmed avian influenza A (H7N9) fatality. APTT, activated partial thromboplastin time; DD2, D-dimer; PT, prothrombin time; TT, thrombin time; PLC, platelet. Reference ranges for each test are listed.

Arterial blood gas analysis

PO_2 remained below normal levels (10.64 kPa) except on March 24, 17:56, 2013, and the $PaCO_2$ (carbon dioxide tension) remained below normal levels (4.65 kPa to 5.98 kPa), which ranged from 2.8 kPa to 4.2 kPa. In the later stage, the $PaCO_2$ increased slowly, reaching 10.13 kPa in the early stage. HCO_3^- also remained below normal levels (21.4 mmol/L to 27.3 mmol/L). SaO_2 (%) ranged from 63% to 99%. SaO_2 (%) remained far lower than normal values except on March 24, 17:56, 2013. See Fig. 3.

Clinical biomarkers

Liver and heart function were damaged, as indicated by the increased levels of lactate dehydrogenase (LDH), creatine kinase (CK), creatine kinase MB (CK-MB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), direct bilirubin (DB), and total bilirubin (TB). However, kidney function was unchanged, as indicated by normal blood urea nitrogen (BUN) and Cr (creatinine). Blood electrolytes such as Na^+ and K^+ were normal. CRP was persistently increased. These clinical findings are summarized in Table 2.

Pleural ultrasonographic examination

Right pleural effusion with a 2.0 cm fluid sonolucent area was observed on March 23, 2013. The pleural effusion increased to 4.8 cm by March 25, 2013, with the addition of left pleural effusion with a 3.6 cm fluid sonolucent area.

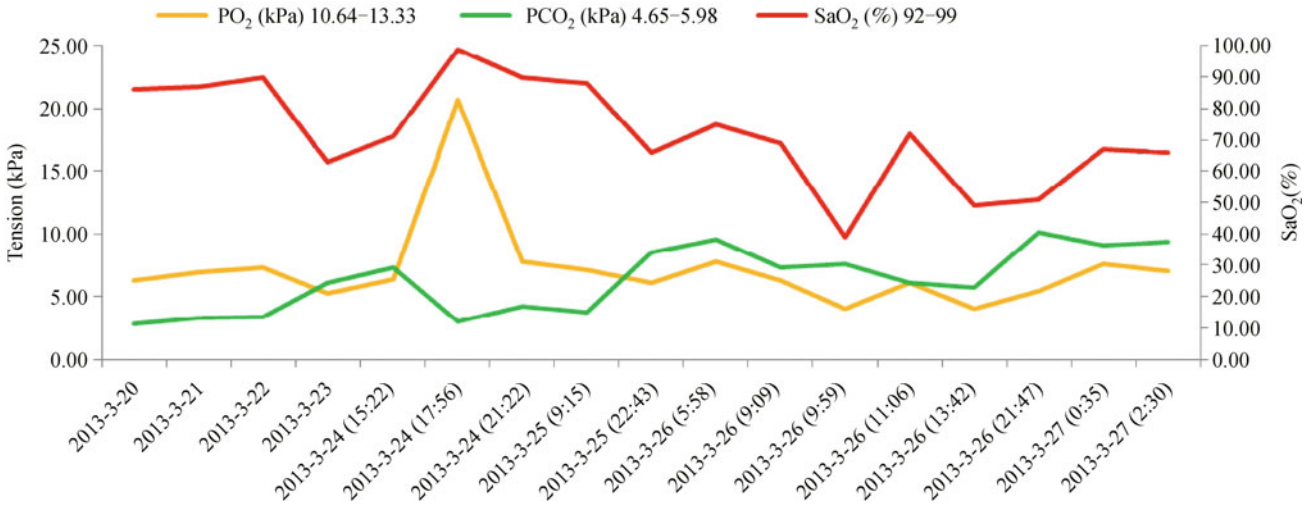

Fig. 3 Arterial blood gas analysis of the first confirmed avian influenza A (H7N9) fatality. PaO_2, arterial oxygen tension; $PaCO_2$, carbon dioxide tension; SaO_2 (%), arterial oxyhemoglobin saturation. Reference ranges for each test are listed.

