Clinical and epidemiological characteristics of a fatal case of avian influenza A H10N8 virus infection: a descriptive study

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Summary

Background **Human infections with different avian influenza viruses—eg, H5N1, H9N2, and H7N9—have raised concerns about pandemic potential worldwide. We report the first human infection with a novel reassortant avian influenza A H10N8 virus.**

Methods **We obtained and analysed clinical, epidemiological, and virological data from a patient from Nanchang City, China. Tracheal aspirate specimens were tested for influenza virus and other possible pathogens by RT-PCR, viral culture, and sequence analyses. A maximum likelihood phylogenetic tree was constructed.**

Findings **A woman aged 73 years presented with fever and was admitted to hospital on Nov 30, 2013. She developed multiple organ failure and died 9 days after illness onset. A novel reassortant avian influenza A H10N8 virus was isolated from the tracheal aspirate specimen obtained from the patient 7 days after onset of illness. Sequence analyses revealed that all the genes of the virus were of avian origin, with six internal genes from avian influenza A H9N2 viruses. The aminoacid motif GlnSerGly at residues 226–228 of the haemagglutinin protein indicated avian-like receptor binding preference. A mixture of glutamic acid and lysine at residue 627 in PB2 protein—which is associated with mammalian adaptation—was detected in the original tracheal aspirate samples. The virus was sensitive to neuraminidase inhibitors. Sputum and blood cultures and deep sequencing analysis indicated no co-infection with bacteria or fungi. Epidemiological investigation established that the patient had visited a live poultry market 4 days before illness onset.**

Interpretation **The novel reassortant H10N8 virus obtained is distinct from previously reported H10N8 viruses. The virus caused human infection and could have been associated with the death of a patient.**

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Introduction

Different subtypes of avian influenza A virus cause sporadic human infections with varied clinical symptoms, such as conjunctivitis (H7N2, H7N3, H7N7, and H10N7), mild respiratory syndrome (H9N2, H7N2, H7N3, H7N7, and H10N7), and severe pneumonia and death (H5N1 and H7N9).[1–9] Avian influenza A H5N1 virus—the first human infection with which was reported in Hong Kong in 1997[5,9]—has caused 648 human infections with 384 deaths in 15 countries since 2003.[10] The potential for person-to-person transmission as a result of mutation or reassortment, or both,[11–13] and the high mortality mean that avian influenza A H5N1 virus has pandemic potential and is of global concern.

Several surveillance systems for pandemic preparedness have been established in China, such as sentinel hospital-based surveillance systems for influenza-like illness and severe acute respiratory illness, the laboratory-based influenza virological surveillance system, and the surveillance system for pneumonia of unknown aetiology that is based in clinical institutions. Overall, the surveillance systems have a crucial role for early detection of human infection with novel influenza viruses. In March, 2013, the novel avian influenza A H7N9 virus was identified through these systems.[6] As of Jan 27, 2014, 250 human infections had been reported, with 70 deaths.[14]

Here, the characteristics of a patient with severe pneumonia of unknown aetiology are described and the clinical investigation is reported.

Methods

Clinical and epidemiological data collection

Information was obtained about a patient who had been admitted to hospital in Nanchang City, Jiangxi Province, on Nov 30, 2013. Field investigators and clinicians used a standardised surveillance reporting form to gather epidemiological and clinical data: demographic characteristics; underlying medical conditions; recent exposures to pigs, poultry, or other animals; recent visits to live animal markets; clinical signs and symptoms; chest radiographic findings; clinical laboratory testing results; antiviral treatment; clinical complications; and outcomes. The National Health and Family Planning Commission deemed the data collection for this case to

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be part of the continuing public health outbreak investigation and exempt from institutional review board assessment.

Close contacts—ie, individuals who had provided care to, had been living with, or had potentially been directly exposed to respiratory secretions or bodily fluids of the patient in the 14 days before illness onset—were identified. The institutional review board of Nanchang Center for Disease Control and Prevention (CDC) approved the assessment of these close contacts. Written informed consent was obtained from the close contacts.

Viral analysis

Tracheal aspirate specimens were obtained from the patient on day 7 and day 9 after illness onset. Real-time RT-PCR or conventional RT-PCR, or both, were used for influenza typing and subtyping by the Nanchang CDC and Jiangxi provincial CDC. The samples were identified as containing influenza A on the basis of the M gene, but could not be subtyped.

