

Epidemiological and virological differences in human clustered and sporadic infections with avian influenza A H7N9



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SUMMARY

Background: Previous research has suggested that avian influenza A H7N9 has a greater potential pandemic risk than influenza A H5N1. This research investigated the difference in human clustered and sporadic cases of H7N9 virus and estimated the relative risk of clustered infections.

Methods: Comparative epidemiology and virology studies were performed among 72 sporadic confirmed cases, 17 family clusters (FCs) caused by human-to-human transmission, and eight live bird market clusters (LCs) caused by co-exposure to the poultry environment.

Results: The case fatality of FCs, LCs and sporadic cases (36%, 26%, and 29%, respectively) did not differ among the three groups ($p > 0.05$). The average age (36 years, 60 years, and 58 years), co-morbidities (31%, 60%, and 54%), exposure to birds (72%, 100%, and 83%), and H7N9-positive rate (20%, 64%, and 35%) in FCs, LCs, and sporadic cases, respectively, differed significantly ($p < 0.05$). These higher risks were associated with increased mortality. There was no difference between primary and secondary cases in LCs ($p > 0.05$). However, exposure to a person with confirmed avian influenza A H7N9 (primary 12% vs. secondary 95%), history of visiting a live bird market (100% vs. 59%), multiple exposures (live bird exposure and human-to-human transmission history) (12% vs. 55%), and median days from onset to antiviral treatment (6 days vs. 3 days) differed significantly between primary and secondary cases in FCs ($p < 0.05$). Mild cases were found in 6% of primary cases vs. 32% of secondary cases in FCs ($p < 0.05$). Twenty-five isolates from the three groups showed 99.1–99.9% homology and increased human adaptation.

Conclusions: There was no statistical difference in the case fatality rate and limited transmission between FCs and LCs. However, the severity of the primary cases in FCs was much higher than that of the secondary cases due to the older age and greater underlying disease of the latter patients.

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1. Introduction

A novel influenza A H7N9 virus emerged in China in March 2013 as an unexpected cause of severe human illness.^{1,2} By January 31, 2016, 721 confirmed cases worldwide, including 285 deaths, had been reported to the World Health Organization.³ Of these, 97% (701/721) were found in mainland China. The other 3% (20/721) were imported cases identified in Hong Kong, Taiwan,

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Malaysia, and Canada, and were attributed to travel in mainland China.⁴ The case fatality rate for H7N9 has been reported as 40% (285/721), which is much lower than that of the H5N1 virus (60%), but significantly higher than that of seasonal influenza (1%). Although most of these cases had no epidemiological link, a few family clusters (FCs) were documented in the provinces of Jiangsu, Guangdong, Shandong, and Zhejiang.^{5–8} Qin et al. reported that the proportion of H7N9 human infections occurring in clusters was 8% of the total cases, which is much lower than the proportion in the H5N1 groups (20%).⁹ Genetic sequencing, glycan array receptor-binding assays, and ferret studies have shown that the H7N9 virus exhibits increased binding to mammalian respiratory cells. New risk assessment tools have also indicated that H7N9 has a greater potential pandemic risk for further mammalian adaptation with possible human-to-human transmission compared to H5N1.²

Two types of human cluster have been identified to date. One is the FC, which involves subjects who have a genetic relationship and share the same living space, and is caused by close physical contact. The other is the live bird market cluster (LC), which is caused by common exposure to an H7N9-positive environment in individuals without a blood relative relationship. However, the epidemiological and virological differences in these two clusters compared to sporadic cases are unclear.^{10–13} Based on large samples of human clusters, the current study investigated the differences in age, sex distribution, case fatality rate, exposure history, underlying diseases, and clinical severity, in addition to hemagglutinin (HA) and neuraminidase (NA) gene mutations. The aim was to identify factors associated with an increased risk of human transmission and the epidemic patterns of these clusters. This information may help control a potential global pandemic of H7N9.

2. Methods

2.1. Ethics statement

The present study was approved by the Zhejiang Medical Ethics Committee and the National Health and Family Planning Commission. Written informed consent was obtained from all patients and/or their families, as well as from individuals who participated in the study. The sampling activities and data collection from human cases were approved by the Medical Ethics Committee of Zhejiang Province.

2.2. Research objectives

The research objectives were to compare the epidemiology and virology of human FCs and LCs of infection with avian influenza A H7N9 virus in China and to identify the risks related to these clusters.

2.3. Case definitions⁹

In accordance with the Chinese guidelines for the diagnosis and treatment of humans infected with H7N9 avian influenza (<http://www.moh.gov.cn/mohwsyjbg/fkzs/list.shtml>), a confirmed H7N9 case was defined as a patient with an influenza-like illness (ILI) or a suspected case with respiratory specimens that tested positive for the H7N9 virus by either (1) the isolation of the H7N9 virus or positive results by real-time reverse-transcriptase PCR (rRT-PCR) assay for H7N9, or (2) a four-fold or greater rise in antibody titre for the H7N9 virus based on testing of an acute serum specimen (collected 7 days or less after symptom onset) and a convalescent serum specimen (collected at least 2 weeks later).

A cluster was defined as two or more persons with an onset of symptoms within the same 14-day period, who were associated

with a specific setting, such as a classroom, workplace, household, extended family, hospital, other residential institution, military barracks or recreational camp, and live bird markets. When the cluster was identified in a household, this was defined as a FC; when the cluster was found in those patients co-exposed to the same live bird market in a 14-day period, this was defined as a LC.