Table 2 Clinical biochemistry findings of the first confirmed death case infected with avian influenza A (H7N9) virus in Zhejiang, China

Index	Reference value	Date of test						
		2013-3-20	2013-3-22	2013-3-24 (4:58)	2013-3-24 (13:25)	2013-3-25 (10:00)	2013-3-25 (15:38)	2013-3-26
AST (U/L)	0–38	199	204	156	257	244	281	319
ALT (U/L)	0–40		134	98		126		124
LDH (U/L)	135–225	495	608		1088	1018	1140	
CK (U/L)	35–200	2533	1015		396	354	2524	
CK-MB (U/L)	0–25	25	30		42	53	37	
Na^+ (mmol/L)	135–145	139	139	146	147	146	148	140
K^+ (mmol/L)	3.5–5.5	4.00	4.40	5.63	5.47	5.50	4.88	4.80
CRP (mg/L)	0–4		74.90	92.20		58.30		49.80
TB (μmol/L)	5.1–22.2		43.80	41.4		53.30		64.20
DB (μmol/L)	0.5–8.3		37	32.6		43.80		49.10

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; CRP, C-reactive protein; DB, direct bilirubin; LDH, lactate dehydrogenase; TB, total bilirubin.

Chest radiography

Chest radiography showed diffused bilateral consolidation with right lung pleural effusion on March 20, 2013, diffuse bilateral consolidation with bilateral pleural effusion on March 23, 2013, and extensive bilateral exudative lesions on March 25, 2013. Slight absorption was observed in the two lungs on March 26, 2013. See Fig. 4.

Treatments

The patient (temperature, SaO_2) continued to deteriorate and eventually died on March 27, 2013 despite the administration of broad-spectrum antibiotics such as piperacillin sodium and tazobactam sodium, imipenem and cilastatin sodium, moxifloxacin hydrochloride and methylprednisolone sodium succinate (ranging from 40 mg to 240 mg) with intubation and continuous ventilation. See Fig. 5.

Virology

The virus was confirmed using fluorescence quantitative PCR for detecting nucleic acids. The phylogenetic tree revealed that the HA, NA, and MP genes of the first patient from Zhejiang and from Shanghai, Anhui in China each constituted one linkage, with identities of 99.2% to 99.8% (HA), 99.4% to 99.9% (NA), and 99.8% to 99.9% (MP), respectively. However, it only had 40.3% to 77.9% identity with other influenza subtypes and vaccine strains. Phylogenetic analysis of the three genes to verify their avian origin indicated that the gene that encodes HA shares the highest identity with A/duck/Thailand/CU-LM7288C/2010 (H7N6). The gene that encodes NA was most closely related to A/wild bird/Korea/A14/2011 (H7N9). The gene that encodes MP was similar to A/chicken/EI-Fayoum/CA125/2011(H9N2). See Figs. 6–8.

The HA of the Zhejiang strain from the first death had three RB sites (loop 135–138, loop 190–198, and loop 221–228), among which loop 190–198 (EQTKLYGSG) was identical to the HA genes from Shanghai and Anhui, but loop 135–138 (ATSA) was only different from Shanghai 01 strain (ATSS), loop 221–228 (PQVNGISG) was different from the Shanghai 01 strain (PQVNGLSG), Shanghai 02 strain, and Anhui 01 strain, which shares the PQVNGLQSG sequence. The HA cleavage site from the four aforementioned strains possesses only a single amino acid arginine (R), indicating low pathogenic effects in poultry. In addition, all strains shared the same five glycosylation sites (30, NGTK; 46, NATE; 249, NDTV; 421, NWTR; 493, NNTY). The NA amino acid sequence of the patient has no H275Y and R294K substitutions, which indicate that it is sensitive to Tamiflu. Amino acids 69 to 73 were deleted in the stalk region of the NA protein. The M1 protein was identified at virulence sites (N30D and T215A). The M2 protein was found to exit S31N substitution, indicating resistance to adamantanes (amantadine and rimantadine). See Table 3.

The patient swab was inoculated into MDCK cell cultures. The typical CPEs of H7N9 were observed under an inverted microscope: infected cells began to swell, became rounded, and developed larger intercellular spaces. The cells ruptured within 24 h after infection (Fig. 9). The ICID50 of the isolated