They were sent to the Chinese National Influenza Center on Dec 6, 2013. The tracheal aspirate samples were maintained in a viral-transport medium, and were propagated in the amniotic cavity of 9-day-old specific pathogen-free embryonated chicken eggs for 72 h at 37°C. The virus titre was established with a haemagglutination test using turkey red blood cells and was recorded as the reciprocal of the highest dilution of the virus that induced haemagglutination.

RNA was extracted from tracheal aspirate samples with QIAamp Viral RNA Mini Kit (Qiagen, Germany), according to the manufacturer's instructions. Specific real-time RT-PCR or conventional RT-PCR assays for seasonal influenza viruses (H1, H3, or B) and avian influenza H1 to H16 and N1 to N9 subtypes were done to verify the viral subtypes. Sequences of the primers and probes are available on request.

The full genome of the virus was amplified with the use of Qiagen OneStep RT-PCR Kit for sequencing (Qiagen, Germany). PCR products were purified from agarose gel with the QIAquick Gel Extraction Kit (Qiagen, Germany). Sequencing was done with the automatic Applied Biosystems 3730xl DNA Analyzer (Life Technologies, USA) and the Applied Biosystems BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies, USA), according to the manufacturer's instructions. Full genome sequences of the viruses were deposited in the Global Initiative on Sharing Avian Influenza Data database (accession number EPI497477-84). A maximum likelihood phylogenetic tree for nucleotide sequences of each gene of selected influenza viruses was constructed with MEGA5.1.

Extracted RNA was reverse transcribed to double-stranded DNA, and deep sequenced on the Ion Torrent platform with the type 318 chip. The resulting sequencing reads were aligned to the National Center for Biotechnology Information non-redundant nucleotide database with BLAST software[15] (version 2.2.22) with parameters "-p blastn –e 1e-5 –b 10 –v 10" after filtering of the adapters and human-origin reads. Alignments that had scores greater than 80 were retained for subsequent analysis. The taxonomic composition (at the species level) of each dataset was identified with MEGAN software (version 4.70.0).[16] The percentages of the identified microbial species were estimated according to the numbers of the sequencing reads of each species normalised by their genome sizes.[17]

To assess the sensitivity of the identified H10N8 virus to neuraminidase inhibitors (oseltamivir and zanamivir), neuraminidase inhibition assays were done as described previously.[18] The serum samples obtained from the patient and six close contacts were assessed with the haemagglutination inhibition assay for antibody titre against the H10N8 virus according to standard protocols, with 0·5% turkey red blood cells and horse red blood cells[19] separately. Before the haemagglutination inhibition assay, serum samples were treated with receptor destroying enzyme (1:4 volume to volume; Denka Seiken, Japan) at 37°C for 18 h, and then heat inactivated at 56°C for 30 min to remove non-specific serum inhibitors. Serum samples were titrated in two-fold dilutions in phosphate-buffered saline and tested at an initial dilution of 1/10.

Throat swabs were obtained from the patient's health-care providers and tested for influenza virus by real-time RT-PCR by Nanchang CDC.

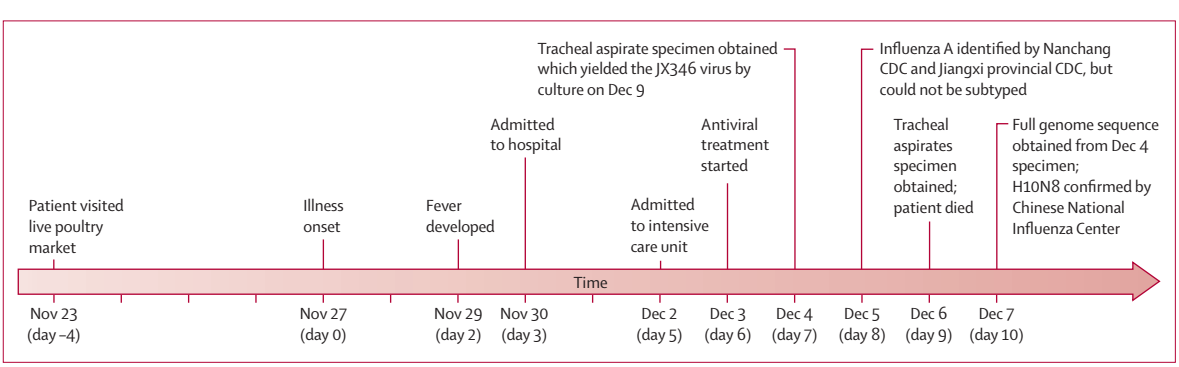

Figure 1: **Timeline of the clinical course of the patient and identification of causative pathogen**
CDC=Center for Disease Control and Prevention.

