Transmission of avian influenza A(H7N9) virus from father to child: a report of limited person-to-person transmission, Guangzhou, China, January 2014

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We investigated a possible person-to-person transmission within a family cluster of two confirmed influenza A(H7N9) patients in Guangzhou, China. The index case, a man in his late twenties, worked in a wet market that was confirmed to be contaminated by the influenza A(H7N9) virus. He developed a consistent fever and severe pneumonia after 4 January 2014. In contrast, the second case, his five-year-old child, who only developed a mild disease 10 days after disease onset of the index case, did not have any contact with poultry and birds but had unprotected and very close contact with the index case. The sequences of the haemagglutinin (HA) genes of the virus stains isolated from the two cases were 100% identical. These findings strongly suggest that the second case might have acquired the infection via transmission of the virus from the sick father. Fortunately, all 40 close contacts, including the other four family members who also had unprotected and very close contact with the cases, did not acquire influenza A(H7N9) virus infection, indicating that the person-to-person transmissibility of the virus remained limited. Our finding underlines the importance of carefully, thoroughly and punctually following-up close contacts of influenza A(H7N9) cases to allow detection of any secondary cases, as these may constitute an early warning signal of the virus's increasing ability to transmit from person-to-person.

Introduction

In February 2013, a novel avian influenza A(H7N9) virus emerged in China [1]. In humans, infection with the virus can result in rapid progressive pneumonia, acute respiratory distress syndrome (ARDS) and even in death [2]. Some young patients (2–4 years-old) nevertheless only develop mild illness or even experience subclinical infections [3,4]. A first epidemic wave of influenza A(H7N9) occurred from February to May 2013, with a total of 132 human infections and 37 deaths reported to the World Health Organization (WHO) [5]. Most cases were sporadically identified in the Yangtze River delta of eastern China [6]. After a period, from June to October 2013 when only four cases were identified [7], the virus re-emerged in the Pearl River delta of southern China in November 2013 resulting in a second epidemic wave. As of 5 April 2014, a total of 98 laboratory-confirmed influenza A(H7N9) cases with 31 deaths, and 411 cases with 145 deaths, have been reported in Guangdong province and mainland China, respectively [8].

Virological research on avian influenza A(H7N9) viruses has demonstrated several characteristic features of mammalian influenza viruses, which are likely to contribute to their infectivity to humans [9]. The amino acid sequence of the receptor-binding site (RBS) of haemagglutinin (HA) determines a preference for human- or avian- type receptors. Mammalian-adapting mutations were found in the RBS of influenza A(H7N9) early strains isolated from Shanghai and Anhui. Furthermore, a T160A substitution in HA of the influenza A(H7N9) virus has been found to result in increased virus binding to human-type receptors [9].

Despite limited person-to-person transmission and few cases of influenza A(H7N9) observed outside China, the further adaptation of influenza A(H7N9) in humans raises serious concerns for a potential pandemic. Previous studies have provided convincing evidence that infected poultry and a contaminated environment might be the key sources of influenza A(H7N9) virus infection in humans [10-13]. However, the risk for a potential pandemic is associated with the person-to-person transmissibility of the virus. Although several family clusters of confirmed influenza A(H7N9) virus

infection were reported previously [2,14,15], only one instance has been strongly suggestive of a possible limited person-to-person transmission of the virus [16]. Clusters of the viral infection may be an early warning sign of the virus adapting to humans, which might result in more efficient person-to-person transmission. In this study, we report the clinical, epidemiological and virological findings of two patients with influenza A(H7N9) in a family cluster identified during the second epidemic wave and provide evidence of a person-to-person transmission of influenza A(H7N9) virus between these two patients.

Methods

Detection and confirmation of influenza A(H7N9) cases

A clinical and laboratory surveillance system for patients with pneumonia of unknown aetiology (PUE), which was designed to identify novel respiratory pathogens like severe acute respiratory syndrome coronavirus (SARS-CoV) and avian influenza A(H5N1) virus, has been in use in China since 2004. All clinical facilities must report any patient lacking a clear diagnosis and meeting the criteria of PUE [17]. Respiratory specimens are collected and sent to the local laboratories of the surveillance network to identify the possible causative pathogens. Since April 2013, these samples are screened for influenza A(H5N1) and A(H7N9), SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) using a reverse transcription-polymerase chain reaction (RT-PCR) assay. The patients reported here were identified using this surveillance system, and the laboratory-confirmed influenza A(H7N9) cases were defined according to the Chinese guidance of diagnosis and treatment for humans infected with avian influenza A(H7N9) [18].