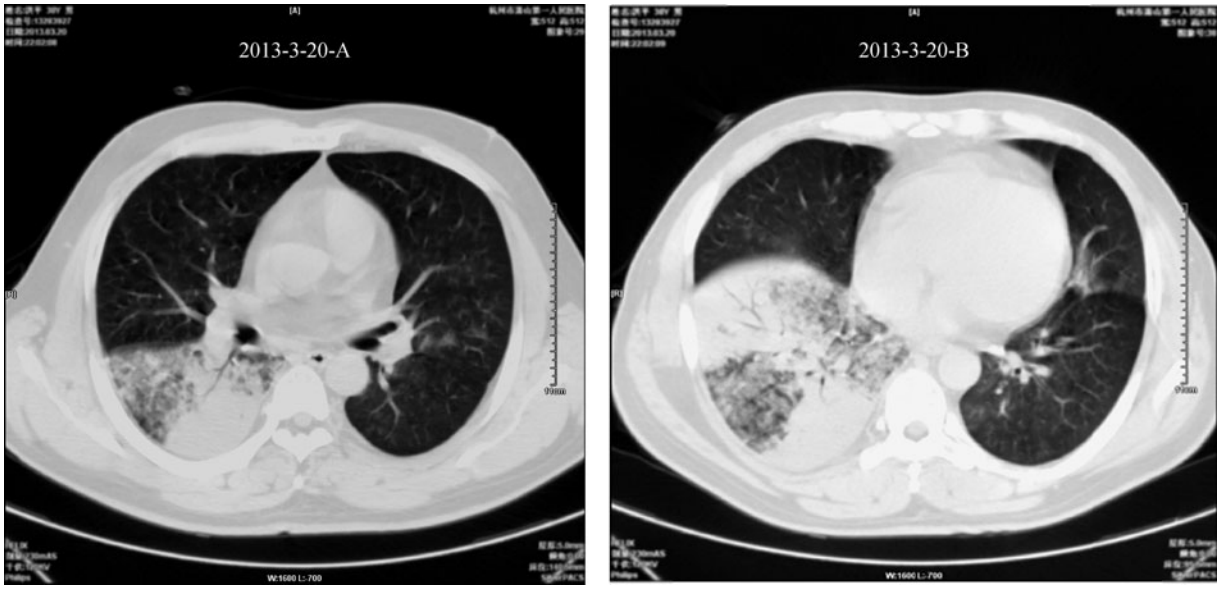

Fig. 4 Chest CT scan images of the first confirmed avian influenza A (H7N9) fatality. 2013-3-20-A: Chest CT scan showed exudation in the dorsal segment of the lower lobe of the right lung; a bronchial air sign was also observed along with patchy consolidation. 2013-3-20-B: Chest CT scan showed large patchy exudation in the basal segment of lower lobe of the right lung.