Role of the funding source
The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The patient was a woman aged 73 years who had hypertension, coronary heart disease, and myasthenia gravis. She had undergone thymectomy in December, 2012. She was reported to have developed initial symptoms of cough and chest tightness on Nov 27, 2013 (figure 1). Fever developed 2 days after illness onset (figure 1, table 1). She was admitted to hospital with fever (38·6°C) on Nov 30, 2013 (figure 1). A chest CT scan showed consolidation of right lung lower lobe and increased density of left lung lower lobe on day 4 (figure 2). The chest radiograph showed that the patient had bilateral pleural effusion on day 6, with rapid progression of ground-glass opacities and consolidation on day 8 (figure 2).

The proportion of white blood cells that were lymphocytes was lower than the normal range on all tests and the proportion that were neutrophils was higher than the normal range from day 5 (table 2). The number of white blood cells was increased on all tests (table 2). Concentrations of C-reactive protein and creatinine were high, and concentrations of aspartate aminotransferase and blood urea nitrogen were increased slightly from day 7, indicating liver and kidney dysfunction. Blood concentrations of alkaline phosphatase, total protein, globins, and albumin were normal on day 4, but were decreased on days 7 and 8 (table 2). Transthyretin concentration was decreased on all tests (table 2). Decreased concentrations of total IgG and complement C3 were recorded on day 8 (table 2). Despite combination antibiotic treatment for prevention of bacterial infection, mechanical ventilation, glucocorticoids, intravenous albumin, and antiviral treatment (table 1), the patient's condition became progressively serious: she developed severe pneumonia, septic shock, and multiple organ failure, and died on day 9.

By means of real-time RT-PCR, conventional RT-PCR, and sequencing, tracheal aspirate samples obtained from the patient on day 7 and day 9 were shown to be positive for avian influenza A H10N8 virus and negative for seasonal influenza viruses (H1, H3 or B), H5N1, H7N9, and H9N2. Viruses were isolated from the specimens obtained on days 7 and 9 after illness onset, with a haemagglutinin titre of 256 on day 7 and 1024 on day 9. The virus isolated from the sample collected on day 7 was designated as A/Jiangxi-Donghu/346/2013(H10N8)—henceforth, JX346—and was used for further analysis. No pathogenic bacteria and fungus were detected in sputum cultures on days 6 and 8, or blood culture on day 6. The deep sequencing data from the tracheal aspirate specimen obtained on days 7 and 9 showed that the avian influenza A H10N8 virus was overwhelmingly dominant (>99%) in microbial species (appendix). The haemagglutination inhibition antibody titre tested with horse blood cells against JX346 virus was less than 10 for the serum obtained from the patient on day 5, and 80 on day 9 (appendix).

See Online for appendix

Homologous comparison in GenBank with BLAST showed that the haemagglutinin gene of JX346 was most closely related to A/duck/Hunan/S11205/2012(H10N3) virus (97% similarity), and the neuraminidase gene was most closely related to A/mallard/Korea/1041/2010(H10N8) virus (98·8%) and A/avian/Japan/8KI0129/2008(H3N8) virus (98·7%; appendix). Phylogenetic analysis based on all the available H10 and N8 subtype sequences in Genbank showed that the haemagglutinin gene of JX346 belonged to the Eurasian avian lineage, and the neuraminidase gene belonged to a subclade of North American avian lineage (figure 3).

All six internal genes of JX346 were closely related to the H9N2 viruses that are circulating in poultry in China. The PB2, acid polymerase, matrix, and nucleoprotein genes were closest to A/environment/Jiangxi/00449/2013 (JX449)-like virus (98·6–99·5%; appendix). The PB1 and non-structural genes were closest to A/environment/Chongqing/00516/2013 (CQ516)-like virus (98·4–99·5%; appendix). JX449 was isolated from environmental samples obtained from live poultry markets in Jiujiang