An index case is defined as the initial patient in the population of an epidemiological investigation, or more generally the first case with the condition or syndrome (not necessarily contagious) to be described in the medical literature, whether or not the patient is thought to be the first person affected. The index case may indicate the source of the disease, the possible spread, and the reservoir that holds the disease between outbreaks; this is the first patient to indicate the existence of an outbreak. A secondary case is defined as the occurrence of a disease due to close contact with a primary case patient in the 2 weeks after onset of illness in the primary case. A secondary case is the next stage to the primary case.

A mild case of H7N9 was defined as an individual with a confirmed H7N9 virus infection who met the respiratory infection criteria and presented with mild respiratory symptoms and no complications (such as acute respiratory distress syndrome (ARDS), multi-organ failure, or hypoxemia) throughout the clinical course.⁹

A severe case of H7N9 was defined as an individual with a confirmed H7N9 virus infection who met any one of the following criteria: presenting with severe respiratory symptoms with any complication (including ARDS, shock, multi-organ failure, or hypoxemia) and requiring hospitalization, intensive care unit admission, or mechanical ventilation for medical reasons.⁹ The objective index is as follows: (1) X-ray showing lesions in multiple lobes or disease progression > 50% within 48 h; (2) dyspnoea with a respiratory rate > 24 breaths per min; (3) hypoxemia with oxygen saturation \leq 92% on oxygen at a flow rate of 3–5 l/min; (4) shock, ARDS, or multiple organ dysfunction syndrome. Patients with a confirmed H7N9 virus infection are critically ill and approximately 20% die of ARDS or multi-organ failure.³

A contact was defined as an individual who (1) did not take protective measures in the diagnosis and treatment of suspected or confirmed cases, or took care of the patient; (2) lived together or was in close contact with a suspected or confirmed case within 14 days of illness onset; or (3) were investigators who had close contact with an index case but did so without any protective gear.

A blood relative contact was defined as parent–offspring, siblings, grandparent–grandchild, and uncle/aunt–niece/nephew, who shared the same living space. A non-blood relative contact was defined as a spouse, healthcare worker, son/daughter-in-law, parent-in-law, and other unrelated household member, who shared the same living space.⁹

2.4. Study design

2.4.1. Data source

In mainland China, all laboratory-confirmed H7N9 cases are reported to the Chinese Centre for Disease Control and Prevention (China CDC) through a national system for reporting notifiable infectious diseases. A total of 17 FCs (five from Zhejiang Province, three from Guangdong Province, two each from Shanghai, Hunan Province, and Shandong Province, and one each from Beijing, Jiangsu Province, and Guangxi Province) involving 39 confirmed H7N9 cases, and a total of eight LCs from Zhejiang Province involving 19 confirmed H7N9 cases were identified in a total of 454 confirmed cases as of December 2014 and included in this research (**Supplementary Material**, Figure S1). In addition, 72 sporadic cases occurring in Zhejiang Province were selected as a control group.

2.4.2. Epidemiological investigation

The epidemiological data of all confirmed cases in Zhejiang Province used in this study were collected by field staff for the purpose of this study. Under the Chinese avian H7N9 influenza surveillance system, once a suspected case is confirmed to be H7N9-positive, a joint field investigation team comprising staff from the local or provincial level CDC and/or the China CDC conduct field investigations of the laboratory-confirmed cases of H7N9 virus infection. Demographic, epidemiological, and basic clinical data on the H7N9 cases are collected using standardized forms. An integrated database is constructed by the China CDC and Zhejiang CDC. Field investigators interview the confirmed case(s) and/or their relatives to determine the exposure history 2 weeks before onset, the source of the infection, clinical course, and epidemiological information including occupation, smoking habit, and history of exposure to birds and symptomatic contacts. In addition, the close contacts are monitored daily for 14 days for symptoms of illness. All available medical records were provided by local clinical healthcare workers.

2.5. Laboratory confirmation

Specific RT-PCR assays for seasonal influenza viruses (H1, H3, and B) and avian influenza (H5N1 and H7N9) were performed as described previously.¹⁴ All specimens positive for viral RNA were inoculated into Madin–Darby canine kidney (MDCK) cell cultures for viral isolation. Viral genetic sequences were obtained directly from clinical specimens or from virus isolates, using an Illumina MiSeq Personal Sequencer, as described by Gao R et al. previously.¹

2.6. Statistical analyses

All statistical analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC, USA). Analysis of variance (*F*-test) was applied to the measurement data. Chi-square tests were applied to compare the distribution of the different variables of qualitative measurements among the three groups. All *p*-values were two-sided and subject to a local significance level of 0.05.

3. Results

3.1. Epidemiological comparison among FCs, LCs, and sporadic cases

A total of 25 clusters (58 confirmed cases) were included, of which 17 were FCs (involving 39 cases) reported across China from March 2013 to December 2014 and eight were LCs (involving 19 cases) reported in Zhejiang Province as the control group. The percentage of cluster cases to total cases was 9% (39/454) for FCs and 4% (19/454) for LCs, and the cluster size ranged from two in the LCs to four in the FCs (Figure 1; **Supplementary Material**, Figure S1, Table S1).

The mean age in the FCs was 36 years (range 1–87 years), in the LCs was 60 years (range 32–86 years), and in the sporadic group (controls) was 58 years (range 5–84 years) ($p > 0.05$) (Figure 2a–c). Significant differences were found in occupation, smoking habits, comorbidities, exposure history, and the percentage of symptomatic contacts among the three groups ($p < 0.05$) (Table 1).

3.2. Epidemiological comparison between the primary and secondary cases

Twenty-five index cases were identified, with 17 primary cases from FCs and eight primary cases from LCs. A total of 33 secondary cases were also found, including 22 from FCs and 11 from LCs. In the LCs groups, the mean age of the eight primary cases was 64 years (range 40–84 years) and of the 11 secondary cases was 58 years (range 32–86 years) ($p > 0.05$, difference not significant).