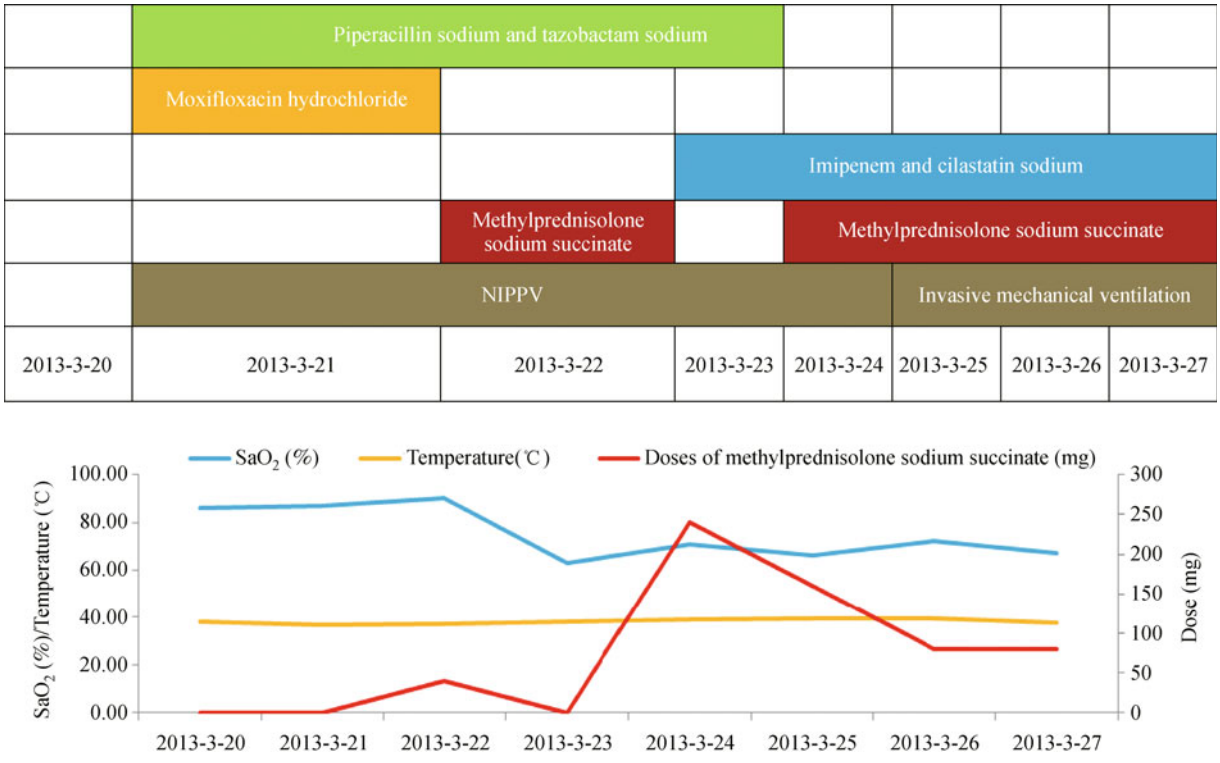

Fig. 5 Timeline of drug administration and dosage for the first confirmed avian influenza A (H7N9) fatality.

virus was between 10^{-4} and 10^{-5}. The presence and subtype was further confirmed as H7N9 via an HAI test and the specific primers used to amplify the H7N9 RNA.

Discussion

Influenza A (H7) virus outbreaks have occurred among avian populations in most European countries. The predominant subtypes circulating in poultry population were H7N1, H7N3, H7N4, H7N7, H7N8, and H7N9 [8]. Human infections with H7 influenza viruses (H7N2, H7N3, and H7N7) were reported in the Netherlands, Italy, Canada, and so on [9–11]. Most of these infections occurred in association with poultry outbreaks. The infections mainly resulted in conjunctivitis, mild upper respiratory symptoms, and moderate illness resulting in hospitalization (lower respiratory tract disease), except for one death [12–14]. Only 100 H7 cases have been confirmed worldwide from 1996 to 2012. The 2013 H7N9 virus reported in China is an avian influenza virus that is the first bird flu subtype (H7N9) found in humans. The novel virus is very different from other H7N9 viruses previously found in birds [1,2].

According to the updated data from 131 cases and 32 deaths, the novel influenza A (H7N9) virus circulating in the urban and rural areas of Beijing and Shanghai, as well as Anhui, Jiangsu, Zhejiang, etc., differs from the avian H5N1 reported in rural areas [15]. The main high-risk population is similar to that of H5N1, which consists of individuals with frequent contact with poultry, those with suppressed immune function, and the elderly with underlying diseases. The initial epidemiology suggests that the confirmed H7N9 cases were isolated, without sustained transmission among people, although two family clusters have been reported, which differs from the limited person-to-person transmission in H5N1 [16]. The source of infection and the mode of transmission are currently unknown. Although some of the confirmed cases were exposed to birds and animals or to outdoor environments, the cases are not associated with disease outbreaks among animals or with direct exposure to animals. About 65% of the confirmed cases had histories of avian exposure, which indicates that contact with live poultry and excreta is a high-risk factor, similar to the H5N1 virus infection in China [17,18]. The confirmed H7N9 cases developed critical and fatal illness, which indicates that the H7N9 virus is more virulent in humans than other H7 viruses and the 2009 H1N1 virus [19]. The current H7N9 case fatality rate is similar to that for reported H5N1 viral infections [20].

The first death was reported in Zhejiang Province, caused by a highly homologous avian H7N9 virus circulating in other areas of China. Phylogenetic analysis showed that this novel virus originated from avian populations. The HA gene sequence from the patient indicated that the virus may be better adapted to infecting mammals than other avian