	Signs and treatment
Fever	Yes
Temperature on admission (°C)	38·6
Highest temperature (°C)	39·4
Complications	
Failure of respiratory function	Yes
Septic shock	Yes
Acute renal damage	Yes
Oxygen treatment	Yes
Bacterial co-infection	No (negative sputum and blood cultures)
Antibiotic treatment	
Day 3	Cefotaxime sodium (two 3 g doses given intravenously)
Day 4	Cefotaxime sodium (two 3 g doses given intravenously), levofloxacin (two 0·3 g doses given intravenously)
Day 5	Vancomycin (500 mg given intravenously every 8 h), meropenem (1 g given intravenously every 8 h)
Days 6–8	Vancomycin (500 mg given intravenously every 8 h), meropenem (1 g given intravenously every 8 h), moxifloxacin (one 0·4 g dose given intravenously)
Glucocorticoids	
Day 5	Methylprednisolone (two 80 mg doses given intravenously)
Day 6	Dexamethasone (two 10 mg doses given intravenously)
Day 7	Dexamethasone (one 10 mg dose given intravenously)
Antiviral treatment	Tamiflu (75 mg twice a day; started on day 6)
Plasma transfusion	500 mL (day 7)
Intravenous albumin therapy	10 g (day 8)

Table 1: **Complications, treatment, and clinical outcome of the patient**

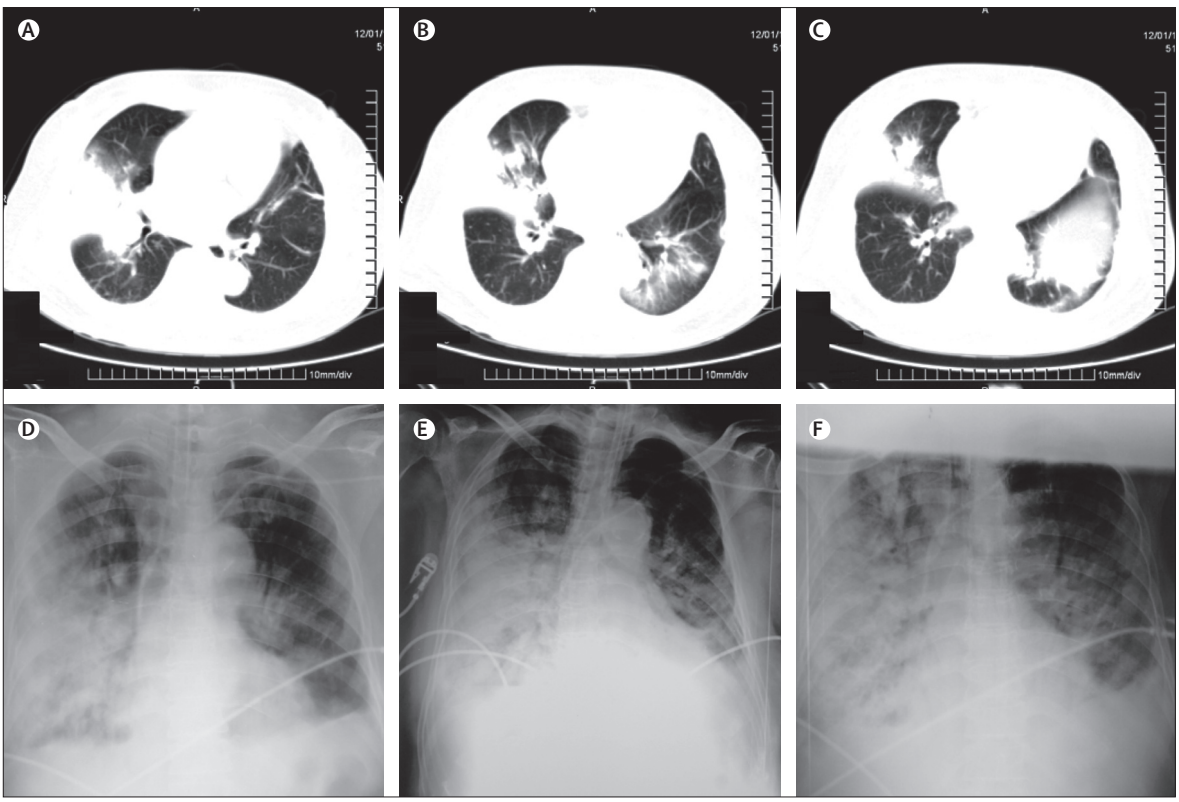

Figure 2: **Imaging of the patient's chest**
(A–C) CT scan obtained on day 4. Chest radiographs (patient's heart on the right) showed mild ground-glass opacities on day 6 (D), and bilateral ground-glass opacities and consolidation on day 8 (E, F).