Epidemiological investigation of patients

Since the index case remained in critical condition when the epidemiological investigation was launched and the second case was too young to understand some of the questions, public health staff from the local Centre for Disease Control and Prevention (CDC) interviewed the remaining four family members to obtain basic information, previous health status, daily life schedule, the timeline of disease and possible close contacts of the two patients. We focused on investigating the poultry and animal exposure history prior to the onset of illnesses of the cases to identify a possible source of infection. We also interviewed some of the healthcare workers who provided medical services for these two cases and reviewed all medical records from the hospitals that these two patients visited to clarify the entire course of disease of the two patients.

Infection source tracing

Once the first patient was laboratory-confirmed to be infected with avian influenza A(H7N9) virus, a field investigation was immediately performed to find the possible infection source. To trace possible exposures, we surveyed the household and the residential district where this index case lived and inspected especially the retail wet market, where he was working before the onset of his disease. We gathered basic information on the wet market, such as the location and floor plan, numbers and distribution of poultry stalls, amounts and species of poultry, poultry transportation and unloading procedure, recent diseases in poultry, and how routine disinfection was performed. Twenty-six environmental samples were also collected from all four live poultry stalls of this market on 10 January 2014 to determine whether the market had been contaminated by the avian influenza A(H7N9) virus. These samples included 11 poultry faecal swabs, four chopping block swabs, three scalding machine swabs, two visceral waste swabs, four bloody sewage samples and two poultry drinking water samples.

Medical observation of close contact

All family members who lived with the two cases, relatives and friends who visited these two cases, as well as healthcare workers who had provided medical services to these two cases and been in contact with them within one meter without proper personal protection, from the onset of the patients' illnesses to when the patients were effectively isolated were recognised as close contacts. The investigators interviewed all close contacts to obtain information, including demographic information, previous health status and immunisation, pattern of contact, duration and frequency of contact, and use of personal protective equipment. All close contacts were monitored for respiratory symptoms and fever (≥38°C) for seven days. We also collected throat swabs from all contacts to detect the influenza A(H7N9) viral RNA within 24 hours after the influenza A(H7N9) cases were confirmed. During a seven-day medical observation, throat swab samples were collected repeatedly if any close contact developed influenza-like symptoms.

Identification of causative pathogen

The Universal Transport Medium (Copan Italia) was used to collect the throat swab and environmental samples. The samples were stored at 4°C and transported to the laboratory within two to four hours. Influenza A(H7N9) viral RNA was detected with a real-time reverse-transcriptase polymerase chain reaction (rRT-PCR) assay using pairs of H7- and N9-specific primers provided by the Chinese National Influenza Center as described previously [19]. The detection limit of the rRT-PCR assay was 300 copies/ml, i.e. the viral RNA was considered to be negative when the viral RNA copies in the throat samples were less than 300 copies/ml.

Virus culture, sequencing and phylogenetic analysis

The viruses were isolated using specific pathogen-free (SPF) chicken embryos as described previously [17]. The full length of HA open reading frame (ORF) gene of isolated strains was amplified using two pairs of primers that we designed (15F-5'-AGC AGG GGA TAC AAA

ATG AAC ACT C-3'/753R-5'-TGT ATC ATT GGG ATT TAG CAT TAG CC-3' and 588F-5'-AAC TGC AGA GCA AAC CAA GCT ATA T-3'/1689R-5'-CCA AAC TTA TAT ACA AAT AGT GCA CCG-3') and the OneStep RT-PCR Kit (Invitrogen, USA). The PCR products were purified and sequenced by Life Technologies Inc. using the same primers described above. The obtained HA sequences were sent to the GenBank database whereby the sequences from the two cases were deposited under accession numbers KJ415822 and KJ415823 respectively, while the HA sequence derived from environmental samples collected in the wet market was given accession number KJ415824. We performed multiple sequence alignments and constructed the phylogenetic tree with MEGA 6.0.6 using a neighbour-joining method with 1,000 bootstrap replicates.