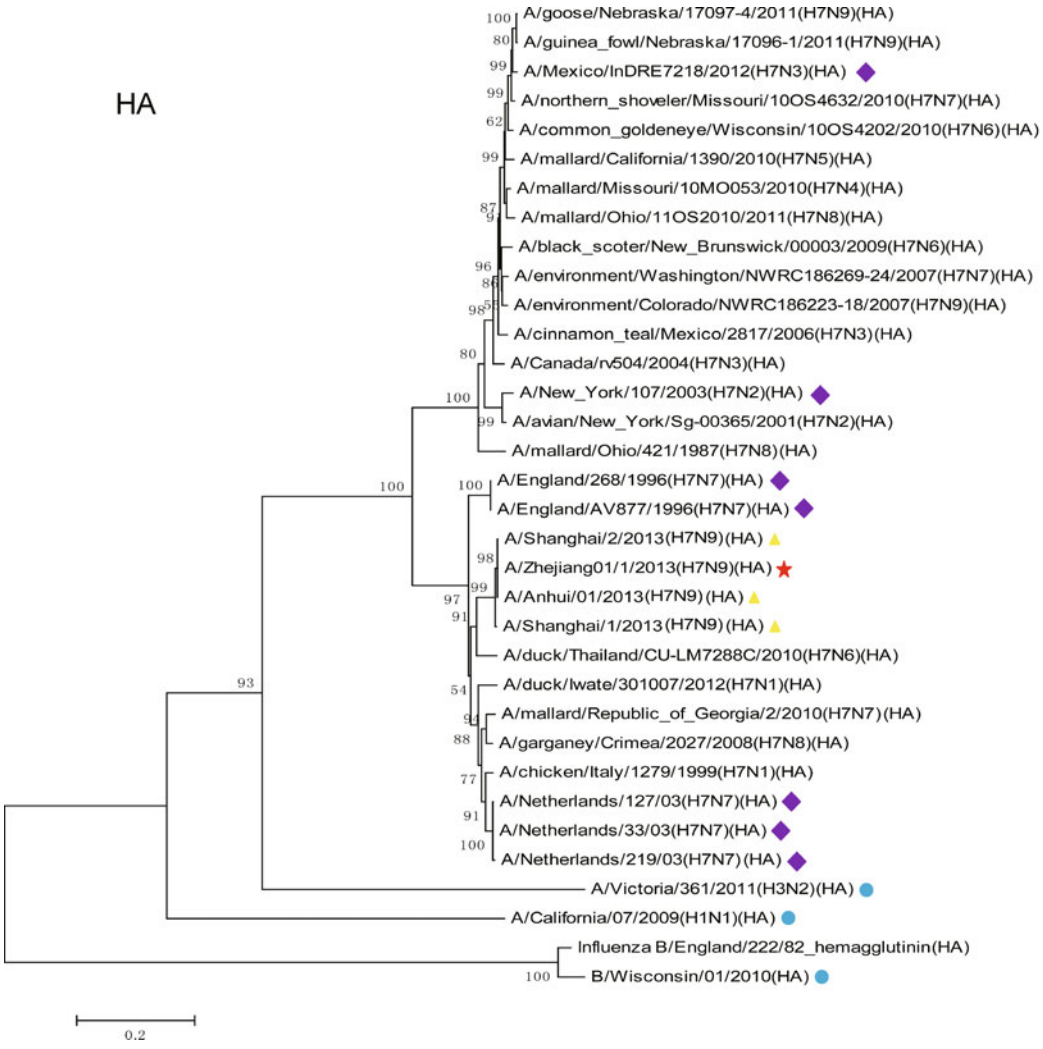

Fig. 6 Phylogenetic tree of NA gene of the first confirmed avian influenza A (H7N9) fatality. ★ : An isolate from the first confirmed H7N9 case in Zhejiang; ▲ : isolates from the confirmed H7N9 patients from Anhui and Shanghai in China; ◆: isolates from human H7 cases; ● : influenza vaccine strains in 2012.

influenza viruses because of the presence of Q226I in the Zhejiang strain and Q226L in the Shanghai 01 strain; the HA protein potentially enhanced the ability to bind to mammalian-like receptors with sialic acid moieties linked to galactose via α-2,6 linkages [21,22]. Cleavage site had only one R, which indicates its low pathogenicity, similar to other Chinese strains. The NA sequence data indicate antiviral resistance to adamantanes and susceptibility to neuraminidase inhibitors. No differences in active sites of MP gene, glycosylation sites, and NA stalk deletion were observed between the Zhejiang strain and other Chinese strains. Additional analyses are needed to understand its significance and the effect of single mutations.

No outbreaks were identified in Zhejiang avian population before the onset of the illness. The source of infection was thought to be exposure to poultry. The virus needs to be tested further to support the exposure in the live poultry market. Although the source of infection and the mode of transmission have not yet been determined, control measures such as closing live poultry markets should be implemented. Although secondary cases developed (the nurse and doctor) during diagnosis and treatment, the attack rate was only 1.6% (2/125) and no close contacts tested positive for H7N9, exclusion of possible person-to-person transmission cannot be considered [13].