	Normal range	Days after illness onset				
		4	5	6	7	8
Number of white blood cells (×10⁹ per L)	4·0–10·0	10·34	12·09	10·45	..	10·90
Proportion of neutrophils (%)	42·0–78·0	76·4	94·3	93·4	..	93·1
Proportion of lymphocytes (%)	11·0–49·0	7·0	2·4	4·2	..	2·3
C-reactive protein (nmol/L)	0–76·19	>1904·80	>1904·80	>1904·80	..	1571·46
Aspartate aminotransferase (U/L)	0–40·0	22·0	..	..	57·0	60·0
Alkaline phosphatase (U/L)	37·0–128·0	51·0	..	..	36·0	34·0
Total protein (g/L)	60·0–86·0	60·0	..	..	47·8	42·9
Globin (g/L)	22·0–39·0	23·8	..	..	21·0	19·2
Albumin (g/L)	35·0–57·0	36·2	..	..	26·8	23·7
Transthyretin (mg/L)	160–400	102	..	..	24	35
Creatine kinase MB (U/L)	0–24·0	..	31·7	..	..	..
IgG (g/L)	7·51–15·60	..	..	..	..	6·32
D-dimer (nmol/L)	0–8·65	17·52	..	96·93	265·59	251·90
Fibrin degradation product (mg/L)	0–5·0	..	..	31·5	84·0	77·0
Creatinine (μmol/L)	31·0–88·0	..	35·3	..	189·0	371·0
Blood urea nitrogen (mmol/L)	2·5–7·1	..	2·44	..	12·2	19·7
Complement C3 (g/L)	0·79–1·52	..	..	..	..	0·49
Complement C4 (g/L)	0·16–0·38	..	..	..	..	0·17

Table 2: **Clinical blood biochemistry tests**

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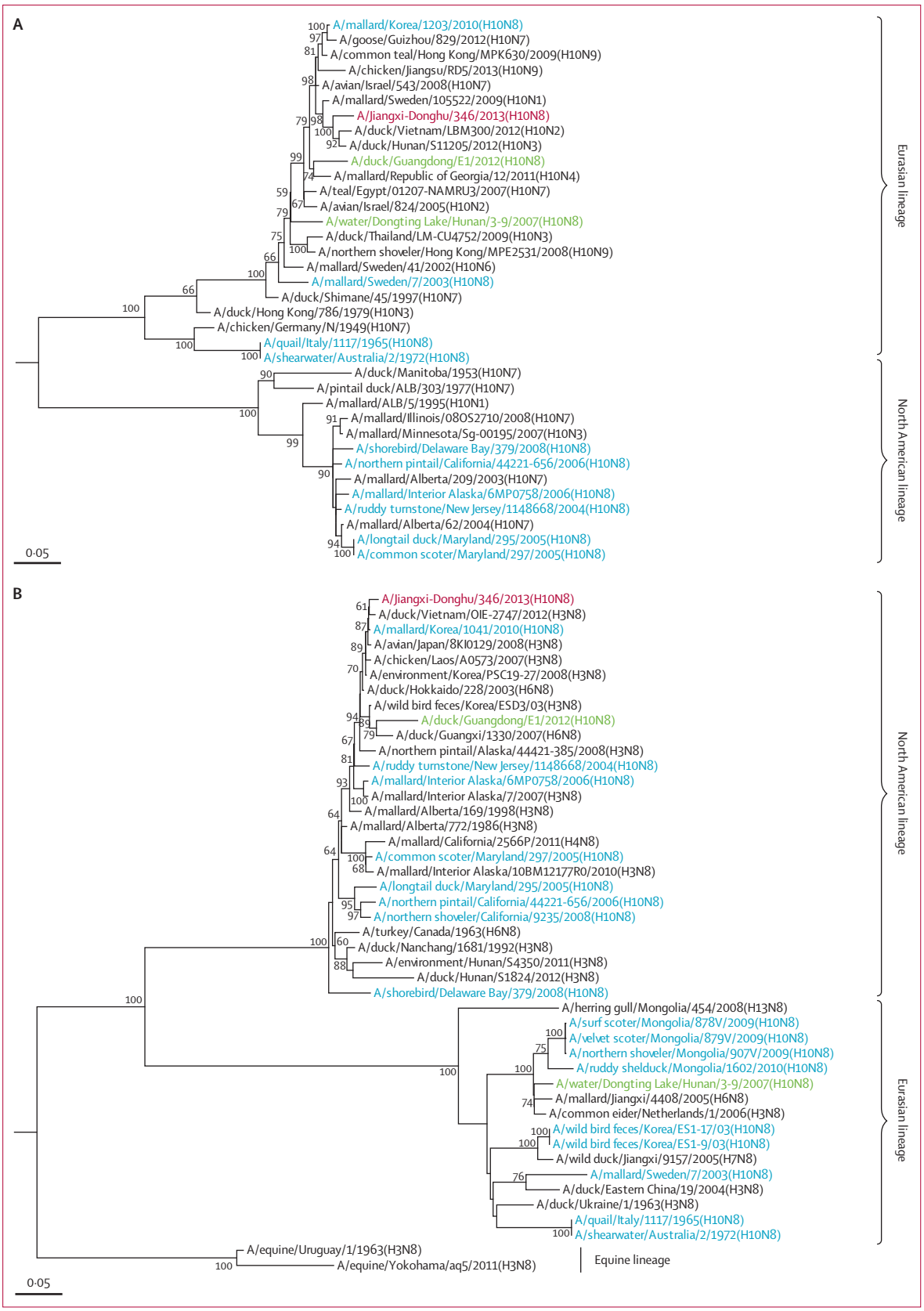