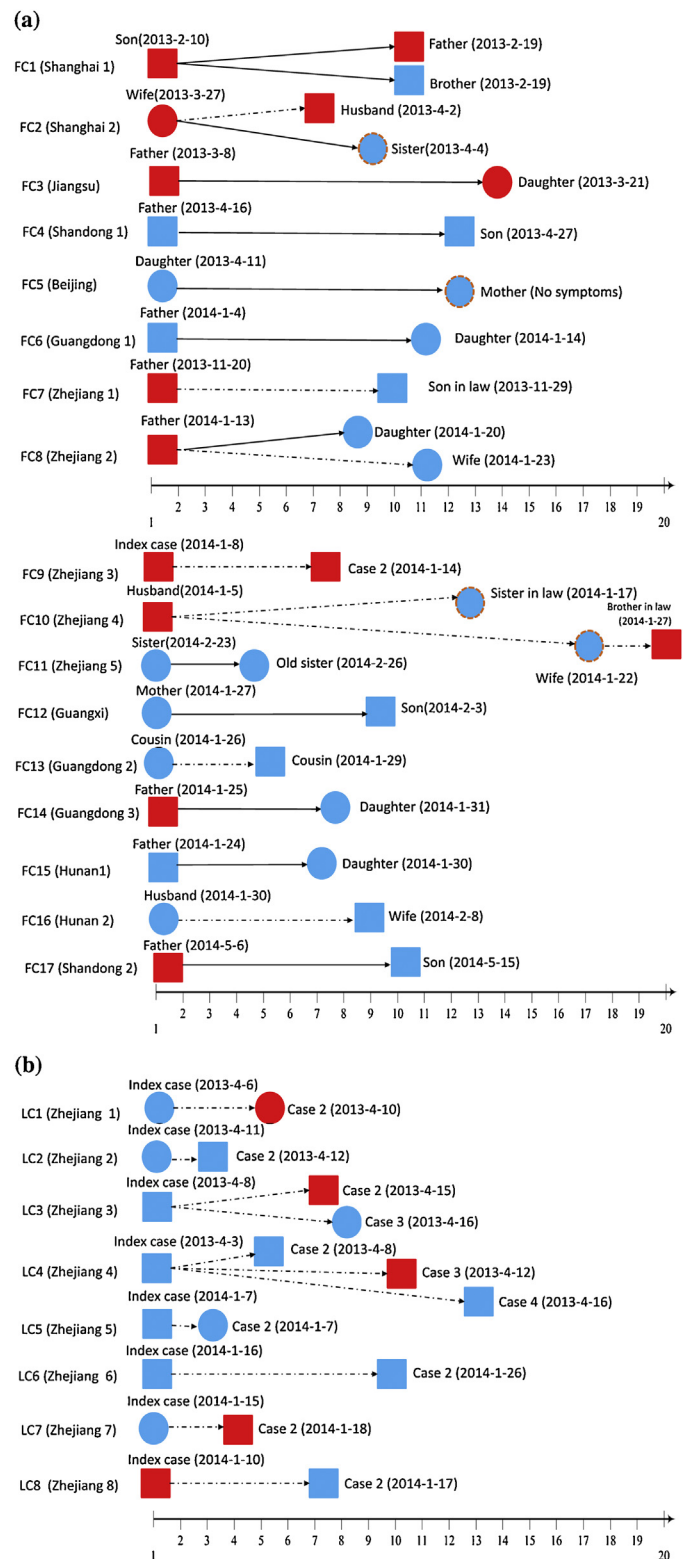


Figure 1. Cluster pedigree showing eight of the live bird market clusters and 17 of the family clusters infected with avian influenza A H7N9 in China from March 2013 to December 2014: (a) 17 family clusters (FCs); (b) eight live bird market clusters (LCs).

In contrast, there was a significant difference in mean age between the 17 primary cases (39 years, range 1–61 years) and the 22 secondary cases (34 years, range 2–87 years) in the FCs group ($p < 0.05$) (Figure 2d, e).