Serological test

Paired serum samples were collected from the two cases and the 40 close contacts one week (10–16 January) and four weeks (15–16 February) after they were recruited, respectively. All serum samples were treated with receptor-destroying enzyme (Denka Seiken) for 18 hours at 37°C and then inactivated for 30 min at 56°C. The haemagglutination inhibition (HI) assay was performed using four HA units of inactivated strain A/Guangzhou/1/2014(H7N9) and 1% horse red blood cells to evaluate the H7-specific antibody titres according to the WHO's protocol [20]. The detection limit was 1:10 dilution of the serum samples, i.e. the samples lacking HI activity at 1:10 dilution were considered to be negative.

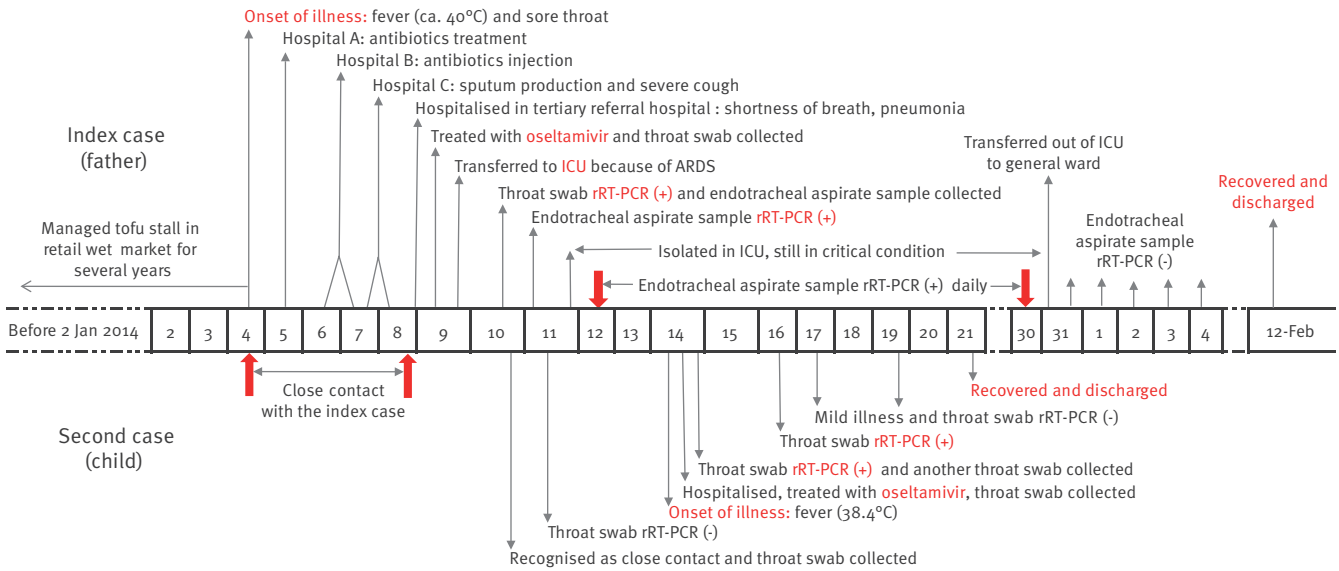
Results

Description of the cases

The family of six members was living in Guangzhou, the capital city of Guangdong province, whose population exceeds 12.7 million (2010 census data). As summarised in Figure 1, the index case, the father in his late 20ies, who had no history of any chronic disease, developed a high fever (approximately 40°C), accompanied by a sore throat and myalgia on 4 January 2014. From 5 to 7 January he visited three local hospitals (hospital A, B and C). At hospital A, he was prescribed oral ciprofloxacin to be taken for two days. He took these on the same day he visited the hospital as well as the next day at home. At hospitals B and C, he was administered ceftriaxone intravenously for two days. The symptoms nevertheless progressed and he experienced a constant high fever, severe cough with heavy sputum and progressive shortness of breath. The patient was therefore admitted to a tertiary referral hospital, and a chest X-ray examination revealed bilateral lower lobe pneumonia on 8 January. Although oral oseltamivir therapy (75 mg twice daily) was started on 9 January, the disease continued to progress, and the patient was transferred to the intensive care unit (ICU) with a diagnosis of viral pneumonia and ARDS. Throat swab and endotracheal aspirate samples collected on 9 and 10 January, respectively, were confirmed to be positive for influenza A(H7N9) viral RNA by rRT-PCR (the Ct values were 30.13 and 28.46, respectively). Thus, the patient was diagnosed with an influenza A(H7N9) virus infection on 10 January. His subsequent specimens were positive for influenza A(H7N9) until

Figure 1

Timeline of disease in two laboratory-confirmed cases of a family cluster of influenza A(H7N9) in Guangzhou, China, January 2014



ARDS: acute respiratory distress syndrome; ICU: intensive care unit; rRT-PCR: real-time reverse-transcriptase polymerase chain reaction.