***Figure 3*: Phylogenetic trees of full-length (A) haemagglutinin and (B) neuraminidase genes of H10 and N8 subtype influenza viruses**
The novel H10N8 isolate that we identified is in red. Other H10N8 viruses from public database are shown in blue, except the two Chinese H10N8 viruses, which are in green. Other previously reported viruses are shown in black. Evolutionary distances were calculated with the maximum likelihood method.

	Mutation*	AH/1 (H7N9)†	JX346 (H10N8)
Haemagglutinin gene			
Connecting peptide	..	ProGluIleProLysGlyArg*Gly	ProGluLeuIleGlnArg*Gly
Favour mammalian adaptation	Ala135Thr	Ala	Thr
	Ser138Ala	Ala	Ala
Receptor binding site	Gly186Val	Val	Ser‡
	Gln226Leu	Leu	Gln
	Gly228Ser	Gly	Gly
Neuraminidase gene			
69–73 deletion	..	Yes	No
Related to drug resistance (N2 numbering)	Ile117Val	Thr	Ile
	Arg118Lys	Arg	Arg
	Glu119Gly	Glu	Glu
	Gln136Lys	Gln	Gln
	Asp151Glu/Asn/Gly	Asp	Asp
	Arg152Lys	Arg	Arg
	Ile222Val/Arg/Lys	Ile	Ile
	Arg224Lys	Arg	Arg
	His274Tyr	His	His
	Glu276Asp	Glu	Glu
	Arg292Lys	Arg	Arg
	Asn294Ser	Asn	Asn
	Ile314Val	Ser‡	Val
	Arg371Lys	Arg	Arg
PB2			
Increased pathogenicity in mice	Leu89Val	Val	Val
	His357Asn	His	His
	Glu627Lys	Lys	Glu/Lys
Enhanced transmission in guinea pigs	Asp701Asn	Asp	Asp
PB1			
Increased replication in mammalian cells	Leu473Val	Val	Val
	Leu598Pro	Leu	Leu
PB1-F2			
Increased pathogenicity in mice	87–90 aminoacids in length	90 aminoacids in length	34 aminoacids in length
Acid polymerase gene			
Increased replication in mice	Ala36Thr	Ala	Ala
M1			
Increased pathogenicity in mice	Asn30Asp	Asp	Asp
	Thr215Ala	Ala	Ala
M2			
Resistant to adamantanes	Ser31Asn	Asn	Asn
NS1			
Increased pathogenicity in mice	Pro42Ser	Ser	Ser
PDZ motif	..	Deleted	Deleted

Data for AH/1 taken from Gao and colleagues.[6] *Numbered from start codon (Met); previously identified in avian influenza A H5N1 virus and other influenza A viruses.[7,20–22] †A/Anhui/1/2013(H7N9), the representative and vaccine virus of H7N9 viruses isolated in China in 2013, as defined by WHO. ‡Function of mutation not established yet.

Table 3: **Mutations in AH/1 and JX346 viruses, by gene**

and CQ516 from markets in Chongqing by the Chinese National Influenza Center according to national guidelines. All the internal genes of JX346 were significantly different from H10 and N8 subtype viruses previously reported, and were in different subclades from avian influenza A H7N9 viruses previously identified (appendix).