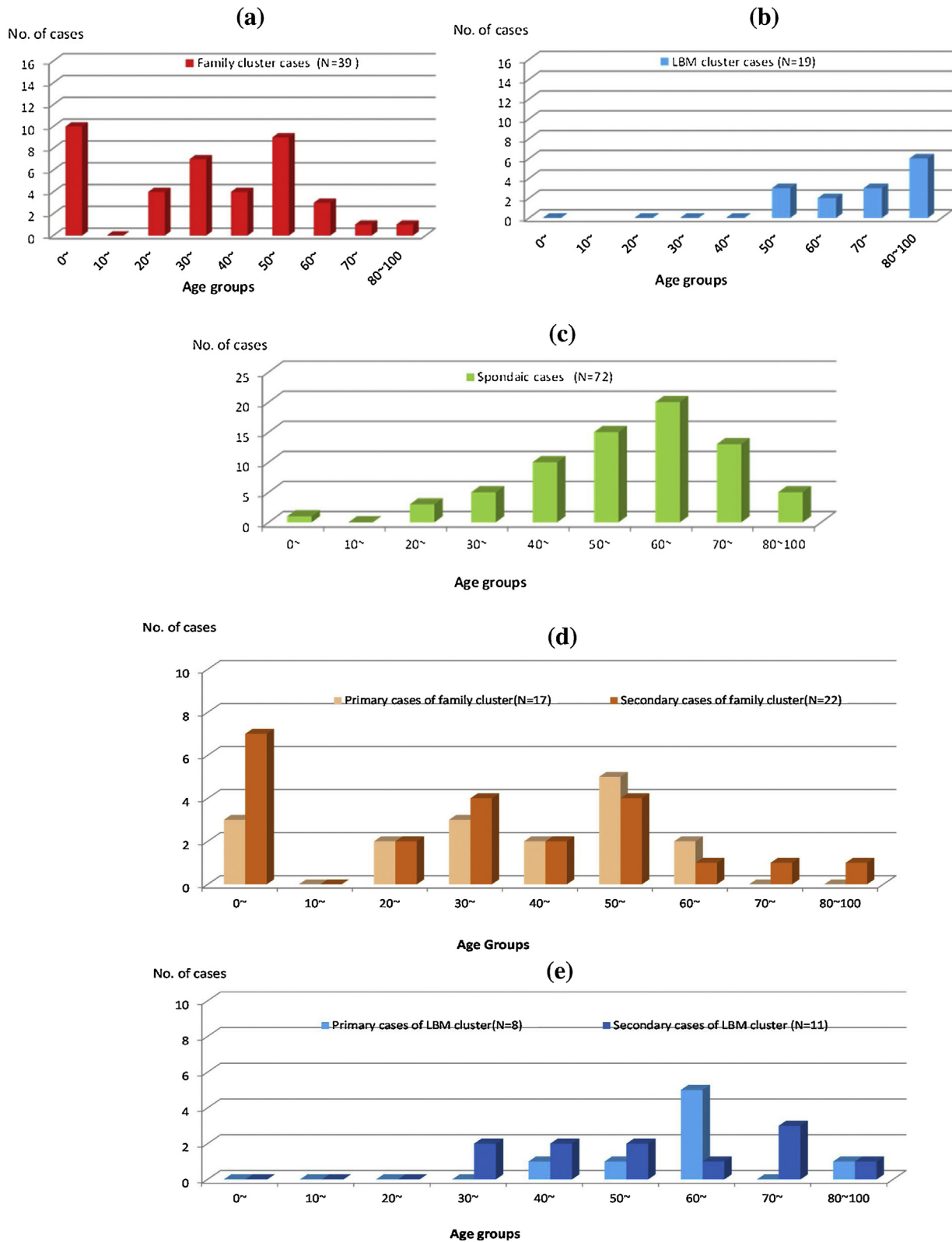


Figure 2. Age distribution among 17 family clusters (including 17 primary cases and 22 secondary cases), eight live bird market clusters (including eight primary cases and 11 secondary cases), and 72 sporadic cases infected with avian influenza A H7N9 in China from March 2013 to December 2014: (a) total family cluster cases; (b) total live bird market cluster cases; (c) total sporadic cases; (d) primary and secondary cases from the family clusters (light yellow denotes primary cases and dark yellow denotes secondary cases); (e) primary and secondary cases from live bird market clusters (light blue denotes primary cases and dark blue denotes secondary cases).

Table 1
Comparison of 17 family clusters (39 cases), eight live market clusters (19 cases), and 72 sporadic cases with avian influenza A H7N9 virus in China

Epidemiological characteristics	Family cluster cases (n = 39)	Live bird market cluster cases (n = 19)	Sporadic cases (n = 72)	F-value/Chi-square value	p-Value
Number of clusters	17	8	0		
Percentage of cluster cases in total cases, n (%)	39/454 (9)	19/454 (4)	-		
Size of cluster, n	2–4	2–5	1		
Source area, n (%)				2.102	0.350
Urban	6 (35)	3 (38)	38 (53)		
Rural	11 (65)	5 (63)	34 (47)		
Age, years, mean (range) ^a	36 (1–87)	60 (32–86)	58 (5–84)	20.099	0.000
Sex, n (%)				1.64	0.440
Male	22 (56)	13 (68)	49 (68)		
Female	17 (44)	6 (32)	23 (32)		
Occupation, n (%) ^a				27.08	0.000
Farmer	10 (26)	4 (21)	34 (47)		
Retired worker	9 (23)	7 (74)	14 (19)		
Preschool/school	11 (28)	0 (0)	1 (1)		
Other	9 (23)	8 (42)	23 (32)		
High risks, n (%)					
Smoking ^a	3 (8)	2 (11)	5 (7)	260	0.000
Alcohol drinking	4 (10)	2 (11)	1 (1)	5.059	0.08
Chronic comorbidities, n (%)	16 (41)	10 (53)	35 (49)	3.579	0.466
One comorbidity	11 (69)	4 (40)	16 (46)		
Two or more comorbidities	5 (31)	6 (60)	19 (54)		
Chronic lung disease (COPD, TB, asthma) ^a	1 (6)	3 (30)	1 (3)	8.677	0.013
Chronic cardiac disease (hypertension, coronary disease) ^a	3 (19)	8 (80)	26 (74)	12.07	0.002
Metabolic disease (diabetes, hyperthyroidism)	3 (19)	2 (2)	14 (40)	3.098	0.212
Chronic immunosuppressive disorder ^a	8 (50)	2 (20)	2 (6)	9.541	0.008
Other	2 (13)	6 (60)	7 (20)	9.279	0.010
Chronic drug history, n (%)	7 (18)	6 (32)	13 (18)	1.865	0.394
History of exposure to birds, n (%) ^a	28 (72)	19 (100)	60 (83)	7.096	0.029
Contacts, n (%)	272	253	717		
Symptomatic contacts ^a	7 (3)	14 (6)	6 (1)	34.85	0.000
Contacts with RNA positive	5 (2)	0 (0)	0 (0)		
Case fatality rate, n (%)	14 (36)	5 (26)	21 (29)	0.745	0.689

COPD, chronic obstructive pulmonary disease; TB, tuberculosis. Analysis of variance (F-test) was applied to weigh the quantity data statistically. Fisher's exact test was used in the analysis of contingency tables when the sample sizes were small. The Chi-square test was applied to compare the distribution of the different variables of qualitative measurements.