30 January. On 31 January, the patient was transferred and isolated in an general ward, and his specimens continued to be negative for influenza A(H7N9) until 4 February. On 4 February, the treatment with oseltamivir, which had been continuous since 9 January was stopped. The patient was discharged from the hospital on 12 February after he had fully recovered.

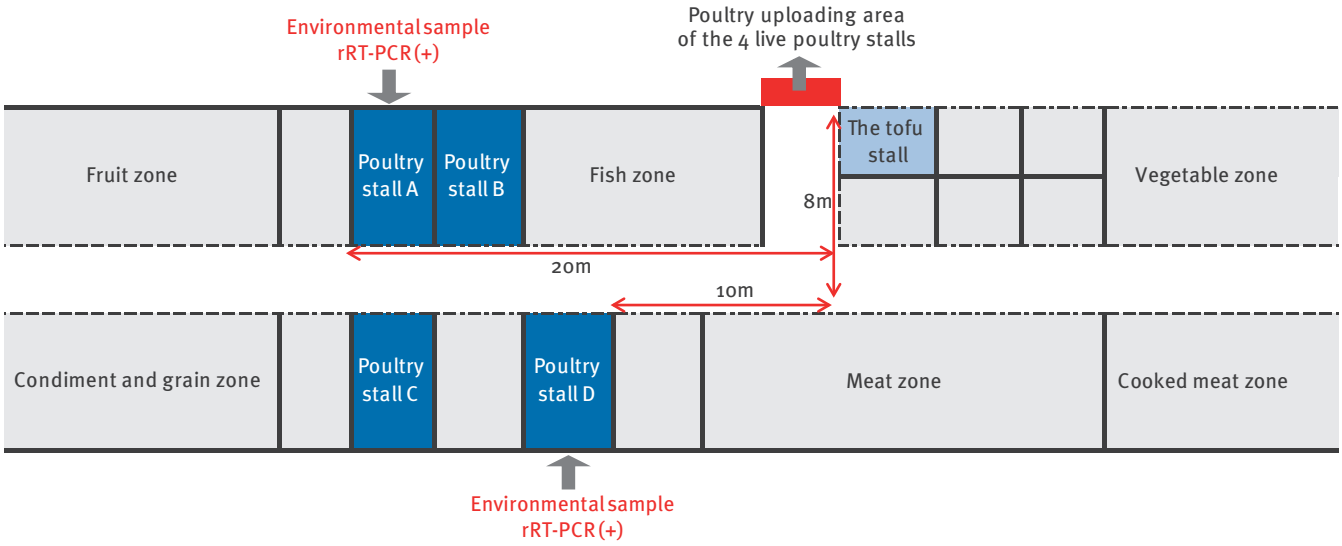
The second patient, the elder child of the index case who was five years-old and healthy without chronic diseases, did not show any influenza symptoms when the father was laboratory-confirmed with influenza A(H7N9). The first throat swab collected on 10 January was negative for influenza A(H7N9) viral RNA, but the child developed a fever (38.4°C) accompanied by cough and fatigue on 14 January. A throat swab sample collected on this day was positive for influenza A(H7N9) viral RNA (Ct value: 31.23). Thus, according to Prevention and Control Protocol for Human Infections with Avian Influenza A(H7N9) (China CDC, 2014), whereby all confirmed cases must be hospitalised in isolation and treated, the child was admitted to an isolated ward of a designated hospital and given oral oseltamivir therapy (45 mg twice daily). A further throat swab sample collected on 16 January was still positive for influenza A(H7N9) viral RNA (Ct value: 32.55) and the patient did not show any other co-morbidities. After 16 January, body temperature returned to normal, and the symptoms subsided. After two throat swab samples collected on 17 and 19 January were negative for influenza A(H7N9) viral RNA, the continuous treatment with oseltamivir from 14 January was stopped and the child was discharged from the hospital on 21 January 2014 with full recovery.