The haemagglutinin cleavage site possesses only one basic aminoacid—arginine—indicating low pathogenic effects in poultry.[20] The aminoacid motif GlnSerGly at residues 226–228 (H3 numbering) of the haemagglutinin protein indicated avian-like receptor binding preference.[21] A mixture of glutamic acid and lysine was detected at residue 627 in PB2 protein from the original trachea aspirate samples by deep sequencing (table 3): frequency of glutamic acid was 87·6% versus 12·4% for lysine in the sample obtained on day 7, and frequency was 3·3% versus 96·7% on day 9. The M2 protein contained the Ser31Asn substitution, indicating resistance to adamantanes. The Ile314Val mutation (N2 numbering)—which was reported to reduce susceptibility of influenza A H5N1 viruses to oseltamivir when combined with Ile117Val mutation[18]—was recorded in the neuraminidase protein of JX346. Further in-vitro neuraminidase function assay showed that the JX346 virus was sensitive to oseltamivir and zanamivir (appendix).

Epidemiological investigation showed that the patient had visited a live poultry market with her carer 4 days before illness onset. She had bought a chicken after a short stay (about 5 min), but the patient did not handle the chicken (appendix). Additionally, she had no other contact with live poultry or with individuals with fever or influenza-like illness in the 2 weeks before illness onset. She had not travelled anywhere in the previous month.

17 close contacts—11 health-care providers, five family members, and one carer—were identified. No signs of influenza-like illness were recorded during the investigation period (2 weeks after contact with the patient). Throat swabs obtained from the 11 health-care providers were negative for influenza virus by real-time RT-PCR test. No antibody titre against JX346 virus was detected in the serum samples obtained from the five family members and the carer by haemagglutination inhibition assay (appendix).

Discussion

A novel reassortant avian influenza A H10N8 virus (JX346) has been isolated in China from a patient with severe pneumonia and multiple organ failure, who deteriorated rapidly and ultimately died. The microbe culture and deep sequencing data showed that the avian influenza A H10N8 virus was overwhelmingly dominant in tracheal aspirate specimens, indicating that the JX346 virus infection was associated with the illness and death of the patient.

At least two features of the virus could have contributed to the severity of the patient's illness. First, JX346 virus preferentially binds avian-like α2,3-linked sialic acid receptor, which is dominant in human lung tissue, potentially causing lung damage, as reported with avian influenza A H5N1 virus infection.[21] Second, an increased percentage of lysine at residue 627 in PB2 was recorded during the clinical course of the patient, and PB2 Glu627Lys mutation could increase virulence in mammalian hosts.[22] However, host factors including the chronic medical conditions could also have had a role in the death of the patient.

Human infection with avian influenza A H10N8 virus has not been reported previously (panel). Avian influenza H10 or N8 subtype viruses circulate widely in birds—mainly H10N7 and H3N8 (panel). Two human infections with avian influenza A H10N7 virus have been previously reported—one in Egypt in 2004, and one in Australia in 2010—with patients presenting with conjunctivitis or mild respiratory symptoms.[2,23] However, no human infection with an N8 subtype influenza virus has been reported previously. Only two H10N8 viruses have been reported in China: one was from a water sample taken from Dongting Lake in Hunan province in 2007,[24] and the other from a live poultry market in Guangdong province in 2012.[25] However, the genomic analysis indicated that the JX346 virus described here differed, especially for the six internal genes.

Notably, H9N2 virus provided the internal genes not only for the H10N8 virus, but also for H7N9 and H5N1 viruses.[6,26] This relation should be studied further to understand the mechanism of how the internal genes of H9N2 virus transfer to viruses that infect people, and how the avian influenza virus transfers between species. Although six internal genes of the JX346 virus were from avian influenza A H9N2 viruses, JX346 was in a different subclade from H7N9 viruses, indicating that the internal genes were not directly from H7N9 viruses.

Phylogenetic data and BLAST results suggested that JX346 might originate from multiple reassortments between different avian influenza viruses. The H10 and H8 gene segments might have derived from different wild bird influenza viruses reassorted to give rise to a hypothetical H10N8 virus in wild birds, which infected poultry and then reassorted with H9N2 viruses in poultry to give rise to the novel reassortant JX346 (H10N8) virus (appendix). However, the absence of the surveillance data means it is difficult to establish the exact origin and evolution process of JX346, and other reassortment processes cannot be excluded.