^a The difference between the two groups was significant ($p < 0.05$).

Interestingly, a genetic relationship compared to a non-genetic relationship was found in 1.8:1 vs. 0:19 in FCs and LCs, respectively ($p < 0.05$). The median number of days from onset of the primary case to onset of the secondary case was 9 days for FCs and 5 days for LCs (Table 2).

3.3. Comparison of exposure history among LCs, FCs, and sporadic cases

Analysis of the infection source indicated that 100% (8/8) of LCs were caused by co-exposure to live bird markets. In contrast, a small fraction (6%, 1/17) of FCs were caused by co-exposure to live bird markets only, whereas 88% (15/17) were caused by person-to-person transmission (i.e., secondary cases had contact with primary cases, such as through bedside care or sharing a bedroom) and 29% (5/17) were caused by both. The difference between the two groups was significant ($p < 0.05$) (Supplementary Material, Table S2). Collectively, there was an obvious difference in the type of exposure to poultry, exposure frequency, and the H7N9-positive rate for those exposed to live bird markets among the three groups (Table 2).

3.4. Clinical comparison among LCs, FCs, and sporadic cases

No statistical difference was identified in the clinical period from onset to outcome (death or discharge), except for the median period from onset to hospitalization, which was 3 days for the FCs, 4 days for the LCs, and 5 days for sporadic cases ($p < 0.05$) (Figure 3a).

There was no statistical difference between the primary and secondary LC cases for the clinical period. There was also no difference between the primary and secondary FC cases except for the median period from onset to antiviral treatment (6 and 3 days, respectively; $p < 0.05$) (Figure 3b).

The clinical spectrum for the LC, FC, and sporadic cases groups were as follows: asymptomatic for 0% (0/19), 3% (1/35), and 0% (0/72), respectively; mild for 0% (0/19), 26% (9/35), and 1% (1/72); severe for 74% (14/19), 37% (13/35), and 69% (50/72); and fatal (death) for 26% (5/19), 34% (12/35), and 29% (21/72) (Figure 4a).

There were no asymptomatic or mild cases in the primary and secondary cases of the LCs. Severe cases accounted for 75% (6/8) and 73% (8/11) and fatal cases (death) for 25% (2/8) and 27% (3/11) in the primary and secondary cases, respectively ($p > 0.05$) (Figure 4b, Table 2).

For the FCs, primary cases were mild (6%, 1/17), severe (35%, 6/17), and fatal (death 47%, 8/17), while secondary cases were asymptomatic (5%, 1/22), mild (32%, 7/22), severe (36%, 8/22), and fatal (death 27%, 6/22) (Figure 4b, Table 2).

3.5. Molecular evolution and mutation among LCs, FCs, and sporadic cases

Whole genome sequencing was performed for 25 representative isolates from the FCs ($n = 13$), LCs ($n = 3$), and sporadic cases ($n = 9$). Alignment of the generated sequences showed 99.1–99.9% homology at the eight genetic segments of all isolates. The clustering pattern at the HA and NA tree showed that all isolates

Table 2
Comparative epidemiology of primary cases and secondary cases among 17 family cluster and eight live market cluster cases infected with H7N9 avian influenza in China during 2013 to 2014.

Epidemiological characteristics	Family clusters (n = 39)			Live bird market cluster (n = 19)		
	Primary cases (n = 17)	Secondary cases (n = 22)	p-Value	Primary cases (n = 8)	Secondary cases (n = 11)	p-Value
Total cases	17	22		8	11	
Age, years, mean (range)	39 (1–61)	34 (2–87)	0.524	64 (40–84)	58 (32–86)	0.472
Male to female ratio	3.3:1	1:1.4	0.109	1:1	2.7:1	0.541
Occupation, n (%)						
Farmer	6 (35)	4 (18)	0.282	2 (25)	2 (18)	1.000
Retired worker	5 (29)	4 (18)	0.465	3 (38)	4 (36)	1.000
Preschool/school	4 (23)	7 (32)	0.725	0 (0)	0 (0)	1.000
Other	2 (12)	7 (32)	0.251	3 (38)	3 (27)	1.000
Exposure history, n (%)	17 (100)	22 (100)	0.725	8 (100)	11 (100)	1.000
Febrile cases ^a	2 (12)	21 (95)	0.000	0 (0)	0 (0)	1.000
Live bird market/birds ^a	17 (100)	13 (59)	0.002	8 (100)	11 (100)	1.000
Multiple exposures ^a	2 (12)	12 (55)	0.008	0 (0)	0 (0)	1.000
Clinical period, median days						
From exposure to onset	2	3	0.433	2	2.5	0.884
From onset to consultation	1	1	0.260	1	1	0.626
From onset to confirmation	8	5	0.845	7.5	6.0	0.170
From onset to admission	3	3	0.983	3.5	4.0	0.307
From onset to outcome	17	13	0.550	19	21	0.319
From onset to antiviral treatment ^a	6	3	0.004	4	4	1.000
Be hospitalized	10.5	14	0.241	19	18	0.448
Comorbidity, n (%)	7 (41)	7 (32)	1.000	5 (63)	4 (36)	0.370
Clinical severity, n (%)						
Asymptomatic	0 (0)	1 (5)	1.000	0 (0)	0 (0)	
Mild	1 (6)	7 (32)	0.106	0 (0)	0 (0)	
Severe	6 (35)	8 (36)	1.000	6 (75)	8 (73)	1.000
Fatal (death)	8 (47)	6 (27)	0.314	2 (25)	3 (27)	1.000
Unknown	2 (12)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Genetic relationship to non-genetic relationship ratio		1.8:1			0:19	
Onset from primary case to secondary cases, days		9			5	

The Chi-square test was applied to compare the distribution of the different variables of qualitative measurements.