Epidemiological investigations of the two cases

The index case lived with his parents, wife and children, in a seven-story building located in a crowded community. He lacked direct contact with known sick persons, and his family did not buy and cook any poultry in the 12 days prior to the onset of illness. According to our investigation, no poultry, birds or pigs were raised in their residential district within a range of approximately 100 metres. However, he and his wife had been working in a tofu stall located in a retail wet market for several years. He worked at his stall for approximately 11 hours per day (from 7 am to 1 pm and 4 pm to 9 pm) and otherwise remained at home every day before 4 January. As shown in Figure 2, four live poultry stalls (A, B, C, D) are located within a 20-metre distance of the tofu stall in this market. Notably, the distance between the poultry unloading area of these four live poultry stalls and the index case's tofu stall was only approximately two metres. Small trucks transporting live poultry were parked near the tofu stall, and the live poultry were unloaded and moved to live poultry stalls by passing the tofu stall every morning for about an hour when the index case was working. Among the 26 environmental samples collected from these four live

Figure 2

Floor plan of the wet market where the index case of a family cluster of influenza A(H7N9) worked, Guangzhou, China, January 2014



rRT-PCR: real-time reverse-transcriptase polymerase chain reaction.

Locations of four live poultry stalls, unloading area of live poultry in the wet market, as well as the tofu stall, where the index case and his wife worked in the past four years are indicated. Twenty-six environmental samples, including 11 poultry faecal swabs, four chopping block swabs, three scalding machine swabs, two visceral waste swabs, four bloody sewage samples and two poultry drinking water samples, were collected from all four live poultry stalls of this market on 10 January 2014. A chopping block swab of stall A and a bloody sewage sample of stall D were positive for influenza A(H7N9) viral RNA.

poultry stalls, one chopping block swab sample from poultry stall A and one bloody sewage sample from poultry stall D were identified as positive for influenza A(H7N9) viral RNA.

The second case, the index case's five-year-old child, was taken care of by the grandparents, who were well aware of the child's daily activities. They confirmed that the child neither visited any wet market nor had contact with any poultry or birds in the 12 days prior to the child's disease onset. However, from 4 January when the father developed symptoms and had to rest at home, to 8 January, when he developed a severe cough with heavy sputum and progressive shortness of breath that resulted in his admission to hospital, the child had very close and unprotected contact with the sick father every day, such as embracing, kissing, playing, talking face to face, watching television, eating and taking naps together.

Close contacts monitoring

A total of 40 close contacts of the two cases, including the other four family members, three additional relatives, four friends, 24 healthcare workers and five patients at the tertiary referral hospital (Table), were recruited for medical monitoring. The younger sibling of the index case, who visited Guangzhou during the 4 to 8 January period, and the other four family members had very close contact with the two cases. Specifically, the index case's wife provided unprotected care to the index case at home and even bedside care at the hospital until the index case was transferred to the ICU. We collected throat swab samples from the index case's wife daily from 10 to 21 January, but all 12 throat swab samples collected were negative for influenza A(H7N9) viral RNA.

During the seven days of medical observation, fever, conjunctivitis, diarrhoea and respiratory symptoms were not detected in any of the 40 close contacts. Throat swab samples (at least two samples were collected from four family members and the index case's younger sibling who had very close contact with the patients) from these close contacts were also negative for influenza A(H7N9) viral RNA. The results of the HI assay showed that the paired serum samples of all 40 contacts were negative, i.e. the influenza A(H7N9) virus HI titre was lower than 10 (Table).

In addition, we expanded our medical monitoring to 15 occupational poultry workers of the four live poultry stalls at the wet market. None of these workers showed abnormal symptoms during the seven-day medical observation, and all 15 throat swabs collected from these poultry workers were also negative for influenza A(H7N9) viral RNA. We also conducted a serological investigation of these poultry workers and found their paired serum samples to be all negative.