Live poultry markets have been shown to be important sources of human infection with avian influenza A H5N1 and H7N9 viruses.[27,28] The field investigation to establish the source of the infection reported here did not reach a clear conclusion. The patient had visited a live poultry market a few days before illness onset and could have acquired the infection during this visit, indicating that the incubation period might be 4 days, similar to other avian influenza virus infections.[29]

Some person-to-person transmission of avian influenza viruses—eg, H5N1[30] and H7N9[31]—has been reported. In this study, the medical observation and laboratory testing of close contacts showed no evidence of person-to-person transmission of JX346. However, a previous study[24] indicated that H10N8 virus can replicate efficiently in mouse lung without previous adaptation, and its pathogenicity in mice increased rapidly during lung adaptation. JX346 contained haemagglutinin Ala135Thr and Ser138Ala mutations, which favour mammalian adaptation;[7] it also contained M1 Asn30Asp and Thr215Ala mutations and NS1 Pro42Ser mutations, which are associated with increased virulence in mice.[7] A search of GenBank showed that all but two of 430 previously reported H9N2 viruses from China submitted between 2003 and 2013, had glutamic acid at residue 627 in PB2. The mixture of glutamic acid and lysine detected at this residue in the patient presented here suggests that Glu627Lys substitution occurred during virus replication in the patient. The virus contained a single basic arginine in the haemagglutinin connecting peptide.

Our study had several limitations. First, the patient's family did not give permission for an autopsy, so an autopsy specimen from the patient cannot be obtained for histopathology or immunohistochemistry experiments to better understand the infection and the pathological lesions. Second, an identical H10N8 virus was not identified in the live poultry market that the patient visited before her illness onset, and so the source of human infection cannot be confirmed.

Pandemic influenza is a public health concern worldwide. Prediction of which viruses could cause a pandemic in the future is challenging. The combined surveillance systems established in China mean that novel influenza viruses causing human infection can be detected as early as possible. The first human infection with novel avian influenza A H10N8 virus further increases the importance of surveillance for pandemic

***Panel:* Research in context**

Systematic review

We searched PubMed for reports with titles or abstracts in English on Dec 22, 2013, with the terms "A(H10N8)", "H10N8", "H10", or "N8". We also searched for reports available online from the websites of international medical and infectious disease journals. We did not identify any reports of previous human infections with avian influenza A H10N8 virus. Avian influenza H10 or N8 subtype viruses are common pathogens in birds, with mainly H10N7 and H3N8 viruses in public databases. Only 26 H10N8 virus sequences were available.

Interpretation

Here, a case of human infection with novel avian-origin reassortant influenza A H10N8 virus in Jiangxi province, China, has been described. The patient deteriorated rapidly and died 9 days after illness onset. The infection with the avian influenza A H10N8 virus and the patient's chronic medical problems contributed to the clinical abnormalities and outcome. Although we cannot predict whether an H10N8 epidemic will occur, our findings suggest that the virus is a potential threat to people. Our study will provide detailed information for clinicians, virologists, and public health experts to guide clinical treatment, laboratory diagnosis and surveillance for pandemic preparedness.

preparedness and response. No poultry outbreak caused by H10N8 virus has been reported previously. The apparently low pathogenicity in poultry could help the H10N8 virus to circulate in poultry without detection. Another human case of infection with H10N8 was reported in Nanchang City on Jan 26, 2014.[32] The first fatal case of avian influenza A H5N1 virus infection in Hong Kong in 1997 preceded the next 17 cases by 6 months,[7] so more human cases of H10N8 infection might occur in the future. The pandemic potential of this novel virus should not be underestimated.

Contributors

YShu designed the study. JZha and JWa treated the patient. HC, HY, JZha, DW, YX, GF, TC, QL, YaZ, HL, TG, YShi, XN, JL, JuZho, JF, JWu, XZho, MH, JWa, and ZF gathered data.. YX transferred samples. JiZho, SZ, and YeZ isolated viruses. JiZho and YL did drug resistance tests. RG, FY, XL, XZha, and Q J did sequencing. LD, HB, and TB did serology tests. RG, DW, LY, TC, ML, and YShu did analyses and wrote the report. JD, SW, WY, DL, GW, GFG, and YW commented on the report. All authors contributed to review and revision and have seen and approved the final version.

Conflicts of interest

We declare that we have no conflicts of interest.

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