^a The difference between the two groups was significant ($p < 0.05$).

were evolutionarily close to the other sporadic isolates, as well as to vaccine strains (A/Anhui/1/2013/H7N9).

For the HA gene, R47K was found in 0/3 isolates from the LCs, 8/13 isolates from the FCs, and 3/9 isolates from the sporadic cases. R47M was only identified in 2/9 sporadic cases and K141R was only detected in one of the isolates from the LCs (A/Zhejiang/8/2013_H7N9). Most of the HA sequences were 'G' at amino acid position 228 and 'L' at amino acid position 226. R304G was found in only one isolate from the LCs (A/Huzhou/5/2013_H7N9 S LBM) (**Supplementary Material**, Figure S2a).

NA sequence analyses showed that E120 was identified in all of the NA sequences but in only one of the FC isolates (A/Jiangsu/02/2013_H7N9), with E120 V. R294K was found in only one isolate from the sporadic cases (A/Zhejiang/LS01/2014_H7N9) and two from the FCs (A/Shanghai/CN01/2013_H7N9 and A/Shanghai/1/2013_H7N9) (**Supplementary Material**, Figure S2b).

Q591K substitutions were not found in the polymerase basic 2 (PB2) segments of the three groups, but were found in one sporadic case (A/Zhejiang/DTID-ZJU08/2013). The E627K mutation was found in 0/3 LC cases, 8/9 FC cases, and 6/9 sporadic cases. D701N was identified in 3/9 sporadic cases (**Supplementary Material**, Table S3).

4. Discussion

A difference in the circulating pattern of LCs and FCs was found. All occurrences of LCs were identified among non-family members in Zhejiang Province, in which a third of severe cases in China have been reported. LCs were found in genetically unrelated persons with a common exposure to the same markets within 2 weeks

of onset. These findings are in contrast to those of the FCs for H7N9 and H5N1, in which 90% of cases occurred in blood relatives, especially in those with a first-degree relationship, suggesting a genetic basis for susceptibility to avian influenza virus infection.^{15,16}

The epidemiological investigation indicated that the average age of those in the LCs was older than that of the H7N9 FC subjects and older than that of the H5N1 FC subjects. However, no obvious difference was found in the sex distribution among LCs and FCs. Three main reasons may explain the age distribution: (1) age-associated practices and norms; (2) biological differences between different ages; and (3) differential healthcare-seeking/access behaviour between the different groups.¹⁷ Although 60% of LC subjects had two or more underlying diseases, only 31% of FC subjects were similarly afflicted.¹⁸ The older population may become infected with H7N9 more easily because of the age-related decline in physical capacity and performance and especially in the immune system.

There were no obvious differences in mortality, source area, alcohol consumption, or positive contacts. In agreement with previous reports, the outbreak size of the two cluster types did not differ and involved two to five members; this also indicates that the reproduction number for human-to-human transmission is well below 1. Disease transmission is very similar to that of H5N1 but less than that of seasonal H1N1 influenza.¹⁹ The present study findings also support the notion that the virus still has a limited and non-sustained transmission capability. In general, the older population with a non-genetic relationship, especially with severe basic conditions, was predominant in the LC population, but transmission was very limited among LCs as well as FCs.¹⁸