The main clinical features of the first patient in Zhejiang Province were similar to those reported in other areas of China. They share features such as acute onset and rapid development to severe and sustained hypoxia accompanied by respiratory failure, ARDS, pleural effusion, and MOF. Despite the administration broad-spectrum antibacterial agents, cortisone, and mechanical ventilation, his condition

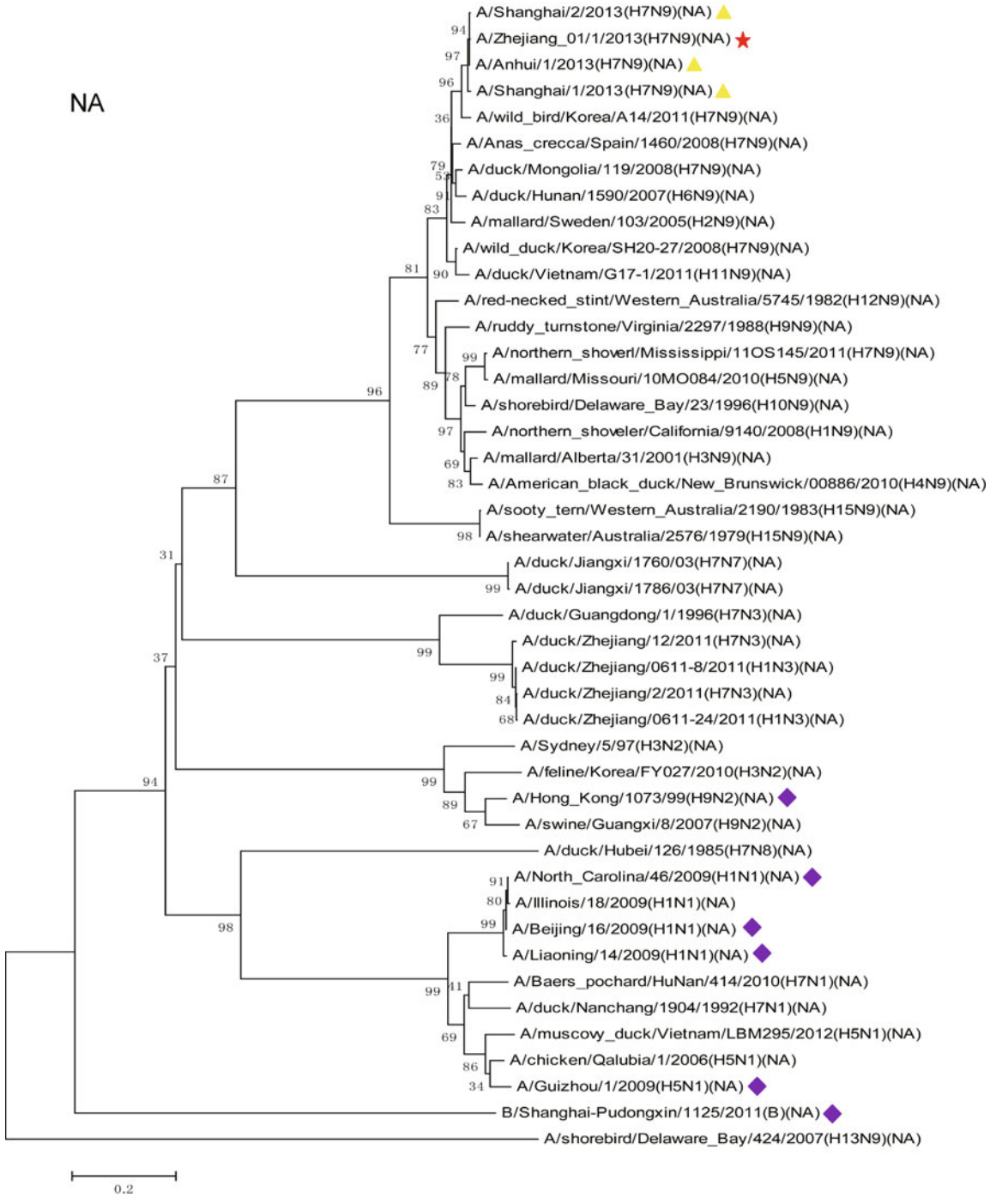

Fig. 7 Phylogenetic tree of NA gene of the first confirmed avian influenza A (H7N9) fatality. ★ : An isolate from the first confirmed H7N9 case in Zhejiang; ▲ : isolates from the confirmed H7N9 patients from Anhui and Shanghai in China; ◆: isolates from human cases .

deteriorated, resulting in death. Considering the patient had chronic hepatitis B, the case significantly differs from other cases in China. The patient presented with hemoptysis, sustained severe liver function, serious coagulation dysfunction, and DIC. Considering the diagnosis was confirmed 5 days after the patient died, he did not receive oral oseltamivir, which contributed greatly to the fatal outcome.