Haemagglutinin gene sequencing and phylogenetic analysis

The influenza A(H7N9) virus was isolated from the throat swab samples collected from the index case (A/Guangzhou/1/2014(H7N9)), the second case (A/Guangzhou/2/2014(H7N9)) and the environmental samples from the wet market (A/environment/Guangzhou/1/2014(H7N9)). The sequencing results of the full length HA ORFs of these three isolated strains showed that the genetic sequences were identical (Figure 3). Moreover, the positions of the cleavage site, RBS and glycosylation motifs did not differ from those of the influenza A(H7N9) virus strains isolated during the first outbreak of avian influenza A(H7N9) virus in eastern China [1-4, 8-14]. However, the similarity of the HA gene was 99.57% between these three isolates and a strain isolated early from chickens in Guangzhou (Figure 3). These virological results strongly supported that the index case acquired the influenza A(H7N9) virus infection in the contaminated environment of the wet market where he worked, and subsequently transmitted the virus to his elder child at home.

Discussion

In the second wave of the influenza A(H7N9) outbreak in China, a family cluster of two influenza A(H7N9) cases was identified. The index case was the first case of influenza A(H7N9) virus infection in Guangzhou because no influenza A(H7N9) case had been previously identified in Guangzhou during the first epidemic wave. He most likely acquired the infection directly from the contaminated environment of the wet market where he worked. This assumption is based on the following findings: (i) he was not exposed to sick persons and had no direct contact with live poultry during the 12 days prior to his disease onset; (ii) the environment of the wet market, where he worked for approximately 11 hours every day prior to his disease onset, was shown to be contaminated by influenza A(H7N9) virus; and (iii) the sequence of the HA gene of the virus strain isolated from the environmental sample completely matched that isolated from this index patient. In fact, 65% (15/23) of influenza A(H7N9) confirmed cases in Guangzhou had a history of wet market exposure, suggesting that contaminated wet markets might be the main source of influenza A(H7N9) virus infection (data not shown). In contrast, the second case, the five-year-old child of the index case, likely acquired the influenza A(H7N9) virus infection from the sick father because of the following facts: (i) the child had not visited any wet market and lacked contact with poultry and birds during the 12 days prior to disease onset; (ii) the child had unprotected and very close contact with the sick father, even after the father's disease onset; (iii) the first throat swab sample collected from the child six days after the father's disease onset was negative for influenza A(H7N9) viral RNA, but the second throat swab sample collected six days after the last exposure to the sick father was positive for the viral RNA; and (iv) the HA gene of the virus strains isolated from father and child were identical.

Table

Information on close contacts of two cases of a family cluster of influenza A(H7N9), Guangzhou, China, January 2014