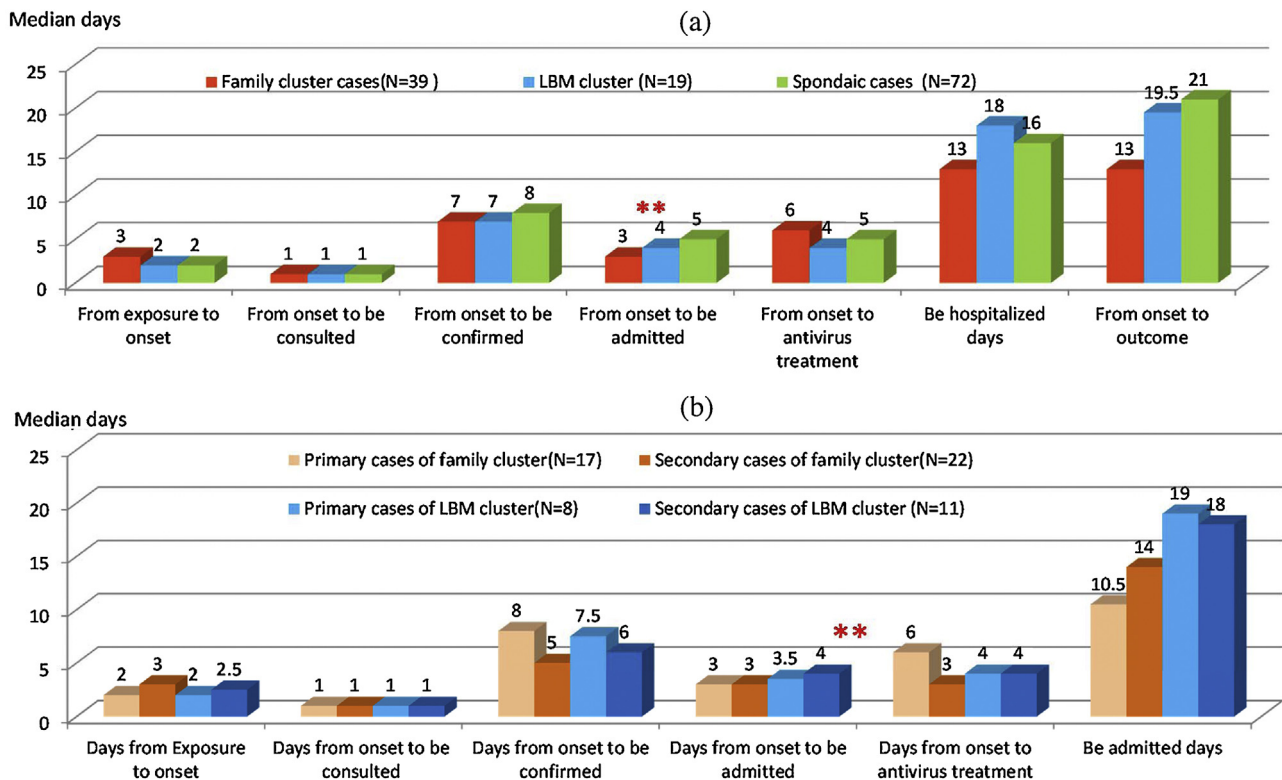


Figure 3. Median days from illness onset to outcome among 17 family clusters (including 17 primary cases and 22 secondary cases), eight live bird markets clusters (including eight primary cases and 11 secondary cases), and 72 sporadic cases infected with avian influenza A H7N9 in China from March 2013 to December 2014. (a) Total cluster cases (red denotes family cluster, blue denotes live bird market cluster, and green denotes sporadic cases): from exposure to onset $F = 0.442$, $p = 0.645$; from onset to consultation $F = 0.026$, $p = 0.974$; from onset to confirmation $F = 1.008$, $p = 0.368$; from onset to admission $F = 3.346$, $p = 0.039$; from onset to antiviral treatment $F = 0.643$, $p = 0.529$; hospitalized days $F = 0.910$, $p = 0.406$; from onset to outcome $F = 1.448$, $p = 0.242$. (b) Primary and secondary cases (for family clusters, light yellow denotes primary cases and dark yellow denotes secondary cases; for live bird market clusters, light blue denotes primary cases and dark blue denotes secondary cases); days from onset to antiviral treatment, $p = 0.004$.

Interestingly, 70% of LC subjects had not had direct contact with birds, which raises questions regarding the source and transmission route of the influenza A H7N9 virus and supports the hypothesis of aerosol transmission.²⁰ A high frequency, high dose, and repeated co-exposure/contact with highly polluted live bird markets led to a high risk for the LCs group. These results implicate wet markets as a causative link with human H7N9 infection.²¹

In terms of primary and secondary cases in the LCs, there was no obvious difference in age, sex, occupation, exposure history and median days, basic diseases, or clinical spectrum. However, in the FCs group, primary cases were infected through the live bird market, while secondary cases were generally due to person-to-person transmission.²² The mean incubation period from the primary to secondary cases in the FCs (9 days) was significantly longer than that for the LCs and other reported sporadic cases (3 days);²³ however, it was significantly shorter than that of H5N1 FCs (14 days). This may be attributed to exposure frequency, dose, and types, as well as with age, sex, susceptibility, and the immune level of the secondary case.

The analysis of clinical features showed a statistical difference in the median days from onset to hospital admission in three groups; this may have been due to delayed consultation being related to illness severity.²³ Additionally, the average number of days from onset to antiviral treatment in the primary cases of the FCs was greater than that of the secondary cases. This could be attributed to the fact that secondary cases, as close contacts, were under investigation by local public health doctors, which facilitated early diagnosis and the start of antiviral treatment once the patient had developed clinical symptoms.²⁴ Interestingly, most of the FC primary cases experienced severe and fatal

manifestations, which is in contrast to the secondary cases, who showed mild conditions. This differs from the H5N1 FCs, in which secondary cases were severe and fatal.²⁵ Furthermore, this result is in agreement with those stated previously based on clinical and epidemiological data.^{26,27}

In agreement with previous studies, sequence analysis of representative isolates from the different groups showed 99.1–99.9% homology at the eight genomic segments and phylogenetic clustering with other Chinese isolates that have been characterized as remaining antigenically similar to the candidate vaccine virus derived from A/Anhui/1/2013-like viruses.²⁷ In this study, the substitution of Q226L and G228 of HA was found in all LCs and FCs, indicating that the virus shares a high affinity and adaptability to humans. The avian influenza H7N9 virus has also shown increased transmissibility in experimentally infected ferrets compared to the H5N1 virus.^{28–31} Most of the FC cases presented with R56K (antigenic E sites) and R312K (antibody binding sites). The role of these substitutions at positions 56 and 312 (H3 numbering) in the HA segment is not well established, but this merits further study. Only three of the isolates had a mutation in the NA gene (R294K) in the three groups; this mutation confers a lack of resistance to oseltamivir and peramivir.³² The PB2 gene encodes proteins that compose the polymerase enzyme complex, which is necessary for viral replication.^{33,34} This amino acid change has been shown to increase virulence in a mouse model, increase transmission in a ferret model, and enhance virus replication efficiency in a pig model.^{35–37} The PB2 proteins isolated from FCs had mutations at position E627K, which leads to enhanced replication in the airway of mammalian hosts and possibly humans.^{1,38,39} In contrast, PB2 from H7N9 viruses isolated from LCs retained 'E' at position 627,