In summary, we report the first human death caused by human avian influenza (H7N9) virus in Zhejiang Province. The patient was exposed to poultry before he initially

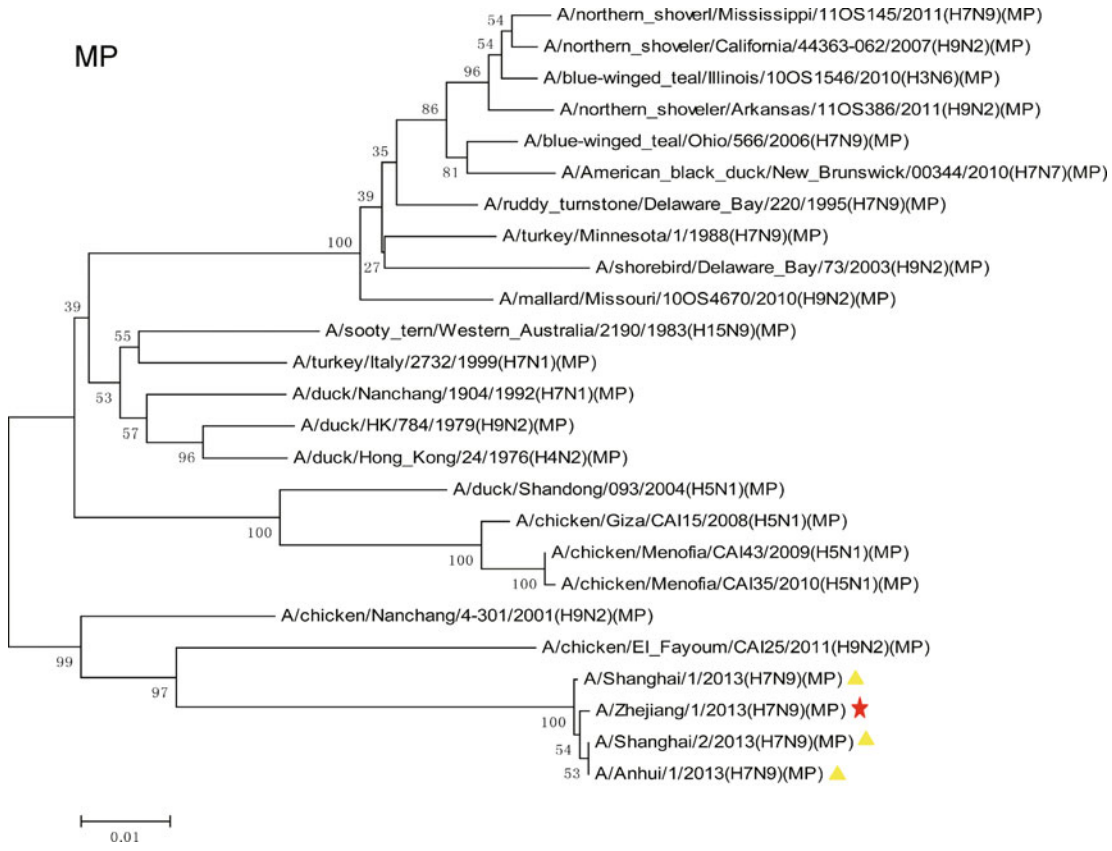

Fig. 8 MP gene phylogenetic tree of the first confirmed avian influenza A(H7N9) fatality. ★ : An isolate from the first confirmed H7N9 case in Zhejiang; ▲ : isolates from the confirmed H7N9 patients in Anhui and Shanghai, China.

Table 3 HA, NA, and MP gene active sites analysis of the H7N9 confirmed cases from Zhejiang, Anhui, and Shanghai in China, 2013

Segments	Active sites	Position	A/Zhejiang /1/2013 (H7N9)	A/Shanghai/1/2013 (H7N9)	A/Shanghai/2/2013 (H7N9)	A/Anhui/1/2013 (H7N9)
HA	RBS	135–138	ATSA	ATSS	ATSA	ATSA
		190–198	EQTKLYGSG	EQTKLYGSG	EQTKLYGSG	EQTKLYGSG
		221–228	PQVNGISG	PQVNGLSG	PQVNGLQSG	PQVNGQSG
	Cleavage site	325–336	PEIPKGRGLFGA	PEIPKGRGLFGA	PEIPKGRGLFGA	PEIPKGRGLFGA
	Glycosylation sites	30	NGTK	NGTK	NGTK	NGTK
		46	NATE	NATE	NATE	NATE
		249	NDTV	NDTV	NDTV	NDTV
		421	NWTR	NWTR	NWTR	NWTR
		493	NNTY	NNTY	NNTY	NNTY
NA	Single substitution	26	I	I	M	I
	Single substitution	40	G	S	G	G
	Stalk	69–73	del	del	del	del
	Antiviral resistance (oseltamivir) H275Y	275	H	H	H	H
	Antiviral resistance (oseltamivir) R294K	294	R	K	R	R
M1	Virulence sites (N30D)	30	D	D	D	D
	Virulence sites (T215A)	215	A	A	A	A
M2	Antiviral resistance (amantadine) S31N	31	N	N	N	N