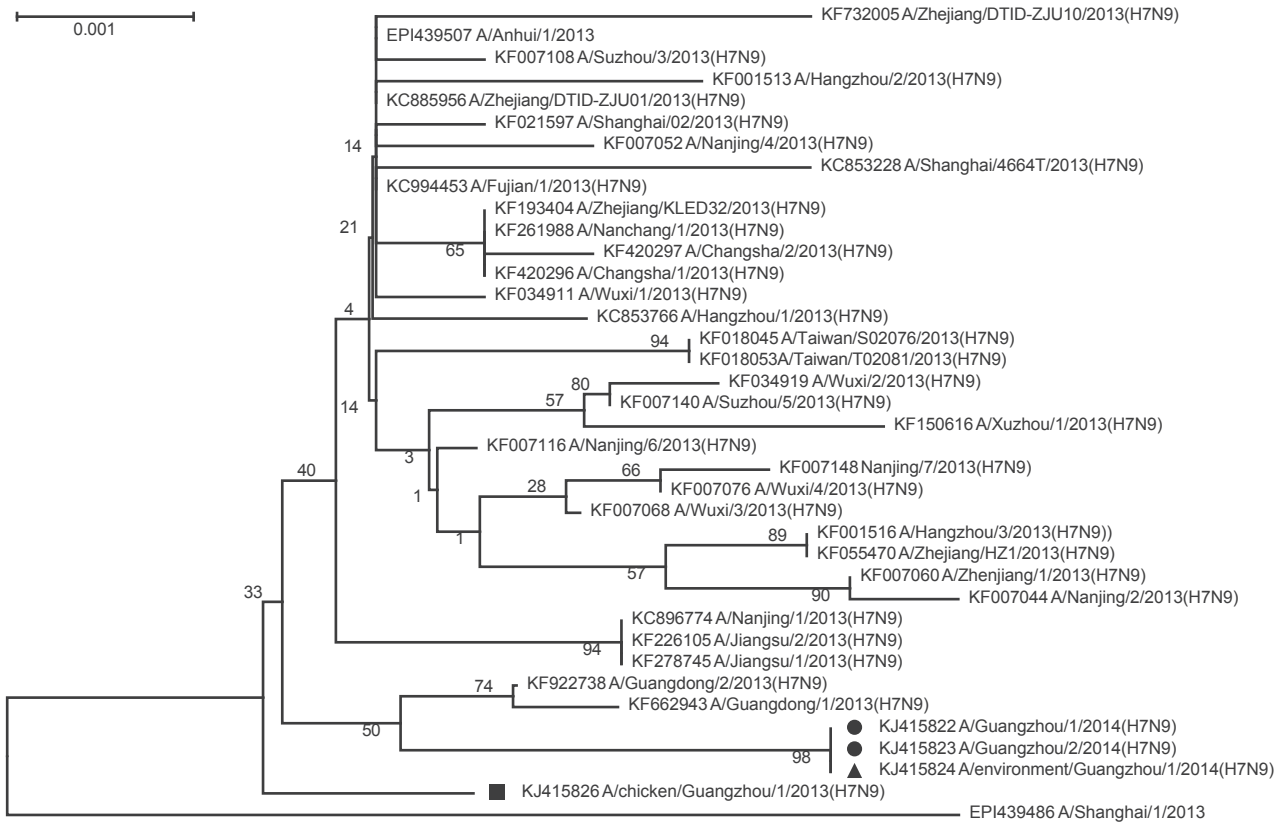
Classification of close contacts	Relations with the index case/ approximate age (years)	Contact with which case	Description of contact	Result of HI assay[a]	
				Date of sampling /titre	Date of Sampling /titre
Five family members of the index case	Wife/late 20s	Both	Provided close care at home and unprotected bedside care at the hospital to the index case; Provided conventional care at home to the second case	10 Jan / <10	15 Feb / <10
	Mother/early 60s	Both	Provided conventional care at home the both cases	10 Jan / <10	15 Feb / <10
	Father/late 60s	Both	Family daily life contact with both cases	10 Jan / <10	15 Feb / < 10
	Child/5	Index case	Family daily life contact with the index cases	10 Jan / <10	15 Feb / 20
	Child/ <5	Both	Family daily life contact with both cases	10 Jan / <10	15 Feb / < 10
Three relatives of the index case	Cousin/early 20s	Index case	Worked with the index case and accompanied him to seek medical care	10 Jan / <10	15 Feb / < 10
	Younger sibling/early 20s	Both	Visited the index case after his hospitalisation; Provided conventional care at home to the second case	10 Jan / <10	15 Feb / < 10
	Uncle/late 50s	Second case	Family daily life contact with the second case	16 Jan / <10	15 Feb / < 10
Four friends of the index case	One friend/late 40s	Second case	Talking and playing with the second case	16 Jan / <10	16 Feb / < 10
	Three other friends/mid to early 30s	Index case	Visited the index case after his hospitalisation	11 Jan / <10	16 Feb / < 10
Twenty-four healthcare workers	Ten staff of hospital A/mid-40s to mid-70s	Index case	Provided various medical services without effective personal protective equipment	11 Jan /<10	16 Feb / < 10
	Two staff of hospital B/early 40s to early 60s			11 Jan / <10	16 Feb / < 10
	Two staff of hospital C/early 20s			11 Jan / <10	16 Feb / < 10
	Ten staff of tertiary referral hospital/ early 20s to mid-50s			10 Jan / <10	16 Feb / < 10
Five patients of tertiary referral hospital	Roommates/early 40s to early 80s	Index case	Shared the same room with the index case before his isolation	10 Jan / <10	16 Feb / < 10

HI: haemagglutination inhibition.

[a] Paired serum samples were collected on 10–16 Jan (week 1) and 15–16 Feb (week 4), respectively.

Figure 3

Phylogenetic analysis of haemagglutinin (HA) genetic sequences obtained in the investigation of a family cluster of avian influenza A(H7N9), Guangzhou, China, January 2014 (n=38 sequences in total in the phylogenetic tree)



● HA genes of two influenza A(H7N9) virus strains isolated from two patients reported in this manuscript
▲ HA gene of the virus strain isolated in this study from environmental samples collected in the wet market (Figure 2)
■ Virus strain isolated from chicken earlier at a wet wholesale poultry market of Guangzhou

Bootstrap values are indicated on the tree nodes.