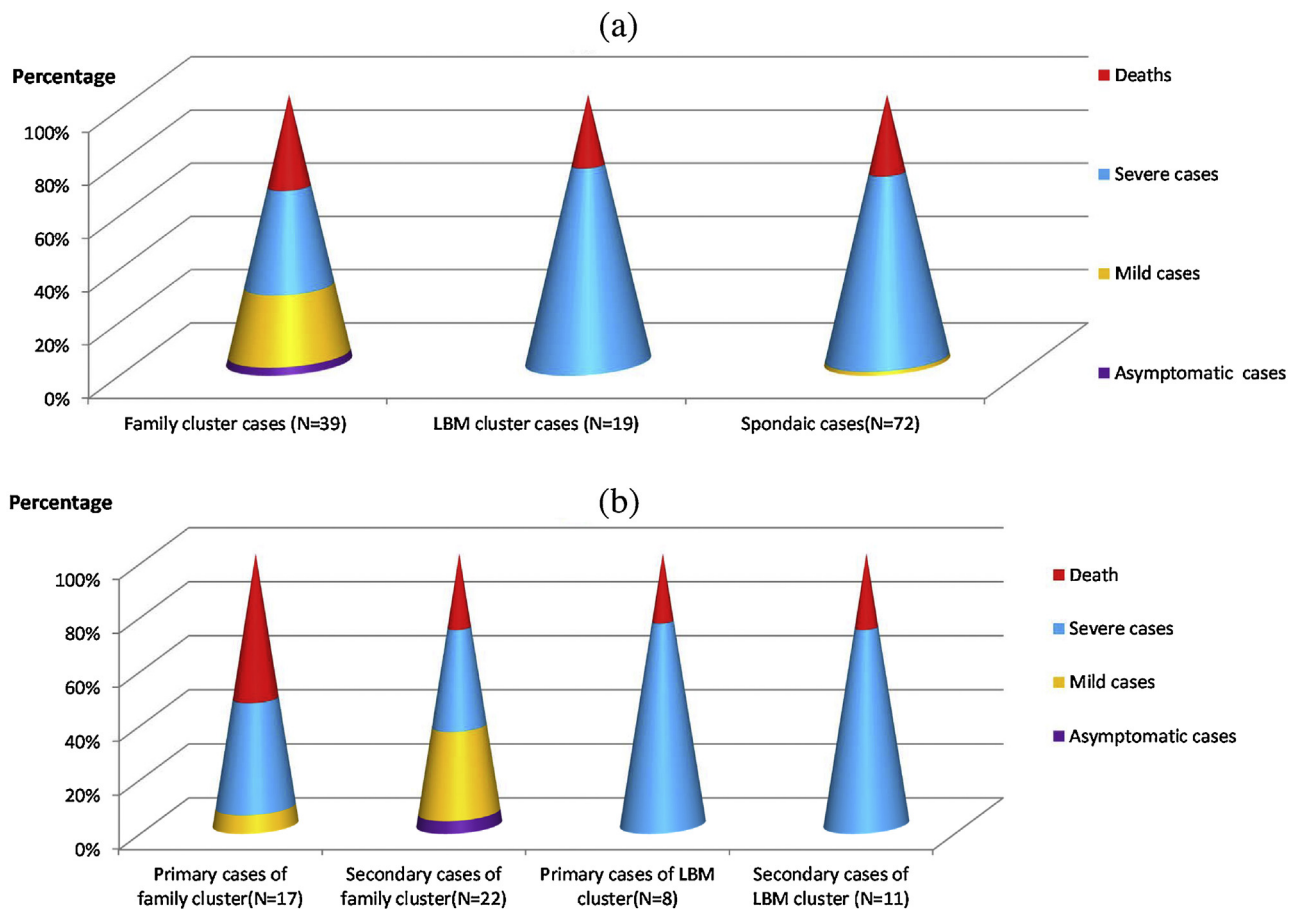


Figure 4. Clinical spectrum among 17 family clusters (including 17 primary cases and 22 secondary cases), eight live bird market clusters (including eight primary cases and 11 secondary cases), and 72 sporadic cases infected with avian influenza A H7N9 in China from March 2013 to December 2014: (a) total clustered cases (red denotes deaths, blue denotes severe cases, yellow denotes mild cases, and purple denotes asymptomatic cases); (b) primary and secondary cases in family clusters and live bird market clusters (red denotes deaths, blue denotes severe cases, yellow denotes mild cases, and purple denotes asymptomatic cases).

suggesting that the mutation is positively selected from an avian origin. Another substitution (D701N) in PB2 was found in some sporadic cases but not in cluster cases; this likely contributes to the increased disease seen in humans with H7N9 infection but does not seem to increase human-to-human transmission.

In conclusion, the findings of the present study showed that the case fatality rate was similar in the FC and LC groups compared to the sporadic cases with avian influenza H7N9 virus. Although the FC and LC cases were caused by human-to-human exposure and co-exposure to the poultry environment, respectively, there was no difference in the extent of transmission. However, the severity of disease in the primary cases in the FCs was much greater than that in the secondary cases due to the older age, more severe underlying diseases, and delayed antiviral treatment in these latter cases.

In the future, the virus will likely continue to circulate in live bird markets, animals, and humans, with the potential to spread beyond China. It is essential to take effective measures to control the source of infection, improve viral surveillance, and strengthen medical observations of close contacts.

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Author contributions: Shelan Liu and Zuqun Wu had full access to all the data in the study and drafted the manuscript. Zhao Yu, Wei Cheng, and Jianping Sha were responsible for the study concept and design. Na Zhao and Ta-Chien Chan were responsible for the analysis and interpretation of the data. Said Amer and Zhiruo Zhang performed the statistical analysis.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ijid.2016.05.022>.

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