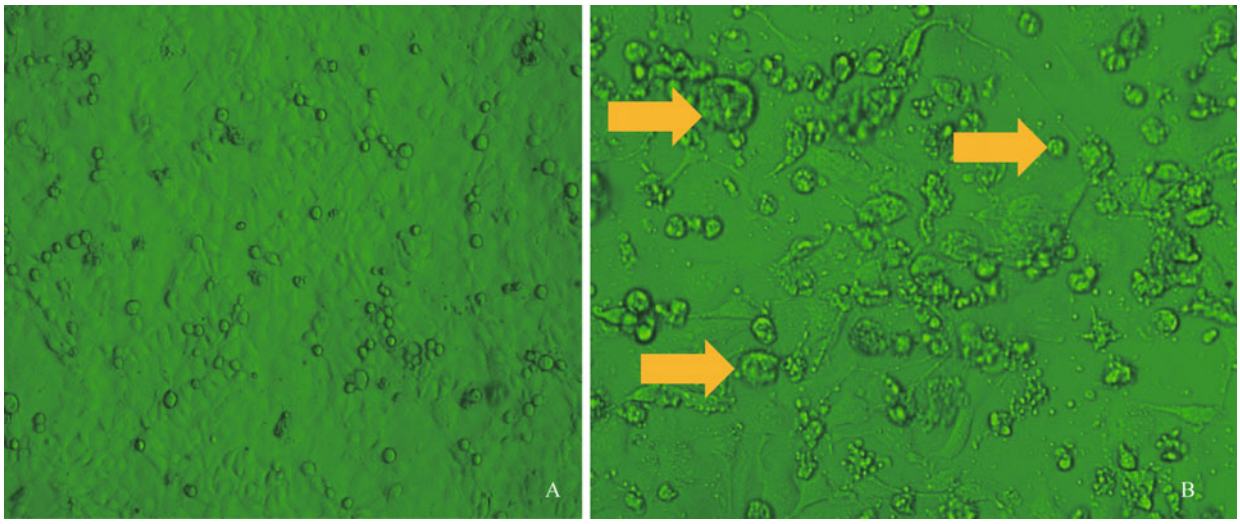

Fig. 9 The MDCK cells exhibited the characteristic cytopathic effect (CPE) when inoculated with the patient's swabs. The CPE presented as cell swelling and becoming rounded, with increased intercellular spaces; the cells ruptured within 24 h after infection (× 100). (A) Control; (B) CPE; Yellow arrow, CPE site.

presented with cough and diarrhea on March 7 and 14, 2013. The patient was hospitalized on March 18 and died on March 27. He was confirmed as infected with a novel avian influenza A (H7N9) virus on April 1, 2013. The major clinical symptoms included fever, cough, and diarrhea, shortness of breath, weakness, poor appetite, cyanosis, coma, and anuria. The main laboratory findings were increasing AST, ALT, LDH, PT, APTT, CPK, and CRP, but decreasing SaO_2% with disease progression. The major complications were ARDS, MOF, and DIC. The patient did not receive any antiviral treatment, although he received antibiotics, glucocorticoid hormone therapy, and invasive mechanical ventilation. The advanced infection control was achieved by testing all contacts, identifying the source of the virus and suspected cases, surveillance in Zhejiang Province, and sterilization.

Considering H7N9 viral infections have not occurred in humans before, persons of all ages might be susceptible. We are uncertain whether the influenza A (H7N9) virus could cause a pandemic. Enhanced and expanded surveillance to ensure immediate discovery, diagnosis, and treatment of suspected cases will reduce the number of severe H7N9 cases and deaths. Information on the emergent H7N9 virus is currently limited; therefore, studies on the source of the virus, transmission models, serologic investigations, vaccines, and drug development are necessary.

Compliance with ethics guidelines

The sampling activities were approved by the Chinese Medical Ethical Committee and the National Health and Family Planning Commission approved the study. This study was supported by the program for Zhejiang Leading Team of Science and Technology Innovation (Grant No. 2011R50021) and for Zhejiang Provincial Health Bureau (2013KYA043 and 2012KYA161). None of the funders had any role in the study design, the collection, analysis, and interpretation of data, the writing of the article, and the decision to submit it for publication. The researchers confirm their independence from funders and sponsors.

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