Several family clusters of confirmed avian influenza A(H7N9) virus infection have been reported previously [2,14-16]. Compared to these reports, our investigation of this family cluster has provided clearer and more solid evidence that the second case acquired the infection of influenza A(H7N9) virus from the sick father. The influenza A(H7N9) virus can reportedly bind to both avian-type (α 2, 3-linked sialic acid) and human-type (α2, 6-linked sialic acid) receptors [21] and replicate efficiently in mammals and human airway cells [22,23], which suggest that the virus possesses the potential for person-to-person transmission. In fact, limited person-to-person transmission of other subtypes of avian influenza, such as influenza A(H7N7) [24] and A(H5N1) [25-28], has been reported previously. Moreover, more than 90% of secondary person-to-person transmission of influenza A(H5N1) virus occurred in blood-related persons, especially in first-degree blood-related persons [29], suggesting a genetic basis for the susceptibility to influenza A(H5N1) virus infection. Similarly, our study and those previously published [2,14-16] have also shown that the person-to-person transmission of influenza A(H7N9) virus occurred in closely related persons. Cases of influenza A(H7N9) transmission between genetically unrelated persons have not yet been reported. Fortunately, the person-to-person transmission of influenza A(H7N9) has been very limited thus far. In this study, the other four family members, i.e. the parents, wife and younger child of the index case, did not acquire the infection with influenza A(H7N9) virus, although they had unprotected and very close contact with him. Moreover, among these family members, the index case's parents and other child, who were genetically closely related to him, were not infected by the virus.

Notably, the symptoms were much milder in the second case than in the index case, and the throat swab samples of the second patient were positive for influenza A(H7N9) viral RNA for only three days after this child's disease onset, while those of the index case were positive for approximately 22 days after his disease onset. In light of the fact that milder disease has

been reported for some young children, the outcome of influenza A(H7N9), in function of age warrants further observation. The symptom duration and virus persistence in patients who acquired the infection from other avian influenza patients may moreover be an index to monitor the adaptation of the virus for infection and growth in humans. In the event reported here, the genetic sequencing data of the virus isolates from the two cases showed no adaptive changes in the RBS and glycosylation motifs of HA. Although we did not investigate internal genes, previous studies on influenza A(H7N9) viral sequences causing human infections only point to the E627K mutation of the polymerase subunit PB2, as potentially contributing to transmission among humans [9]. Thus, the influenza A(H7N9) virus might not yet be well adapted for human infection, and the risk for community-level spread of this virus is still considered to be very low.

In summary, our clinical, epidemiological and virological findings strongly implicate that the contaminated live poultry market might be the most possible source of influenza A(H7N9) virus infection for the index case. As a tofu seller, the index case had a good habit of hand hygiene. However if sufficient contaminated aerosolised materials were inhaled by this case, airborne transmission of influenza A(H7N9) virus could have occurred. Remarkably, our study indicated that the second case most likely acquired the virus from the index case. Fortunately, the investigation also suggested that the person-to-person transmission of influenza A(H7N9) was still highly limited. However, the influenza A(H7N9) virus reportedly continues to undergo reassortment [30], the strict surveillance of the influenza A(H7N9) virus infection, including monitoring the symptoms and viral load in patients who acquired the infection from person-to-person transmission and genetically unrelated persons who have had close contact with patients, should be continued.

Acknowledgments

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Conflict of interest

None declared.

Authors' contributions

Ming Wang and Zhicong Yang conceptualised the study design and supervised the study. Ming Wang was responsible for epidemiological survey, Xincai Xiao, Zongqiu Chen, Jun Yuan, Shuanglang Ye, Hui Liu and Jianyun Lu participated in field investigation and sample collection. Hongbing Luo, Zhi Nie and Xiaoping Tang acquired clinical data, Zhicong Yang,Kuibiao Li and Biao Di performed the laboratory testing, including rRT-PCR, genome sequence and phylogenetic tree analysis. Xincai Xiao, Zongqiu Chen and Bojian Zheng drafted the manuscript and other co-authors contributed to review and approved the final version.

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